LONG-TERM SKELETAL UNLOADING INDUCES A FULL-THICKNESS PATELLAR CARTILAGE DEFECT WITH INCREASE OF URINARY COLLAGEN II CTx DEGRADATION MARKER

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Introduction:
Much effort has been made to clarify the effects of overloading on articular cartilage and bone. However, little is known about the effects of reduced loading despite its possible contribution to the pathogenesis of certain clinical diseases, such as so-called chondromalacia patellae. In addition, the skeletal response to long-term space flight has not been elucidated even though such effects will become more crucial in the near future, especially with regard to articular cartilage. The purpose of this study is to investigate the effects of long-term skeletal unloading on articular cartilage and bone using the tail suspension model in growing rats.

Materials and Methods:
Forty-eight 9-week-old male F344/N rats were randomly divided into two groups: caged control (C) and tail suspended (TS). Hindlimbs of the TS rats were subjected to unloading for up to 12 weeks by the Morey-Holton method (1, 2) with some modification. The rats were sacrificed at 3, 6, 9, and 12 weeks. The sequential changes of the patellar cartilage and bone were analysed macroscopically and by pathological findings using hematoxylin-eosin stain and alkaline phosphatase (ALP) stain. Total cartilage area (TCA), calcified cartilage area (CCA)/TCA, bone volume (BV)/tissue volume (TV) both in the medial and lateral facets and the ALP-positive area in articular cartilage were assessed in the section at the level of the distal one-third of the patella by personal computer using NIH image 1.62. The values of cross-linked C-telopeptide of type II collagen (Col2CTx), which may be a useful index of cartilage degradation in patients with joint disease (3, 4), in 24-h urine obtained at 0, 1, 2, 3, 6, 9, and 12 weeks were also measured by ELISA. All of the levels were normalized to the creatinine level. The protocol for this study was approved by the Guiding Committee of the Center for Laboratory Animal Science, National Defense Medical College.

Results:
Macroscopic findings revealed that in the TS group the surface of the distal patellar cartilage became purlush, particularly in the medial margin at 3 weeks, suggesting a decrease in cartilage thickness. The purple color intensified at 6 weeks, and after 9 weeks a patellar cartilage defect was found. Pathological findings demonstrated that in the TS group, both the non-calcified and calcified layer thinned in the medial facet, indicating a significant decrease in thickness of the entire articular cartilage. On the other hand, the articular surface did not show any fibrillation. A full-thickness patellar cartilage defect at the margin of the medial facet was found in 94% of the TS animals after 9 weeks or more of tail suspension. In the medial facet of the patella, at 3 and 6 weeks, most of the subchondral bone marrow was directly in contact with the calcified cartilage where there was an increase in hypertrophic chondrocytes; a decrease in thickness of the entire articular cartilage was the result. TCA in the medial facet in the TS group was significantly decreased compared with the C group at 3, 6, 9, and 12 weeks (p<0.01), but no such significant differences were found in the lateral facet. CCA/TCA in the TS group significantly increased at 3, 6, 9, and 12 weeks compared with the C group. Bone atrophy was observed in the TS group, with significant decreases in BV/TV in the TS group compared with the C group at 3, 6, 9, and 12 weeks. These findings were more marked in the medial than in the lateral facet. In the TS group, the ALP-positive area in patellar cartilage increased in comparison with the C group (Fig. 1). This increase was particularly marked in the deep zone above the tidemark. Urinary Col2CTx excretion in the C group slowly decreased during the experimental period (Fig. 2) Excretion in the TS group was highest at the first week and remained significantly elevated compared with the C group; however, the difference between the two groups decreased with time (Fig. 2).

Discussion:
In the lateral facet, TCA did not show a significant change, although CCA/TCA significantly increased with skeletal unloading. These results suggested that skeletal unloading accelerated advancement of the tidemark toward the articular surface, which coincided with O’Connor’s report on the effect of skeletal unloading for four weeks on medial femoral and tibial articular cartilage (5). On the other hand, our results with regard to the medial facet demonstrated acceleration of not only advancement of the tidemark, but also of the subchondral ossification front. This study, in which unloading was applied for 12 weeks by modification of a standard method, indicated that skeletal unloading accelerated not only advancement of the tidemark, which was preceded by a high level of ALP expression in chondrocytes in the deep zone, but also that of the subchondral ossification front. Also revealed was a full-thickness cartilage defect in the medial margin of the patella without any fibrillation at the surface of the articular cartilage. These results suggested that long-term skeletal unloading could cause destruction of the articular cartilage in a way that differed from destruction induced by overloading. In addition, our results demonstrated that skeletal unloading increased the degradation of type II collagen especially at the early phase, and this degradation coincided with histological findings.

Conclusion:
1. Skeletal unloading caused destruction of articular cartilage without any fibrillation at the surface of articular cartilage.
2. Skeletal unloading increased ALP activity in articular cartilage and the degradation of type II collagen.
3. Skeletal unloading induced patellar bone atrophy rapidly in the first 3 weeks, particularly in the medial part, and the atrophy progressed slowly thereafter.

References: