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CHANGES IN JOINT STIFFNESS ASSOCIATED WITH IMMOBILIZATION AND SUBSEQUENT REMOBILIZATION -A STUDY USING A RAT KNEE JOINT CONTRACTURE MODEL

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LEVEL OF EVIDENCE: N/A (animal study)

ABSTRACT

Objectives: This study aimed to clarify changes in the range of motion and joint stiffness following joint immobilization and remobilization using a rat knee joint contracture model.

Methods: Male Wistar rats were divided into two immobilization groups (knee joint immobilization for 4 or 8 weeks), two remobilization groups (immobilization followed by an equivalent period of remobilization), and two age-matched control groups (normal rearing). After the rearing period, each group was allowed to extend the knee joint until a torque value of 120 mNm was reached. Torque curves were then created from the data collected, and the knee extension angles and slope of the approximate straight line of the linear region were compared between the groups.

Results: At 8 weeks, the knee extension angle at 10 and 30 mNm differed significantly between the three groups, with the immobile group having the lowest value, followed by the remobilized and control groups. Although knee extension angles at other torque levels and at 4 weeks were significantly lower in the immobile and remobilized groups than in the control group, no significant differences were observed between the immobile and remobilized groups. The slope of the linear region differed significantly between the three groups at 4 and 8 weeks, with the immobile group having the lowest values, followed by the remobilized and control groups.

Conclusions: Joint immobilization caused the torque curve to begin its linear region at an earlier stage, with a slower slope. Remobilization over the same duration did not sufficiently improve these changes.

KEYWORDS

Animal model, immobilization, remobilization, joint stiffness, torque curve

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INTRODUCTION

Joint contracture is one of the primary factors affecting activities of daily living and quality of life. Joint range of motion (ROM) limitations can be classified as contracture or ankylosis, depending on whether the limiting factor resides within or outside the joint structures. However, in practice, limiting factors rarely exist in isolation. Since, at least in cases of joint immobilization, ROM limitations are associated with factors both within and outside the joint structures, this study uses contracture to broadly indicate any state in which normal joint movement is restricted. Considerable data on joint contractures have been accumulated through histological and biochemical verification using laboratory animals, which have clarified the changes in joint components after immobilization or remobilization[1-9]. For context, most functional verifications cease after determining the ROM of the joints, leading to insufficient verification of joint stiffness, which can be considered a qualitative change in joint motion. In clinical settings, the resistance felt in the final region of joint motion (e.g., end feel[10]) and situations where resistance is felt earlier than normal throughout the ROM, characterized by spasticity or rigidity, reflect changes in stiffness. This has not received much clinical attention due to the difficulty of objective evaluation. However, clarifying how stiffness changes with contracture and whether these changes are reversible is important to restore joint contracture. Severe joint contractures that are difficult to restore to their full ROM are often associated with prolonged immobility or joint structure damage. Therefore, clarifying changes in stiffness caused by these joint structures is particularly necessary.

Recently, several studies[11–18] reported the torque curves for joint motion using joint contracture models. However, no consensus has been reached. Furthermore, these models employed immobilization following surgical or hyperextension trauma, and verification was not performed using immobilization alone. Immobilization models involving damage to joint structures result in more severe contractures[6]. Therefore, verification using a simple immobilization model that excludes inflammation effects is essential for simulating immobilization associated with cast immobilization or prolonged bed rest.

Therefore, the current study aimed to clarify how the ROM and torque curves of the knee joint change after joint immobilization and remobilization using a rat model of joint contracture in which the knee joint was immobilized for 4 or 8 weeks followed by measurements using a torque machine.

MATERIALS AND METHODS

A total of 31, 8-week-old male Wistar rats (175–200 g) and 6 retired rats (generally 24 weeks old, 370–455 g) were used for experimentation. All experiments were conducted in accordance with the Nagoya Gakuin University Animal Experimentation Regulations and were approved by the University's Animal Experimentation Committee (Approval No. 2021-001).

All 31 animals were randomly assigned to five groups: a 4-week immobilization group (Im4W, n = 5), whose knee joints were kept internally fixed for 4 weeks, a 4-week remobilization group who received 4 weeks of immobilization followed by 4 weeks of remobilization (Re4W, n = 7), a 4-week control group who received normal rearing for 8 weeks (Con4W, n = 6), an 8-week immobilization group who received 8 weeks of immobilization (Im8W, n = 7), or an 8-week remobilization group who received 8 weeks of immobilization followed by 8 weeks of remobilization (Re8W, n = 6) *(Fig. 1)*. Retired rats served as the control group for the 8-week model *(Fig. 1)*. ROM limitations induced by joint immobility show a greater increase in joint-related restrictions after 4 weeks of immobilization[19]. Therefore, the 4-week and 8-week models were employed to verify the joint stiffness. Each group was kept in plastic cages and had free access to food and water. A 12-h light-dark cycle was used to maintain a regular diurnal rhythm. The rearing temperature was kept at 25°C to minimize diurnal variations as much as possible.

The knee joint was immobilized through internal fixation with a plate according to previous studies[19,20]. Under isoflurane inhalation anesthesia, a pre-drilled hole was made in the right lateral tibia and right lateral femur using a 0.8-mm diameter Kirschner steel wire, after which a 1.0-mm diameter screw was used to fix the plastic plate. The plate was passed under the biceps femoris muscle, and the knee joint was placed in approximately 135° flexion.

Stiffness indicates the degree of displacement relative to load, and an approximate straight line/curve can be plotted from information on its increase. In biological tissues, the increase in stiffness is often expressed as a curve *(Fig. 2)*. In the initial phase of the load-displacement curve, a phase called the toe region, in which the increase in load is smaller than the increase in displacement, can be observed. After a

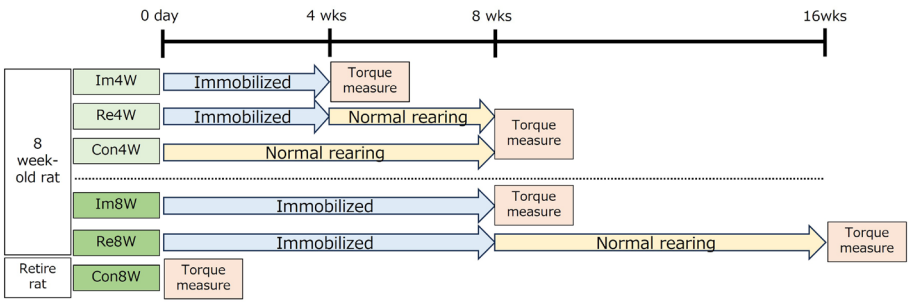


Figure 1. *Protocols and ages for each group. Control groups were age-matched to their respective remobilization groups. Retired rats are generally 24 weeks old.*

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certain point, a phase called the linear region emerges, in which displacement and load increase linearly[21]. Should the load continue to be applied, tissue rupture follows, during which the amount of load relative to the amount of displacement decreases rapidly. The load-displacement curve in joint motion is represented by the torque-angle curve, which is treated as the torque curve in the current study.

The joint torque during knee joint extension was measured at the end of the rearing period for each group. A small animal exercise device[22] (NDH-1; Bio Research Center, Co., Ltd., Nagoya, Japan) was used for the measurements, and a self-made attachment for the knee was used *(Fig. 3)*. After euthanasia via isoflurane overdose, all skin and posterior muscles across the knee joint (hamstrings, adductor musculature, gastrocnemius, and plantaris muscles) were immediately removed. No movement restriction in the knee flexion direction occurs in the contracture model[20]. Therefore, the structural changes in the anterior knee associated with immobilization were minor and would not affect the torque during knee extension movement. The anterior quadriceps femoris muscle was not removed. The lateral malleolus and fibular head, which indicate the lower leg axis, were marked. The rats were then placed on the measuring device, which was adjusted so that the center of the right lateral epicondyle was at the center of the motor's axis of rotation, and the femur was fixed. Thereafter, the lower leg was moved in the direction of knee joint extension at 3° per second while measuring the torque value required for this movement. The maximum torque intensity was set at 120 mNm based on a previous study[7]. A digital video camera positioned directly above the measuring device was used to capture a video of the torque measurement. Still images were extracted from the video every 200 ms using motion capture software (Kinovea® version 0.9.5). The knee joint extension angle was then measured from the obtained images using image editing software (Photoshop®, Adobe Systems, San Jose, CA, USA). The angle between the femur long axis and fibula long axis was measured, and the state in which the fibula reached the extension of the femur long axis (full knee joint extension) was expressed as 180°.

From the collected data, a torque-angle curve was created with the vertical axis as the torque value and the horizontal axis as the knee joint extension angle. Thereafter, the slope of the approximate straight line in the linear region was calculated using the least-squares method. With reference to the torque-angle curve of a normal knee *(Fig. 2)*, which was determined preliminarily prior to this study, the point at which the torque required for joint motion exceeded 10 mNm was considered to be the start of the linear region, whereas the region prior to that point was treated as the toe region. The slope of the approximate line and knee extension angle values (at four time points: 10, 30, 60, and 120 mNm) were then compared between the three groups. After checking each value for normality, a one-way analysis of variance was performed, followed by multiple comparison tests if significant differences were found. All analyses were performed using R version 4.0.2 (CRAN, freeware) for Windows, with the significance level set at 5%.

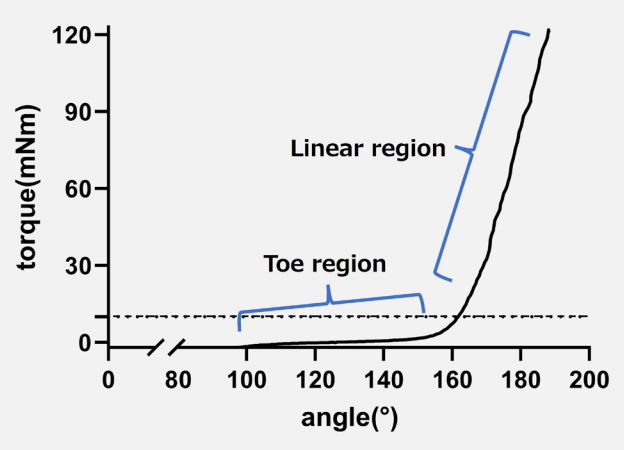


Figure 2. *Example of a torque curve. The amount of variation in knee extension angle after applying knee extension torque to normal 12-week-old Wistar rat knee joints (excluding skin and muscle). The toe region is the range wherein the load amount increases less than the initial increase in the displacement amount, whereas the linear region is the range wherein the subsequent displacement amount and load amount increase linearly.*

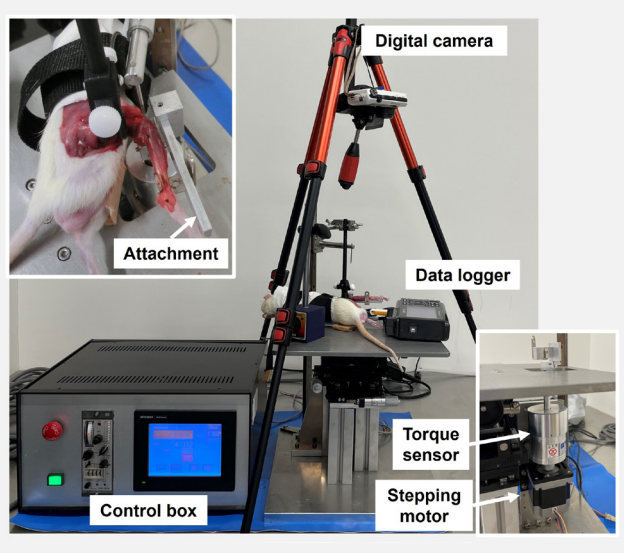


Figure 3. *Torque measuring device. The upper left panel shows an enlarged view of the stage, whereas the lower right panel shows a side view of the stage. The stepping motor rotates the attachment of the hind limb at 3°/s and measures the torque required at that time. Torque was recorded on a data logger (sampling frequency 500 Hz). The motor rotates until it registers 120 mNm.*

RESULTS

In the Im4W, Re4W, and Con4W groups, the knee extension angle at 10 mNm, which indicated the start of the linear region, was 136.8° ± 3.4°, 140.7° ± 5.0°, and 158.5° ± 2.5°, respectively. Although no significant difference was observed between the Im4W and Re4W groups, a significant difference was noted between the Im4W and Re4W groups and the Con4W group, with the linear

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regions starting earlier in the former two groups *(Table 1 and Fig. 4A)*. The knee extension angles in the Im4W, Re4W, and Con4W groups were 150.5° ± 2.6°, 151.0° ± 5.9°, and 165.8° ± 3.4° at 30 mNm and 160.3° ± 3.2°, 160.3° ± 6.9°, and 172.1° ± 2.6° at 60 mNm, respectively. Although a significant difference was noted between the Con4W group and the other groups at both torque levels, no significant difference was found between the Im4W and Re4W groups. At the final 120 mNm, the knee extension angles in the Im4W, Re4W, and Con4W groups were 180.7° ± 2.6°, 174.6° ± 5.9°, and 182.5° ± 2.5°, respectively, with a significant difference having been observed only between the Re4W and Con4W groups *(Table 1)*. The knee extension angles of the Im8W, Re8W, and Con8W groups at 10 mNm were 118.7° ± 3.9°, 134.8° ± 7.5°, and 152.9° ± 2.3°, respectively, with a significant difference having been observed between all the groups. The linear region started earliest in the Im8W group, followed by the Re8W group and then the Con8W group *(Table 1 and Fig. 4B)*.

The knee extension angles at 30 mNm were 133.3 ± 2.2°, 142.1 ± 7.2°, and 158.4 ± 1.4° for the Im8W, Re8W, and Con8W groups, respectively, with significant differences observed between the groups. The knee extension angles in the Im8W, Re8W, and Con8W groups were 143.4° ± 2.8°, 147.6° ± 7.4°, and 163.7° ± 1.1° at 60 mNm and 161.1° ± 3.8°, 158.6° ± 7.0°, and 172.8° ± 1.9° at 120 mNm, respectively. Although a significant difference was observed between the Im8W and Con8W groups at all time points, no significant difference was found between the Im8W and Re8W groups *(Table 1)*.

		Knee extension ROM			ANOVA p value (n^2)	Multiple comparison tests p value (95% Confidential Interval)		
		Im G	Re G	Con G		Im G vs Re G	Im G vs Con G	Re G vs Con G
4 W	10 mNm	136.8 ± 3.4*	140.7 ± 5.0*	158.5 ± 2.5	<0.001 (0.873)	0.229 (-2.0 - 9.8)	<0.001 (15.6 - 27.8)	<0.001 (12.2 - 23.4)
	30 mNm	150.5 ± 2.6*	151.0 ± 5.9*	165.8 ± 3.4	<0.001 (0.753)	0.981 (-6.3 - 7.2)	<0.001 (8.3 - 22.2)	<0.001 (8.4 - 21.2)
	60 mNm	160.3 ± 3.2*	160.3 ± 6.9*	172.1 ± 2.6	<0.001 (0.639)	0.999 (-7.4 - 7.4)	0.002 (4.9–20.2)	<0.001 (5.5–19.6)
	120 mNm	180.7 ± 2.6	174.6 ± 5.9*	182.5 ± 2.5	0.011 (0.452)	0.063 (-12.6 - 0.3)	0.783 (-4.9 - 8.4)	0.012 (1.7 - 14.0)
8 W	10 mNm	118.7 ± 3.9*†	134.8 ± 7.5*	152.9 ± 2.3	<0.001 (0.889)	0.002 (7.1 - 25.1)	<0.001 (28.9 - 39.5)	0.001 (9.5 - 26.8)
	30 mNm	133.3 ± 2.2*†	142.1 ± 7.2*	158.4 ± 1.4	<0.001 (0.849)	0.041 (0.4 - 17.2)	<0.001 (22.1 - 28.0)	0.002 (7.9 - 24.6)
	60 mNm	143.4 ± 2.8*	147.6 ± 7.4*	163.7 ± 1.1	<0.001 (0.789)	0.377 (-4.4 - 12.9)	<0.001 (16.7 - 23.9)	0.003 (7.5 - 24.6)
	120 mNm	161.1 ± 3.8*	158.6 ± 7.0*	172.8 ± 1.9	<0.001 (0.651)	0.704 (-11.0 - 6.0)	0.001 (6.7 - 17.0)	0.003 (6.0 - 22.0)

Table 1. *Knee extension angle at each torque (°). The angle values are presented as mean ± standard deviation. η2 is an effect size measure for one-way ANOVA. A) *Significant difference compared with the Con group, p <0.05. †Significant difference compared with the Re group, p <0.05. Abbreviations: Im G, immobilization group; Re G, remobilization group; Con G, control group.*

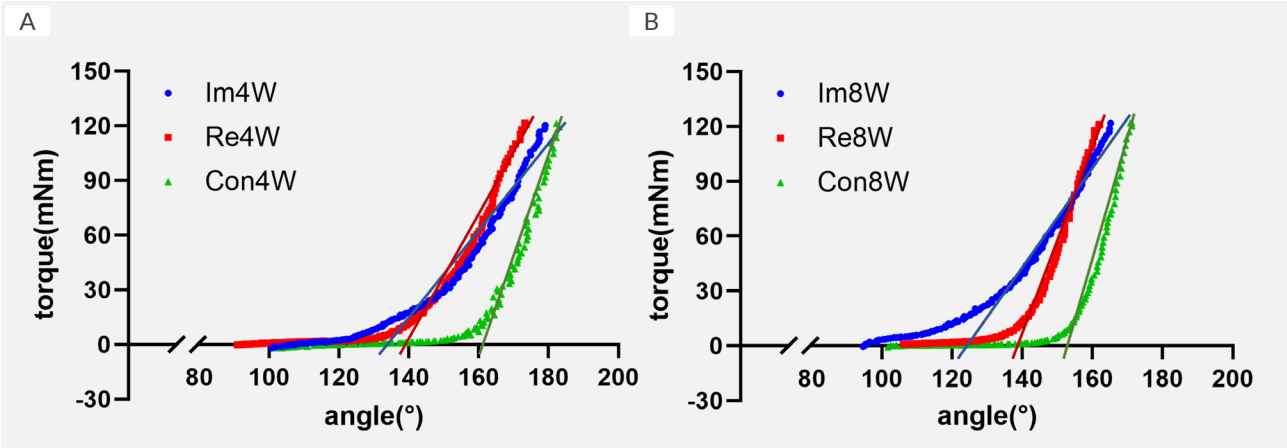


Figure 4. *Representative examples of torque curves. Torque curve after rat knee joint immobilization and subsequent remobilization over the same duration. A) Comparison at 4 weeks. B) Comparison at 8 weeks. Each line represents an approximate linear region. Blue, red, and green colors indicate the immobilization, remobilization, and control groups, respectively. A remobilization period over of the same duration did not lead to normalization.*

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The slope of the linear region was 2.6 ± 0.3, 3.4 ± 0.4, and 4.7 ± 0.3 in the Im4W, Re4W, and Con4W groups, respectively, with significant differences having been observed between the mentioned groups. In the Im8W, Re8W, and Con8W groups, the slope values were 2.8 ± 0.3, 5.0 ± 0.2, and 5.8 ± 0.5, respectively, with significant differences also having been observed between the groups. Furthermore, the mean coefficient of determination for the calculated approximation lines was ≥0.95 in all groups *(Table 2)*.

DISCUSSION

The current study used rat knee joints to verify how the torque curve of the joint changes after joint immobility and remobilization. Notably, our results showed that the torque curve of a normal knee joint extends for quite some time leading up to the linear region, after which the resistance increases rapidly, whereas the torque curve after immobilization showed an early increase in joint resistance, followed by a gradual increase in resistance. Although the start of the linear region slowed down and the slope became steeper as it approached normal, it did not return to the normal torque curve after the same period of remobilization. No significant differences in knee extension angle values were found between the immobile and remobilized groups, except at the 10 and 30 mNm point in the 8-week model, indicating that changes originating from joint components are difficult to reverse even with a remobilization period. This is the first study to clarify changes in the torque curves of joint structures following simple immobilization, excluding the effects of inflammation, and subsequent remobilization.

Although several reports have examined how ROM restriction changes using joint contracture models, few have examined changes in joint torque. A series of studies from one research group[11-13] used a contracture model in which the elbow joints of Long-Evans rats weighing 320-370 g were immobilized for 6 weeks to examine changes in torque after immobilization and after remobilization for the same duration. They measured the elbow joint during both flexion and extension and compared the torque curves divided into extension stiffness (Nmm/deg), flexion stiffness (Nmm/deg), and the neutral zone (NZ) in between. Their findings showed that immobilization did not significantly change the stiffness during extension/flexion but significantly shortened the length of the NZ and increased stiffness. Their NZ corresponds to the toe region in the present study, whereas their stiffness is expressed in Nmm/deg, which corresponds to the slope of the approximate line in the present study. The shorter toe region and earlier start point of linear region due to immobilization observed in the current seemed to support the findings of the aforementioned study, but our results on the change in the slope of the linear region did not coincide with that reported in the aforementioned study. In this regard, the present study tentatively defined the point at which 10 mNm of force was required for joint motion as the boundary between the toe region and the linear region, but the aforementioned study defined the NZ as "the flatter region of the curve that falls between linear fits of flexion and extension stiffness." Despite the lack of clarity owing to the absence of any further description, we believe that the difference in the definitions used was one of the reasons for discrepancy in results. Another study[12] that examined the changes after remobilization for the same duration found no significant difference in NZ length (no mention of stiffness in the NZ), although ROM limitations remained, indicating improvement due to remobilization. The changes due to remobilization in the mentioned study also differed from those reported in the present study. This could be attributed to the difference in joint morphology and differences in the contracture model, with the aforementioned study immobilizing the joint after damaging the joint capsule; however, we believe that their failure to remove muscle tissue during torque measurement was the most significant cause of the discrepancy in results. Although they also reported torque values without the muscle or the joint capsule[14], they did not focus on the extension stiffness, and their findings did not clarify whether or not a clear difference was present. However, from the figures presented in their study, there seems to be a difference in the slope.

Alternatively, another research group[15-18], who used a model immobilized for 8 weeks after rabbit knee joint trauma (hyperextension), reported differences in the slope of the torque curves drawn after interventions, such as joint injection of celecoxib and subcutaneous injection of ketotifen. Moreover, torque measurements were

	Slope at linear region: ΔNm/Δ° (coefficient of determination)			ANOVA p value (n^2)	Multiple comparison tests p value (95% Confidential Interval)		
	Im G	Re G	Con G		Im G vs Re G	Im G vs Con G	Re G vs Con G
4 W	2.6 ± 0.3*† (0.977 ± 0.02)	3.4 ± 0.4* (0.973 ± 0.02)	4.7 ± 0.3 (0.959 ± 0.03)	<0.001 (0.850)	0.006 (0.2 - 1.4)	<0.001 (1.5 - 2.6)	<0.001 (0.7 - 1.8)
8 W	2.8 ± 0.3*† (0.969 ± 0.01)	5.0 ± 0.2* (0.981 ± 0.01)	5.8 ± 0.5 (0.979 ± 0.01)	<0.001 (0.938)	<0.001 (1.7 - 2.7)	<0.001 (2.5 - 3.5)	0.001 (0.3 - 1.3)

Table 2. *Slope of the approximate line in the linear region. The slope values are presented as mean ± standard deviation. n^2 is an effect size measure for one-way ANOVA. *Significant difference compared with the Con group, p <0.05. †Significant difference compared with the Re group, p <0.05. Abbreviations: Im G, immobilization group; Re G, remobilization group; Con G, control group.*

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performed with all periarticular knee muscles resected. In a 2019 study[15], the authors reported that celecoxib injections during the immobilization period significantly reduced "capsular stiffness" (corresponding to the slope of the linear region in the present study) in the torque curve after 16 weeks of remobilization compared to the absence of an intervention. Although the aforementioned study failed to mention the toe region, the figure clearly shows a prolongation of the toe region (by approximately 30°) in the intervention group. Another study in 2020[16] that used a similar model and compared it with ketotifen or saline administration found no significant difference in "capsular stiffness" between both interventions, but the figures presented in their study showed that the start of the toe region in the ketotifen group was prolonged by around 60°. A study in 2022[18], which tested a similar model using a combination of intra-articular celecoxib and capsular release, again reported a significantly lower "capsular stiffness" among those who received the intervention than among those who did not receive the intervention. Although not the focus of the mentioned report, their figure clearly shows a prolonged toe region in the intervention group. Other reports from their group showed that treatment interventions reduced the slope of the linear region, which they considered an improvement. Of course, it is natural to consider this as an improvement if one also includes the enlargement of ROM and the obvious prolongation of the toe region, despite the lack of clear numerical comparisons. The aforementioned report did not report the torque curves of the normal knee and the knee immediately after immobilization, and the "capsular stiffness" of these knees was unknown, precluding a direct comparison. However, in the current study, the slope of the linear region was the largest in the normal knee, and the increase in slope after immobilization, which was lowered by immobilization, can be considered as an improvement. Thus, the results regarding the slope of the liner region different from those presented in the aforementioned study. This difference may be attributed not only to differences in animal species but also to the fact that the joint capsule was injured before immobilization. This is because the presence or absence of inflammation in the joint capsule has been reported to cause more severe contractures[6]. Kaneguchi et al.[23] reported that joint laxity was increased by joint capsule vulnerability after immobilization of rat knee joints for 3 weeks. The decrease in the slope of the torque curve after immobilization in the current study may also be caused by changes in the joint capsule tissue. Regarding the failure to restore the torque curve after remobilization, reports have shown that subsequent remobilization was not sufficient to improve capsular tissue after a period of immobilization[6,24,25], with the present study seemingly supporting this from a functional aspect.

The present findings demonstrate that, during the progression of contracture induced by joint immobilization, quantitative limitations in range of motion are accompanied by qualitative changes in resistance during joint movement, and that these changes are not fully reversed during a comparable period of remobilization. These findings provide insight into the functional pathophysiology of joint contracture and may serve as a foundation for the future development of its treatment. Although the direct applicability of these findings to clinical practice is limited, they at least highlight the importance of understanding contracture from both quantitative and qualitative perspectives, as well as the need for careful risk management to prevent tissue injury during therapeutic interventions. If individuals experience discomfort during movement despite regaining a normal range of motion after contracture, unresolved changes in torque during joint movement may contribute to this sensation. These results highlight the need for further investigation to improve recovery strategies for joint contracture.

However, this study has several limitations. First, the validity of the contracture model used in this study was not confirmed. Direct extrapolation from rats to humans is not possible due to anatomical differences in the knee joint. Although this study did not involve direct damage to the joint structures, the joint was immobilized by surgically damaging the femur and tibia. Therefore, further studies are needed to determine the extent to which this can be extrapolated to clinical situations involving joint immobilization, such as those associated with plaster cast immobilization, prolonged bed rest, and paralysis. Additionally, when selecting a contracture model, it is also necessary to consider not only joint immobilization but also whether the joint is maintained in a weight-bearing or non–weight-bearing condition. The contracture model used in this study was selected because it is widely used, allows weight-bearing on the hindlimb during the immobilization period, and enables observation of changes purely due to immobilization. However, immobilization is often accompanied by no-weight-bearing in clinical settings. It has been reported that even short-term unloading, such as 20 minutes, can improve pain and muscle strength in patients with knee osteoarthritis[26]; therefore, further studies are needed to examine differences based on the presence of weight-bearing. Second, the influence of resecting the posterior knee muscles while maintaining the quadriceps intact on torque curve measurements remains unclear. Although reports indicate that similar immobilization models do not cause restriction in flexion[20], suggesting that the anterior knee structures have no or only a minor effect on knee extension torque, it remains necessary to examine whether the results truly reflect only the articular components. Regarding the threshold setting for the linear region of the torque curve, it was determined only tentatively with reference to the torque curve of a normal knee joint, and no clear justification exists for this choice. However, because the linear region represents a linear relationship between displacement and

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load, the coefficients of determination of the approximate straight lines calculated for each group in this study were all ≥0.95. This suggests that the criterion was generally satisfied, although further investigation is required. Finally, we did not perform histological and biochemical verification of the joint components; therefore, causal relationships regarding the changes in the torque curve cannot be inferred. Although several studies have reported changes in the joint capsule following joint immobilization, there are also reports indicating that torque curves do not return to normal even after joint capsule excision[14], as well as reports showing that the mechanical strength of the posterior joint capsule does not change even after immobilization based on tensile testing[27]. These findings suggest that further investigation is needed to clarify the contribution of the joint capsule.

CONCLUSION

Joint immobilization causes a decrease in joint ROM and an earlier onset of the linear region of the torque curve, with its slope becoming gradual. Moreover, we found that remobilization over the same duration may fail to sufficiently improve these changes, highlighting the difficulty of restoring the mechanical properties of the joint components.

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