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Changes in biomechanical properties of tendons and ligaments from joint disuse

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Summary

Objective: The purpose of this paper is to review changes in the biomechanical properties of tendons and ligaments from joint disuse.

Method: We have reviewed 37 experimental studies on joint disuse, which have been carried out with various models of disuse and with various animals.

Results: Immobilization of joints has most commonly been used as a model of disuse. Immobilization of the joint deteriorates the mechanical properties of tendons and ligaments, and reduces their cross-sectional area, although there are some differences in the speed of deterioration among tissues. Remobilization returns the mechanical properties once reduced by immobilization to nearly normal quickly, although the structural properties of the bone–ligament–bone complex continue to lag behind those of the controls. Stress deprivation has been regarded as an essential causative factor in joint disuse. Even if joint motion is allowed, stress deprivation rapidly reduces the mechanical properties of the tendon and ligament tissues, and increases the cross-sectional area of them. These effects appear time- and dose-dependent. Restressing increases the mechanical properties once reduced by stress deprivation, although it takes much time to completely recover them. The reduction of the ultimate stress may be explained by the reduction of the total area of collagen fibrils in tendon cross-section and the increase of thin and immature fibrils.

Key words: Joint disuse, Tendon, Ligament, Biomechanical properties.

Introduction

Disuse of diarthrodial joints is accompanied not only by many musculoskeletal problems including trauma, degenerative disorders, rheumatologic diseases, neuromuscular diseases, infection and congenital deformities, but also by treatments for various disorders, such as immobilization, prohibition of weight-bearing, and bed rest. The clinical manifestations due to joint disuse are characterized by the chronic edema, joint stiffness, muscle atrophy, and osteoporosis. In serious cases, joint disuse results in permanent dysfunction of the joint. Numerous experimental studies on joint disuse have been carried out with various means for obtaining disuse including immobilization with internal and external fixation methods, denervation, and disarticulation, and with various animal models including rats, rabbits, dogs, and monkeys.

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Immobilization has been the most common experimental model of joint disuse, since Menzel first used it in 1871, according to Evans [1]. The morphological and histological changes in joints following immobilization have been well established by many investigators [1–4]: these joints appear less glistening in gross inspection and more 'woody' on palpation and dissection. Histologically, ligaments show disorganization of the parallel arrays of fibrils and cells. Changes in the matrix of the tendon and ligament tissues from immobilization have been analyzed from the biochemical aspect since the 1960s. Changes of collagen include increased turnover with increases of both synthesis and degradation, increased reducible collagen cross-links, and slight loss of total mass [5–9]. Changes of proteoglycan involve reduced total glycosaminoglycans, reduced hyaluronic acid, reduced chondroitin 4 and 6 sulfate, reduced dermatan sulfate [10, 11]. Water content decreased by 3–4% [2, 11].

Biomechanical analysis on changes in tendons and ligaments from joint disuse started a little later, mainly using the immobilization model. In

Table I
Effects of joint immobilization on the biomechanical properties of ligaments. The number shows the immobilization/control ratio

Authors	Subject	Method of immobilization (Period)	Cross-sectional area	Mechanical properties		Stractural properties	
				Stress at failure	Modulus	Load at failure	Stiffness
Amiel *et al.* [7]	Rabbit LCL	Internal (9 Wks)	NS	—	50%*	—	—
Binkley *et al.* [23]	Rat MCL	Internal (40 days)	NS	38%	43%	—	—
Woo *et al.* [18]	Rabbit MCL	Internal (9 Wks)	78%	—	50%*	31%	60%*
Noyes *et al.* [15]	Primate ACL	Cast (8 W)	—	—	—	61%	60%
Klein *et al.* [9]	Canine ACL	Internal (12 W)	—	—	—	44%	73%
Larsen *et al.* [24]	Rat ACL	Cast (4 W)	—	—	—	75%	75%
Larsen *et al.* [24]	Rat PCL	Cast (4 Wks)	—	—	—	NS	67%
Newton *et al.* [19]	Rabbit ACL	Internal (9 Wks)	74%	NS	NS	—	—

*The value was approximately calculated by us from the stress-strain curve shown in the paper; NS: the effect of immobilization was not significant; —: data was not shown in the paper.

early phase of the history, changes in the structural properties of the bone–ligament–bone complex from immobilization were studied with tensile testing [3, 12–16]. Recently, advances in technology enabled to precisely measure changes in the stress–strain characteristics of the ligament and tendon substance from immobilization [17–19]. Biomechanical effects of stress deprivation has been a focus in studies on joint disuse, because it has been regarded as the most essential causative factor in joint disuse [8]. In the immobilization model, however, it is difficult to determine the exact amount of stress deprivation. Most recently, a few experimental models were developed to clarify effects of stress deprivation, which were isolated from the other unknown causative factors in joint disuse, on the biomechanical properties of tendon and ligament tissues [20–22].

The purpose of this paper is to review those studies on changes in the biomechanical properties of tendons and ligaments from joint disuse.

Changes from immobilization of the joint

REVIEW OF LITERATURE

Collateral ligaments

Obvious changes in the mechanical properties of ligaments from immobilization have been demonstrated with the collateral ligaments (Table I). Amiel *et al.* [7] described on tensile tests of the rabbit femur–lateral collateral ligament–fibula complex following 9 weeks of immobilization with internal skeletal fixation. They stated that the immobilization resulted in great changes of the stress–strain curve of the ligament. Specifically, they noted that the tangent modulus of the ligament substance significantly decreased approximately one half. Regarding the cross-sectional area of the ligament, there were no significant differences between the immobilized and control ligaments. Binkley *et al.* [23] also reported on tensile tests of bone-medial collateral ligament (MCL)-bone preparations in rats following 40 days of internal skeletal immobilization. The failure mode of all ligaments was by avulsion of the ligament from the tibia. They described that the modulus of the ligaments obtained from the immobilized knees significantly decreased 57%, although there were no significant differences in the strain at failure. They also found no significant differences in the cross-sectional area.

Woo *et al.* [18] documented a precise biomechanical study on effects of internal skeletal immobilization on the mechanical and structural properties of the femur-MCL-tibia complex in rabbits. After 9 weeks of immobilization, the cross-sectional area of the MCL obtained from the immobilized knee significantly decreased into 78% of the control value. Almost of all specimens failed by tibial avulsion. They found significant differences in the stress–strain characteristics of the ligament substance at low and middle loads between the control and the immobilized specimens. Specifically, the

tangent modulus in the immobilized specimens was approximately one half of the control value. Regarding the structural properties, the stiffness and the load at failure of the bone-ligament complex significantly decreased into approximately 50% and 30% of the control value, respectively. They also compared changes after 9 weeks and 12 weeks of immobilization, and implied a time-dependent effect of immobilization on the mechanical properties of the MCL.

Cruciate ligaments

The cruciate ligaments have been also one of the foci in experimental studies on joint disuse (Table I). Noyes *et al.* [15] measured changes of the structural properties of the femur-ACL-tibia complex after 8 weeks of cast immobilization in primates. Regarding modes of failure, 60% failed in the substance of the ligament in the specimens obtained from the control limbs, while only 44% failed in the substance failed in the tibia in the specimens obtained from the immobilized limbs. Femoral avulsion fractures increased in the specimens from immobilized limbs. The load at failure and the energy absorbed to failure of the immobilized specimens significantly decreased into 61% and 68%, of the control value respectively. Klein *et al.* [9] performed tensile tests of the canine femur–ACL–tibia complex from the knees immobilized with internal skeletal fixation for 12 weeks. They described that failure occurred in the tibial insertion (avulsion) for both the experimental and control limbs. In specimens obtained from the immobilized limbs, the load at failure was significantly reduced into 44% of specimens obtained from the control limbs. The stiffness was also significantly decreased into 73% in the immobilized side. Larsen *et al.* [24] examined the influence of cast immobilization on the structural properties of the femur-ACL or PCL-tibia complexes in rats. After 4 weeks of immobilization, the majority of failures for the ACL occurred at the tibia. Conversely, 66% of PCL failures occurred at the femoral insertion site. Both the load at failure and the stiffness of the bone–ACL–bone preparation from the immobilized limbs decreased into 75% of the control value. The stiffness of the bone–PCL–bone preparation was reduced into 67%, although no changes occurred in the load at failure.

In the above-described studies, little attention was paid to the changes in the mechanical properties of the ligament substance. Most recently, Newton *et al.* [19] determined the effects of internal skeletal immobilization of the knee joint on the mechanical properties of the ACL in rabbits, specifically using the laser micrometer technique to determine the cross-sectional area. In specimens from immobilized knees, the cross-sectional area of the ACL significantly decreased into 74% of the control value. Several modes of failure were observed during tensile testing. The modes did not correlate with the treatment group. The strain at failure in the immobilized group significantly increased 32–40% in the groups, compared with the control group. The modulus of the ligament substance showed slight, but not significant, decreases. However, the ultimate stress of the ligament substance was not obtained in every specimen, because the ligament was not always the site of failure.

Effects of remobilization following immobilization

To understand the essence of immobilization, it is important to clarify changes in ligaments from by remobilization for the joint that has been immobilized. In 1960, Evans *et al.* [1] first reported effects of remobilization on the immobilized knee in rats using gross and histological observations. Noyes *et al.* [15] described that 12 weeks of resumed activity following 8 weeks of immobilization partially recovered the load at failure of the primate ACL, although ligament stiffness had nearly returned to normal. They also stated that the remobilized group showed a return to the control distribution of failure modes. According to Larsen *et al.* [24], the load at failure and the stiffness of the rat bone–ACL–bone complex in the knee that had been immobilized for 40 days returned to control levels following 6 weeks of remobilization. Additionally, there was a transition from avulsion type failures in the immobilized animals to insertion site and intraligament failures in the remobilized animals. Woo *et al.* [18] reported biomechanical and morphological changes in the rabbit MCL caused by remobilization following immobilization. With 9 weeks of immobilization followed by 9 weeks of remobilization, the load at failure of the bone–ligament complex improved into 80% of the control value but did not return to normal, and failure by tibial avulsion became less frequent. Regarding the mechanical properties of the MCL, the stress–strain characteristics returned to normal following remobilization. The cross-sectional areas of the MCL also returned to the equivalent value to the controls.

DISCUSSION ON EFFECTS OF IMMOBILIZATION

One of the major problems in investigating changes in the biomechanical properties of the

ligament substance from immobilization has been that the bone–ligament–bone complex does not always fail in the substance in tensile testing. In all reports, the load at failure of the complex was reduced after immobilization. However, the reduction of the load was related not only to the deterioration in ligament substance itself but also to changes in bony insertion sites. Regarding the latter, osteoclasts that were activated during immobilization resorted the subperiosteal bone; thus, the ligament was secured by only its superficial periosteal attachment [4, 15, 18]. Therefore, we have to say that no studies have completely clarified changes in the ultimate load or stress of the ligament substance from immobilization.

Concerning the collateral ligaments, specifically the MCL, we can inspect effects of joint immobilization on the mechanical and structural properties of the ligament substance as the following, based on approximate agreement among the previous studies [7, 23, 18] (Table I): immobilization of the knee joint may significantly reduce the ultimate stress and the tangent modulus of the collateral ligaments, with a decrease of the cross-sectional area of the ligaments. Thus, the structural properties of the collateral ligaments may be drastically reduced by immobilization of the knee joint. In addition, the effects of immobilization may be time-dependent.

Regarding effects of immobilization on the biomechanical properties of the ACL, Newton *et al.* [19] measured the mechanical properties of the medial portion on the ACL with the most sophisticated measuring system. Therefore, it could not be directly compared with the structural properties of the femur-whole ACL-tibia complex reported by the other investigators [9, 15, 19, 24] (Table I). Newton *et al.* pointed out that the modulus of the ACL did not change following immobilization; whereas, the cross-sectional area was reduced. A reduction in stiffness values which was obtained by others may be explained in part by the reduction of the cross-sectional area. Of course, it should be noted that the stiffness of the femur–ACL–tibia complex has contributions from the insertion site. In addition, because the ACL has extremely complex collagen fiber orientation and attachment [25], it is difficult to precisely determine not only the ultimate stress but also the cross-sectional area of the ACL. Therefore, the discrepancy in measured data between the reports may be explained by the differences of technique for determining strain and cross-sectional area. Furthermore, Keira *et al.* [21] reported that effects of chronic relaxation of the ACL on its mechanical properties were significant at 12 weeks, but not at 6 weeks, as below-described in detail in the present paper. Therefore, the ACL may be more resistant to stress deprivation than the MCL. These facts may explain the discrepancy between the previous reports.

We believe that immobilization of joints may essentially deteriorate the mechanical properties of ligaments despite some differences in the velocity between the MCL and the ACL. A number of studies have shown differences in the cellular, ultrastructural, biochemical, and biomechanical properties of the ACL and the MCL [26–28]. The differential effects of immobilization on the mechanical properties of the ligaments may be explained by the inherent differences in the fibroblasts of these two ligaments.

Concerning mechanisms of the mechanical deterioration in the ligament substance, many studies have shown a correlation of the biochemical and biomechanical aspects in the effects of immobilization [7, 9, 15, 16, 29]. Immobilization increases the rate of collagen turnover with an increased rate of both collagen synthesis and degradation [5, 9, 30]. Thus, a net increase in the amount of newly synthesized collagen occurs. Less mature collagen fibers may be less stiff against tensile loads. Histological and ultrastructural observations have demonstrated changes in the shape and intracellular structures of the fibroblasts obtained from the ACL after immobilization [19]. These changes in the fibroblasts may result in the biochemical alterations in the ligaments and tendons. Regarding the time-dependent effect of immobilization, Amiel *et al.* [8] reported that degradation produced minor net decrease in total collagen mass (2%) after 9 weeks of immobilization, but a large decrease in total collagen mass (27%) was observed at the end of 12 weeks. This fact may be correlated to the time-dependent effect of immobilization on the mechanical properties. However, we also should note controversies among the previous studies: for example, Binkley *et al.* [23] examined the cross-sectional area of collagen fibrils of the MCL obtained from the immobilized knee of the rat using electron microscope. There was a significant decrease in the proportion of smaller fibrils and a significant increase in the proportion of larger fibrils following immobilization. They attributed this finding to a decreased synthesis and decreased degradation of collagen during immobilization. Thus, we can say that effects of immobilization on ligaments and tendons have not been completely clarified.

Regarding changes following remobilization, the previous studies implied that the mechanical properties of the ligaments that have been immobilized

return to nearly normal quickly. However, the loads at failure of the bone–ligament complex continue to lag behind those of the controls. The recovery of the ligament–bone junction is much slower, and it will probably be many months before the ligamentous insertion sites completely return to normal. Woo *et al.* [18] proposed the following hypothesis. With immobilization, there are rapid declines in both the structural and the mechanical properties of the bone–ligament complex and of the substance of the ligament, respectively. However, during remobilization, the mechanical integrity of an immobilized ligament returns to normal quite rapidly.

Changes from stress deprivation with joint motion

REVIEW OF LITERATURE

Patellar tendon

In the immobilization model, we cannot distinguish effects of stress deprivation from effects of other unknown factors caused by loss of joint motion. To quantitatively determine effects of stress deprivation with joint motion on tendons and ligaments, we have developed a few experimental models and have conducted a series of biomechanical studies [20–22, 33–36).

Effects of complete stress shielding on the mechanical properties of the patellar tendon were determined in the rabbit knee [20]. Stress shielding was accomplished by stretching a stainless-steel wire installed between the patella and tibial tubercle and thus, completely releasing the tension in the patellar tendon. No postoperative immobilization was applied to the knee joint. Complete stress shielding significantly changed the mechanical properties of the patellar tendon: it decreased the tangent modulus and ultimate stress of the tendon substance to only 9% of the control values at 3 weeks. There was a 131% increase in the cross-sectional area. Histologically, the stress shielding increased the number of fibroblasts and decreased the longitudinally aligned collagen bundles.

The dose-dependent effects of stress shielding on the mechanical properties of the patellar tendon was shown with a similar model in rabbits [22]. In the experimental groups, tension applied to the patellar tendon was 0%, approximately 30%, and 100% of the normal tension, respectively, with a polyester artificial ligament. The ultimate stress of the tendon substance decreased in comparison with the sham-operation group to 50.2, 13.5, and 9.7% in the completely stress-shielded group and to 75.2, 57.6, 59.6, and 57.3% in the partially stress-shielded group at 1, 2, and 3 weeks after surgery, respectively. The ultimate stress in the completely stress-shielded group was significantly less than that in the partially stress-shielded group at each period. The cross-sectional area of the patellar tendon significantly increased to 132, 206, and 237% in the completely stress-shielded group at 1, 2, and 3 weeks, respectively, and to 136, 170, and 175% in the partially stress-shielded group, compared with the sham-operation group. The cross-sectional area of the completely stress-shielded tendon was significantly larger than that of the partially stress-shielded tendon. This study demonstrated that effects of stress shielding on the mechanical properties of the patellar tendon are dependent on the degree of stress shielding.

Anterior cruciate ligament

The effects of stress deprivation with joint motion on the mechanical properties of the anterior cruciate ligament were studied in a canine model [21]. The tibial insertion of the ACL in the right knee of each animal was made free from the tibia with a cylindrical bone block. In the relaxed group, the bone block was translocated toward the femoral attachment site of the anterior cruciate ligament by 3 mm and then fixed by a cortical screw. The left knee of each dog was given no treatment. No postoperative immobilization was applied to the knee joints. To simplify data analysis, the treat/non-treat ratio (the ratio of the data obtained from the treated knee to that from the non-treated knee) was used. The cross-sectional area of the ligament in the relaxed group (average treat/non-treat ratio=1.37) was significantly larger than that in the sham-operated group (1.16) at 6 weeks but not at 12 weeks. The treat/non-treat ratio of ultimate stress of the ligament substance in the relaxed group significantly decreased to 0.67 and 0.58 at 6 and 12 weeks, respectively; in the sham group, it significantly decreased to 0.79 and 0.87, respectively. Only at 12 weeks, a significant difference was observed between the two groups. This study demonstrated that, in the ACL, stress deprivation results in a rapid increase in the cross-sectional area, although this effect disappears by 12 weeks, and in a decease in mechanical properties, although a relatively long period of more than 6 weeks is required for the deterioration.

Effects of resumption of stressing following stress-shielding

To quantitatively determine effects of resumption of stressing (restressing) on the patellar tendon that had been completely shielded from stress,

Table II
Effects of stress deprivation with joint motion on the mechanical properties of tendons and ligaments. The number shows the treated/sham ratio

Authors	Tissues	Degree of deprivation (Period)	Cross-sectional area	Mechanical properties	
				Tensile strength	Modulus
Yamamoto *et al.* [20]	Rabbit PT	Complete (3 w)	131%	9%	10%*
Majima *et al.* 22]	Rabbit PT	Partial (3 w)	175%	57%	59%
		Complete (3 w)	237%	10%	9%
Keira *et al.* [21]	Canine ACL	Complete (6 w)	115%	NS	NS
		Complete (12 w)	NS	66%	61%

*The value was approximately calculated by us from the stress-strain curve shown in the paper; NS: the effect of immobilization was not significant.

we conducted a biomechanical study with the above-described rabbit model [34]. After completely unloading the patellar tendon for 1–3 weeks, tension was again applied to the tendon for the subsequent 3–12 weeks. Although the stress shielding markedly decreased the tangent modulus and the ultimate stress of the tendon, restressing significantly increased them. However, the mechanical properties of the tendon were not completely recovered even after a prolonged period of restressing. The microstructure of the tendon was also restored by restressing, although the recovery was incomplete.

DISCUSSION ON EFFECTS OF STRESS DEPRIVATION WITH JOINT MOTION

These studies [20–22] clearly demonstrated the following facts (Table II): The mechanical properties and morphology of tendons and ligaments change in response to mechanical demands. Stress deprivation rapidly and strongly reduces the mechanical properties of the tendon and ligament tissues, and increases the cross-sectional area of the tissues. The effects of stress deprivation appears time- and dose-dependent.

Mechanism of the effects of complete stress shielding on the patellar tendon were studied by means of transmission electron microscopy [33]. Stress shielding decreased the average number of collagen fibrils in a unit area(1 μm^2) at 3 weeks (17.6 fibrils), compared to the control (24.4 fibrils), possibly because of the increased number of thin fibrils. The ratio of the total area of collagen fibrils to the whole visualized area was significantly reduced by stress shielding from 68.0% (the control value) into 49.0% at 3 weeks. These results suggested that a decrease in the ultimate stress of the stress-shielded patellar tendon may be explained by the reduction of the total area of fibrils in tendon cross section and the increase of thin and immature fibrils.

It is noted that the reduction of the mechanical properties from stress deprivation appears more slowly in the canine ACL in comparison to the rabbit patellar tendon (Table II). Concerning the cross-sectional area, there are some minor differences between the rabbit patellar tendon and the canine ACL. Amiel *et al.* [37] showed that there are many differences in morphology and biochemistry between the tendon and ligament tissues. In addition, these tissues are located in different environmental conditions, biologically, biochemically, and biomechanically. These differences between tendons and ligaments may affect the phenomena induced by stress deprivation.

It is important to compare the effects of stress deprivation with those of immobilization in order to understand joint disuse (Table I and II). As described previously, immobilization reduces the mechanical and structural properties of ligaments in various degrees. We can find that the degree of the reduction in the immobilized models is extremely less than that in the completely stress-shielded models, and comparable to that in the models stress-shielded partially in some degree. This fact shows that the joint immobilization is not equivalent to the complete stress-shielding. As Klein *et al.* [39] described in their study with the ankle-disarticulation model, effects of immobilization may involve not only effects of stress deprivation but also various unknown effects that derive from loss of joint motion.

The study on restressing reported by Yamamoto *et al.* [34] implied that, if the tendon tissue is once exposed to a non-stress condition even for a short period of time, it takes a long time to recover the reduced mechanical strength with restressing, although the deterioration may be essentially

reversible. The recovery in the complete stress-shielding model appears slower than that after remobilization in the immobilization model. This phenomenon implies that changes in the tendon substance from complete stress-shielding are greater than those from immobilization. Histologically, complete stress shielding for 2–3 weeks increased the number of round-shaped fibroblasts and decreased the number of longitudinally aligned collagen fibers [20, 22]. Stress resumption restored microstructure toward that of the control, although the number of fibroblasts did not reach the control level [34]. Such a change in the microstructure corresponds well with the recovery of ultimate stress induced by restressing. These results imply that the collagen fibers newly synthesized from fibroblasts during complete stress shielding are gradually matured and therefore strengthened by subsequent stress resumption.

Conclusion

The previous experimental studies implied that joint disuse may reduce the mechanical and structural properties of tendons and ligaments around the joint. In addition, they also showed that the degree of the reduction is various, depending the type and period of disuse, the degree of unloading, biological and biomechanical conditions of tissues, and species. Changes in the cross-sectional area of tendon and ligament tissues from joint disuse may be different, depending the type of disuse. Clinically, it is important to understand that the biomechanical properties of the bone–tendon/ligament–bone complex are strongly affected by changes in changes of tendon/ligament attachment site from joint disuse. However, changes in the biomechanical properties of tendons and ligaments from joint disuse have not entirely clarified yet. Moreover, pathomechanisms of the changes in the biomechanical properties of tendon and ligament tissues remain unknown. It may be necessary to investigate them at molecular level. In near future, further studies using various disuse models should be conducted to determine effects of each causative factor in joint disuse on the biomechanical properties of tendon and ligament tissues, and, to clarify pathomechanism of the effects from biomechanical, ultrastructural, biochemical, and molecular–biological aspect.

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