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Original Article

Daily and short-term application of joint movement for the prevention of infrapatellar fat pad atrophy due to immobilization

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Abstract. [Purpose] To mobilize the knee joint during cast fixation and to determine whether infrapatellar fat pad changes can be prevented. [Materials and Methods] We randomly allocated Wistar rats into 3 groups as follows: normal group, raised in normal conditions (n=5); contracture group, immobilized with cast fixation (n=5); and prevention group, treated with joint movement during immobilization (n=5). We immobilized the right hindlimb using cast fixation. Joint movement in the prevention group was accomplished by repeatedly pulling the right hindlimb caudally and then returning the leg to the bent position for 10 minutes every day for 2 weeks. We used a metronome to maintain a constant speed, with one set lasting 2 seconds (1-second traction and 1-second return). [Results] The contracture group had adipose cells of various sizes and fibrosis in the infrapatellar fat pad. These changes were also found in milder forms in the prevention group. We found significant differences in the cross section of adipose cells and in knee extension restriction between the groups. [Conclusion] Promoting joint movement may not only have a therapeutic effect on adipose cells but also a preventative effect.

Key words: Prevention, Infrapatellar fat pad, Joint movement

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INTRODUCTION

Joint contractures limit movement during activities of daily living. Prevention and treatment of joint contractures is the responsibility of the physical therapist. From an orthopedic surgery perspective, the cause of limited range of joint motion is conventionally classified as inside the joint capsule (ankylosis) or outside the joint (contracture). However, in rehabilitation medicine, limited range of motion of the joint is denominated as joint contracture irrespective of the location of the responsible lesion[1)]. In practice, it is difficult to identify the responsible lesion that causes limited range of motion of a joint. In this article, the latter definition will be used.

Studies using histological observations of joint contracture using a knee fixation model in rats[2–4)] reported atrophy, fibrosis and blood congestion of adipose cells in the anterior part of the joint. It was confirmed that the infrapatellar fat pad (IPFP) moves during joint movement (hereafter referred to as "ROM-ex")[5)]. Researchers have reported that the functional role of the IPFP is 1) to promote efficient joint movement: the fat body occupies a dead space within the joint, thereby maintaining the

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joint space and promoting efficient and lubricated joint movement, and 2) to protect the joint by cushioning for joint load[6–11].

Atrophy and fibrosis of the IPFP are predicted to result in dysfunction. Promoting joint movement effectively improves joint contractures[12]. A number of reports observed this healing process, including reports on remobilization after joint fixation[13–16] and reports on treatments for joint contracture[17–19]. Studies that observed the treatment process demonstrated the therapeutic effect of joint movement with improvement in the degree of atrophy or loss of adipose cells in the anterior part of the joint by implementing ROM-ex[17] and joint mobilization[18]. Nevertheless, there are no reports on the effect of preventative interventions on the body or on histological changes. There are also reports that more advanced joint contracture makes spontaneous improvement more difficult[20]; it is important to prevent contracture before it becomes too advanced. Therefore, in this study, we performed joint movement during cast immobilization with the aim of investigating whether this can prevent IPFP changes.

MATERIALS AND METHODS

We used 15 9-week-old male Wistar rats (256–304 g). The rats were brought in at 8 weeks old, acclimatized for one week and then randomly allocated into 3 groups: one group subjected to normal rearing conditions only (hereafter referred to as "normal group") (n=5); one group subjected to immobilization with cast fixation only (hereafter referred to as "contracture group") (n=5); and one group subjected to joint movement during the immobilization period (hereafter referred to as "prevention group") (n=5). The right hindlimb was immobilized with cast fixation. Cast fixation was performed under isoflurane inhalation anesthesia, as described[2, 3, 16–18], and after fitting a home-made jacket made of urethane foam, the entire hindlimb was covered in gauze, mainly around the knee joint, to prevent scratching. The hindlimb was fixed from the pelvic girdle to the distal part of the ankle joint with the leg in the position of maximum extension of the hip joint, maximum flexion of the knee joint and maximum plantar flexion of the ankle joint (Fig. 1). The fixed limb was exposed from the distal part of the ankle joint to the toes to check for the presence of edema. The cast was rewound every day for cast fixation in both the contracture and prevention groups. The left hindlimb was left free and the rats were able to move freely around the cage using both forelegs, with full access to water and food.

Joint movement in the prevention group was performed by removing the cast fixation each day during the experiment period, and moving the right hindlimb for 10 minutes under isoflurane inhalation anesthesia. Previous studies[21] indicated that approximately 1 N of traction is required to completely extend the knee joint in rats; therefore, 1 N was adopted as the traction force for joint movement. The right hindlimb was repeatedly pulled caudally with approximately 1 N of traction, and then returned to the bent position for 10 minutes (Fig. 2). A metronome was used to keep the speed constant, with one set taking 2 seconds (1 second traction, 1 second return). The experiment period was set as 2 weeks after referencing a report indicating that atrophy and fibrosis of the IPFP were confirmed through immobilization of the joint in all groups[3].

The range of joint motion was measured under anesthesia on the final day of the experiment, after performing the intervention for the prevention group. The measurements were taken with the femur set as the basic axis and the midline of the lower leg set as the movement axis, based on a previous study[16]. The measurement method involved placing the rat in a lateral position, pulling the right hindlimb caudally with approximately 1 N of traction force and measuring the knee joint extension restriction angle using a goniometer designed for human fingers.

After completion of the experiment period, the rats were euthanized with an intraperitoneal overdose of pentobarbital sodium and the hip joint was rapidly transected to isolate the right hindlimb knee joint. The collected lower limb underwent tissue fixation for 72 hours in 10% neutral buffered formalin solution, and was then decalcified for 72 hours using Plank-

Fig. 1. Cast fixation.
Fixed from the pelvic girdle to the distal part of the ankle joint with the leg in the position of maximum extension of the hip joint, maximum flexion of the knee joint and maximum plantar flexion of the ankle joint.

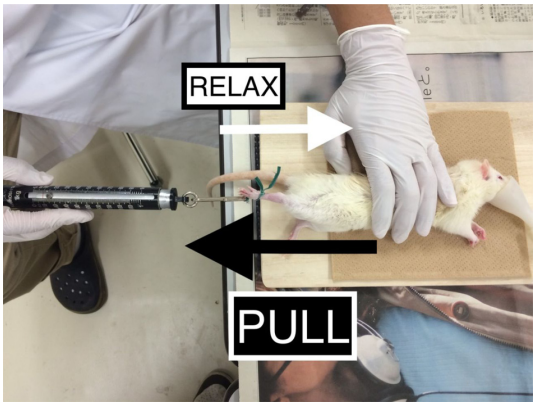

Fig. 2. Joint movement.
Joint movement involved repeatedly pulling the leg caudally with approximately 1 N of traction, and then returning the leg to the bent position.

Rychlo's solution. After decalcification, the knee joint was excised, dissecting approximately 1 cm above and below the joint. The knee joint was cut along the center of the sagittal plane to enable observation of the IPFP. The sample was then neutralized with 5% anhydrous sodium sulfate solution for 72 hours, washed in running water for 30 minutes and then immersed in 100% alcohol for approximately 3 hours for degreasing. Paraffin embedding was performed with an automatic paraffin dewatering and embedding machine. The paraffin blocks were thinly sliced to approximately 3 μm with a microtome (SM2000R, Leica) and affixed to slide glass. The samples were then stained with hematoxylin and eosin to create tissue specimens. The specimens were observed under an optical microscope (Olympus BX-51) and the IPFP between the anterior aspect of the femur and the patellar ligament was imaged with a digital camera (Olympus DP50). The observation site was set as the IPFP at the anterior aspect of the condyle of the femur based on a previous study[18], and the sample was observed at 200× magnification to ensure the adipose cells fit within the screen. Measurement of the cross-sectional area of the adipose cells involved random selection of 100 adipose cells from the captured image, referencing previous studies[4, 22, 23], and the cross-sectional area of the cells was measured using Image J (ver. 1.49). Comparison of the joint range of motion and comparison of the cross-sectional area of the adipose cells were conducted using one-way variance of analysis using SPSS (ver.25.0.0.0) and the Tukey method was used for multiple comparison tests. The level of significance was set as 5%. Changes in the adipose tissue were determined by having several observers view blinded histology images, basing determinations on the presence or absence of fibrosis in the adipose cells.

The animal rearing methods and experiments were approved by the Kanazawa University Animal Care and Use Committee (Approval number: AP-153662).

RESULTS

In the normal group, adipose cells were densely aligned in the IPFP on the anterior aspect of the femur. In a similar site in the contracture group, the adipose cells in the IPFP varied in size and had become fibrotic. Diverse sizes and fibrosis were also observed in the adipose cells in the prevention group; but the changes were milder than those seen in the contracture group (Fig. 3). The number of animals with fibrosis in the adipose tissue is shown in Table 1.

The cross-sectional area of the adipose cells was significantly different among all groups (Table 2, $p<0.05$). The limit of knee joint extension was significantly different among all groups (Table 2, $p<0.05$).

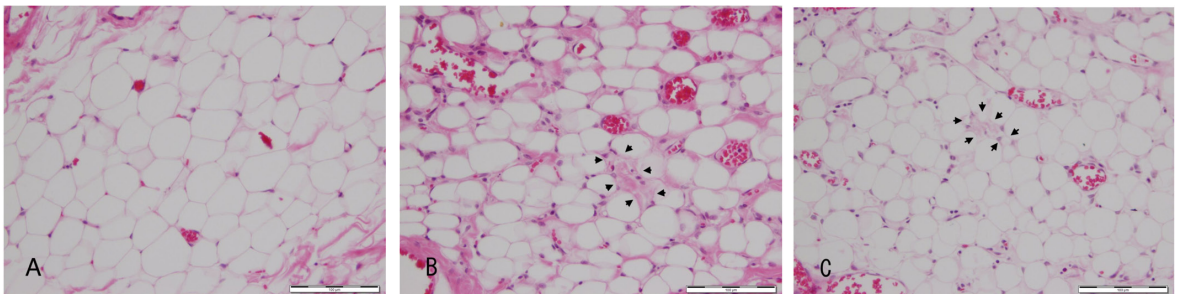

Fig. 3. Changes in adipose cells.
A: normal group, B: contracture group, C: prevention group (200× magnification).
Arrows: Typical example of fibrotic.

Table 1. Number of cases with fibrosis

	Absent	Present
Normal group	5	0
Contracture group	1	4
Prevention group	2	3

Table 2. Angle of extension limit and cross-sectional area of adipose cells

	Angle of extension limit (°)	Cross-sectional area of adipose cells (μm^2)
Normal group	19.4 ± 3.0	1,356.3 ± 275.1
Contracture group	75.4 ± 7.8*	954.6 ± 287.7*
Prevention group	60.7 ± 8.4**	1,165.0 ± 316.6**

There was a significant difference in the range of motion between all groups (normal group vs. contracture group $p<0.001$, normal group vs. prevention group $p<0.001$, contracture group vs. prevention group $p=0.01$). There was a significant difference in the cross-sectional area of adipose cells between all groups (normal group vs. contracture group, $p<0.001$, normal group vs. prevention group $p<0.001$, contracture group vs. prevention group, $p<0.001$).
*vs. normal group.
**vs. normal group, vs. contracture group.

DISCUSSION

The IPFP, also known as the Hoffa fat pad, is located within the joint capsule, but has an extra-synovial structure[24], and is located directly under the patella tendon, between the condyle of the femur and the tibial plateau[25]. The exact role of the IPFP is unknown; however, recently, the IPFP has attracted attention in areas outside the field of biomechanics. There have been reports that the IPFP plays an important role in the progression of osteoarthritis by causing changes in the cartilage and synovial membrane due to production of adipokines, cytokines, chemokines and growth factors[25]. Opinions are divided regarding whether the IPFP should be resected or preserved in total knee arthroplasty for osteoarthritis of the knee. Ye et al.[26] concluded that preserving the IPFP may provide superior outcomes to those after resecting the IPFP, in terms of the incidence of anterior knee pain and shortening of the patella tendon. White et al.[27] also stated that there was no evidence that preserving the IPFP caused harm or adverse outcomes for the patient, and they concluded that it should be preserved whenever possible. On the other hand, opinions are divided with respect to the relationship between joint range of motion and resecting or preserving the IPFP. Pinsornsak et al.[28] reported that there was no difference in postoperative joint range of motion, and Tanaka et al.[29] found that the preserved group had significantly better range of motion. In the present study, there was less change in the range of motion and in the atrophy and fibrosis of adipose cells following preventative intervention with joint movement than in the non-intervention group; however, the relationship between these two factors is unknown because other joint structural factors are also included.

In animal models, Clements et al.[30] found adipose cell necrosis and IPFP fibrosis in a rat monoiodoacetate osteoarthritis model, while Kitagawa et al.[31] found IPFP fibrosis in a rat patella tendonitis model. IPFP fibrosis occurs in arthritis models. A previous study that reported histological observations using a knee fixation model in rats, as used in this study, reported that the changes in adipose cells presented as atrophy and fibrosis[2–4]. There was atrophy of the adipose cells after joint fixation in this study as well; therefore, it is assumed that adipose cells atrophy due to immobilization. There are also a number of reports on remobilization of the joint after joint fixation that also mention adipose cells. In a study that implemented unrestricted rearing after removal of fixation in a knee fixation model in rats, Matsuzaki et al.[4] reported that no change was seen in the histology imaging even after 2 weeks of unrestricted rearing after removal of fixation in a 2-week knee fixation model in rats. Kojima et al.[16] conducted unrestricted rearing after removal of fixation in a 4-week knee fixation model in rats and found that joint range of motion had completely improved within 6 weeks; however, they reported that there was no improvement in the changes in the adipose cells, and both reported that there was no improvement in the changes in adipose cells caused by contracture. Watanabe et al.[18] reported an increase in the atrophy and loss of adipose cells when treatment intervention for joint movement was implemented after the end of fixation. Matsuzaki et al.[21] reported that preventative intervention for joint contracture, which was implemented through preventative intervention with ROM-ex for a joint fixation model, maintains the range of motion and enables reduction in the changes to the joint structure. However, that study did not mention adipose cells. Therefore, our study is the first to demonstrate the effect of preventative intervention on adipose cells. We demonstrated that joint movement reduced atrophy of adipose cells compared to non-intervention group, suggesting that encouraging joint movement not only has a therapeutic effect on adipose cells, but may also have a prophylactic effect.

We found that joint movement reduces atrophy of adipose cells in the IPFP. However, the effect mechanism remains unknown. The limitations of this study include that the observed site was limited to the IPFP located at the anterior aspect of the condyle of the femur in the center of the knee joint, and measurements were not taken at more distal, medial or lateral locations. Furthermore, the suitable time, intensity and frequency of joint movement was not investigated. These are issues for future investigation to obtain more effective therapeutic effects.

Presentation at a conference

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Funding and Conflict of interest

There are no conflicts of interest relating to this study.

REFERENCES

1) Koizumi K, Hoso M, Hamade S, et al.: Prevention and treatment of contracture, 2nd ed. Tokyo: Igakusyoin, 2008, pp 19–22 (in Japanese).
2) Yoshikubo H, Hoso M, Takemura K, et al.: Histopathological changes in synovium after two-week knee joint immobileity in rats. Rigakuryoho Kagaku, 2001, 16: 77–82 (in Japanese). [CrossRef]
3) Watanabe M, Hoso M, Takemura K, et al.: Histopathological changes in joint components during contracture—a study of the long term knee joint immobility model in the rat. Rigakuryoho Kagaku, 2007, 22: 67–75 (in Japanese). [CrossRef]
4) Matsuzaki T, Kojima S, Watanabe M, et al.: Histopathological changes in the infrapatellar fat-pad of rats with knee contracture. Ishikawa J Phys Ther, 2012, 12: 11–14 (in Japanese).

5) Hannon J, Bardenett S, Singleton S, et al.: Evaluation, treatment, and rehabilitation implications of the infrapatellar fat pad. Sports Health, 2016, 8: 167–171. [Medline] [CrossRef]
6) Belluzzi E, El Hadi H, Granzotto M, et al.: Systemic and local adipose tissue in knee osteoarthritis. J Cell Physiol, 2017, 232: 1971–1978. [Medline] [CrossRef]
7) MacConaill MA: The movements of bones and joints; the synovial fluid and its assistants. J Bone Joint Surg Br, 1950, 32-B: 244–252. [Medline] [CrossRef]
8) Teichtahl AJ, Wulidasari E, Brady SR, et al.: A large infrapatellar fat pad protects against knee pain and lateral tibial cartilage volume loss. Arthritis Res Ther, 2015, 17: 318. [Medline] [CrossRef]
9) Guilak F, Fermor B, Keefe FJ, et al.: The role of biomechanics and inflammation in cartilage injury and repair. Clin Orthop Relat Res, 2004, (423): 17–26. [Medline] [CrossRef]
10) Gallagher J, Tierney P, Murray P, et al.: The infrapatellar fat pad: anatomy and clinical correlations. Knee Surg Sports Traumatol Arthrosc, 2005, 13: 268–272. [Medline] [CrossRef]
11) Macchi V, Porzionato A, Sarasin G, et al.: The infrapatellar adipose body: a histotopographic study. Cells Tissues Organs, 2016, 201: 220–231. [Medline] [CrossRef]
12) Farmer SE, James M: Contractures in orthopaedic and neurological conditions: a review of causes and treatment. Disabil Rehabil, 2001, 23: 549–558. [Medline] [CrossRef]
13) Evans EB, Eggers GW, James K, et al.: Experimental immobilization and remobilization of rat knee joints. J Bone Joint Surg, 1960, 42A: 737–758. [CrossRef]
14) Yaoita M: [Experimental study on the genesis of knee joint immobilization induced by long-term fixation an d its remobilization]. Nippon Seikeigeka Gakkai Zasshi, 1966, 40: 431–453 (in Japanese). [Medline]
15) Trudel G, Zhou J, Uhthoff HK, et al.: Four weeks of mobility after 8 weeks of immobility fails to restore normal motion: a preliminary study. Clin Orthop Relat Res, 2008, 466: 1239–1244. [Medline] [CrossRef]
16) Kojima S, Hoso M, Watanabe M, et al.: Experimental joint immobilization and remobilization in the rats. J Phys Ther Sci, 2014, 26: 865–871. [Medline] [CrossRef]
17) Takemura K, Hoso M, Yoshikubo H, et al.: Histopathological effects of the stretching on joint components after two-week knee joint immobilization in rats. Rigakuryohogaku, 2004, 31: 76–85 (in Japanese).
18) Watanabe M, Hoso M, Kojima S, et al.: Histopathological changes in Joint components in a rat knee joint contracture model following mobilization. J Phys Ther Sci, 2012, 24: 1199–1203. [CrossRef]
19) Kaneguchi A, Ozawa J, Minamimoto K, et al.: Active exercise on immobilization-induced contractured rat knees develops arthrogenic joint contracture with pathological changes. J Appl Physiol 1985, 2018, 124: 291–301. [Medline] [CrossRef]
20) Trudel G, Uhthoff HK, Goudreau L, et al.: Quantitative analysis of the reversibility of knee flexion contractures with time: an experimental study using the rat model. BMC Musculoskelet Disord, 2014, 15: 338. [Medline] [CrossRef]
21) Matsuzaki T, Yoshida S, Kojima S, et al.: Influence of ROM exercise on the joint components during immobilization. J Phys Ther Sci, 2013, 25: 1547–1551. [Medline] [CrossRef]
22) Dupont-Versteegden EE, Houlé JD, Gurley CM, et al.: Early changes in muscle fiber size and gene expression in response to spinal cord transection and exercise. Am J Physiol, 1998, 275: C1124–C1133. [Medline] [CrossRef]
23) Gallegly JC, Turesky NA, Strotman BA, et al.: Satellite cell regulation of muscle mass is altered at old age. J Appl Physiol 1985, 2004, 97: 1082–1090. [Medline] [CrossRef]
24) Saddik D, McNally EG, Richardson M: MRI of Hoffa's fat pad. Skeletal Radiol, 2004, 33: 433–444. [Medline] [CrossRef]
25) Clockaerts S, Bastiaansen-Jenniskens YM, Runhaar J, et al.: The infrapatellar fat pad should be considered as an active osteoarthritic joint tissue: a narrative review. Osteoarthritis Cartilage, 2010, 18: 876–882. [Medline] [CrossRef]
26) Ye C, Zhang W, Wu W, et al.: Influence of the infrapatellar fat pad resection during total knee arthroplasty: a systematic review and meta-analysis. PLoS One, 2016, 11: e0163515. [Medline] [CrossRef]
27) White LD, Melhuish TM: The role of infrapatellar fat pad resection in total knee arthroplasty. Ann Rheum Dis, 2016, 75: e66. [Medline] [CrossRef]
28) Pinsornsak P, Naratrikun K, Chumchuen S: The effect of infrapatellar fat pad excision on complications after minimally invasive TKA: a randomized controlled trial. Clin Orthop Relat Res, 2014, 472: 695–701. [Medline] [CrossRef]
29) Tanaka N, Sakahashi H, Sato E, et al.: Influence of the infrapatellar fat pad resection in a synovectomy during total knee arthroplasty in patients with rheumatoid arthritis. J Arthroplasty, 2003, 18: 897–902. [Medline] [CrossRef]
30) Clements KM, Ball AD, Jones HB, et al.: Cellular and histopathological changes in the infrapatellar fat pad in the monoiodoacetate model of osteoarthritis pain. Osteoarthritis Cartilage, 2009, 17: 805–812. [Medline] [CrossRef]
31) Kitagawa T, Nakase J, Takata Y, et al.: Histopathological study of the infrapatellar fat pad in the rat model of patellar tendinopathy: a basic study. Knee, 2019, 26: 14–19. [Medline] [CrossRef]