Introduction: In biomechanics of tendon and ligament tissues, it has been an important focus to clarify the effect of mechanical environment on these tissues. In previous studies, we developed a unique stress-shielding technique without immobilization of the joint for the rabbit patellar tendon (PT), and reported that stress deprivation dramatically reduces the mechanical properties of the rabbit PT, depending on the degree of shielded stress. However, these studies have been conducted to clarify the mechanisms of this effect on the tendon tissue. We have hypothesized that stress deprivation significantly affects expression of various cytokines in fibroblasts of the PT. In order to test this hypothesis, we have developed a rat model in which the PT is shielded from stress, because this model has advantages in examining expression of cytokines with immunohistochemical methods. The purpose of this study is to clarify the effect of complete stress deprivation not only on expression of IL-1beta, TGF-beta, bFGF, and PDGF-B in fibroblasts but also on the mechanical properties of the extracellular matrix of the PT using the same model.

Materials and Methods: Forty male Wistar rats aged 14 to 16 weeks old were divided into two groups of 20 animals each. In Stress-shielded (SS) group, the right PT was shielded from stress by drawing the patella toward the tibial tubercle with a flexible stainless steel wire [1]. In Sham group, the sham surgery was carried out with the same stainless steel wire. No immobilization was applied after operation. In each group, 10 rats were sacrificed at 2 and 6 weeks after the operation, respectively. At each period, 5 of the 10 rats were used for immunohistochemical evaluation and the remaining 5 rats were used for biomechanical examination. To obtain normal control data, 10 PTs were randomly harvested from the left knees of all the rats. For immunostaining, the PT was excised and fixed with 10% neutral buffered formalin, and paraffin sections were prepared. The sections were incubated with each primary antibody for IL-1beta, TGF-beta, bFGF, and PDGF-B. The reaction products were detected with diaminobenzidine. Finally, the sections were counterstained with hematoxylin. The number of total cells was counted in a unit rectangular area (220 x 330 micrometers) that was randomly chosen in a microscopic visual field. Then, we defined the ratio of the number of positively stained cells to the number of total cells as “stained cell ratio”. In biomechanical examination, the cross-sectional area of the PT was measured with an area micrometer. Then, each patella-PT-tibia complex was mounted on a tensile tester. After preconditioning with 10 cycles of 3% strain, the complex was stretched until failure at a crosshead speed of 20 mm/min. The strain in the tendon substance was determined using a video dimension analyzer. Tangent modulus was determined from the stress-strain curve. The two-way ANOVA was used for statistical analysis.

Results: 1) Immunohistochemical evaluation (Fig. 1): The stained cell ratio on IL-1beta significantly increased from 2 to 6 weeks in SS group (p<0.05), but the ratio did not significantly changed in Sham group. The stained cell ratio on IL-1beta was significantly higher in SS group than in Sham group at each period (p<0.05, Fig.2-a). Concerning TGF-beta, the stained cell ratio significantly increased from 2 to 6 weeks in SS group (p<0.05), but the ratio did not significantly changed in Sham group. The stained cell ratio was significantly higher in SS group than in Sham group at each period (p<0.05, Fig.2-b). Regarding bFGF and PDGF-B, we could find only few positively stained cells at each period in both groups.

2) Biomechanical evaluation: At each period, the cross-sectional area of SS group was significantly larger than that of Sham group (p<0.05, Fig.3-a). The tendon modulus of SS group was significantly lower than that of Sham group at each period (p<0.05, Fig.3-b).

Discussion: This study using a new experimental model clearly demonstrates that complete stress deprivation simultaneously induces not only significant overexpression of IL-1beta and TGF-beta in fibroblasts but also significant reduction of the mechanical properties of the extracellular matrix of the PT. Siwik et al. [2] reported that IL-1beta activates matrix metalloproteinases that degrade collagen. Therefore, this fact may explain the mechanical and dimensional changes that occurred simultaneously with the overexpression in the PT. On the other hand, Varga et al. [3] described that TGF-beta promotes the collagen synthesis in fibroblasts. Tsuchida et al. [4], however, found that stress-shielding significantly increases thin collagen fibrils in the extracellular matrix. Therefore, there is a possibility that overexpression of TGF-beta may result in the reduction of the mechanical properties due to the thin fibrils. Thus, this study showed that stress deprivation significantly affects fibroblast activities, and implied that there is a direct relationship between the expression of IL-1beta and TGF-beta and the mechanical deterioration of the extracellular matrix.


**Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan.
***Molecular and Cellular Pathology, Hokkaido University School of Medicine, Sapporo, Hokkaido, Japan.