Amount of Torque and Duration of Stretching Affects Correction of Knee Contracture in a Rat Model of Spinal Cord Injury

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Abstract

Background Joint contractures are a common complication of many neurologic conditions, and stretching often is advocated to prevent and treat these contractures. However, the magnitude and duration of the stretching done in practice usually are guided by subjective clinical impressions.

Questions/purposes Using an established T8 spinal cord injury rat model of knee contracture, we sought to

determine what combination of static or intermittent stretching, varied by magnitude (high or low) and duration (long or short), leads to the best (1) improvement in the limitation in ROM; (2) restoration of the muscular and articular factors leading to contractures; and (3) prevention and treatment of contracture-associated histologic alterations of joint capsule and articular cartilage.

Methods Using a rat animal model, the spinal cord was transected completely at the level of T8. The rats were randomly assigned to seven treatment groups (n = 4 per group), which were composed of static or intermittent stretching in combination with different amounts of applied torque magnitude and duration. We assessed the effect of stretching by measuring the ROM and evaluating the histologic alteration of the capsule and cartilage.

Results Contractures improved in all treated groups except for the low-torque and short-duration static stretching conditions. High-torque stretching was effective against shortening of the synovial membrane and adhesions in the posterosuperior regions. Collagen Type II and VEGF in the cartilage were increased by stretching.

Conclusions High-torque and long-duration static stretching led to greater restoration of ROM than the other torque and duration treatment groups. Stretching was more effective in improving articular components of contractures compared with the muscular components. Stretching in this rat model prevented shortening and adhesion of the joint capsule, and affected biochemical composition, but did not change morphologic features of the cartilage.

Clinical Relevance This animal study tends to support the ideas that static stretching can influence joint ROM and histologic qualities of joint tissues, and that the way stretching is performed influences its efficacy. However, further studies are warranted to determine if our findings are clinically applicable.

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Introduction

Contractures are a common complication of neurologic conditions including stroke, spinal cord injury, traumatic brain injury, and cerebral palsy [4, 7, 12, 15, 42]. They are characterized by limitations in passive ROM of the affected joints [20, 34]. Most importantly, contractures limit the function and independence of patients with these conditions, and they predispose individuals to other complications such as pressure sores, sleep disturbances, and pain [4, 12, 15, 42]. Consequently, attention has been directed toward preventing and treating contractures in patients with neurologic conditions [10]. Nevertheless, we continue to see patients who have contractures that limit limb function [6].

Stretching (that is, the application of tension to soft tissues) has been advocated as an effective means of preventing and treating contractures in patients with neurologic conditions [9, 10, 12, 15, 16]. Additionally, it is believed that contractures can be prevented more readily than treated, and that less stretching is required to maintain rather than to increase the extensibility of soft tissues [12]. Considerable resources [12], including some rigorously designed clinical studies [1, 10, 11, 13], have investigated the efficacy of stretching. However, it remains unclear whether the effects observed in these trials are clinically worthwhile, and the therapeutic effect of stretching on the pathophysiology of contractures has received little attention. In addition, the magnitude and duration of the stretching force applied have been guided mainly by subjective clinical impressions and the patient's subjective feedback [39].

The use of stretching to prevent and treat contractures after immobilization is supported by numerous animal studies [3, 8, 12, 30, 32, 39, 40]. Ample evidence from these animal studies suggests that contracture management can be achieved through repeated or sustained stretching in the case of contractures not attributable to injury to the central nervous system [3, 8, 10, 39, 40]. In contrast, contractures after central nervous system injuries are the result of neural and nonneural factors, including paralysis and structural changes in soft tissues [15, 19]. Therefore, contracture produced experimentally by immobilizing joints does not model specific contractures in humans with neurologic conditions. We therefore developed a unique animal model of contractures using rats with spinal cord injuries, with the goal of having this model be representative of animals with central nervous system injuries [25, 26]. Previous studies have shown that the articular component (bone, cartilage, synovial membrane, capsule, and ligaments) plays a larger role in pathogenesis of contractures after spinal cord injury than does the muscular component (muscle, tendon, and fascia) [22–24, 26]. The two major pathologic changes in joint contractures are joint capsule stiffness and cartilage degeneration [5, 22–24, 34].

Based on this, the overall goal of our study was to define an effective stretching protocol for a joint (knee) contracture

after a central nervous system injury, using an established T8 spinal cord injury rat model of knee contracture. Specifically, we sought to determine what combination of static or intermittent stretching, varied by magnitude (high or low) and duration (long or short), leads to the best (1) improvement in limitation in ROM; (2) restoration of muscular and articular factors leading to contractures; and (3) prevention and treatment of contracture-associated histologic alterations of the joint capsule and articular cartilage.

Materials and Methods

Experimental Design

The protocol for this experiment was approved by the University Animal Care Committee. In total, 32 male Wistar rats (CLEA Japan Inc, Tokyo, Japan; age, 10 weeks; weight, 240–268 g) were used for this study. Surgical procedures and postoperative care conformed to our previous studies [22–26]. Twenty-eight of the 32 rats were anesthetized by intraperitoneal administration of 40 mg/kg sodium pentobarbital. The spinal cord was transected completely at the level of T8. This procedure led to the development of a knee flexion contracture [25, 26]. These rats were randomly divided into seven equal treatment groups ($n = 4$) (Table 1): (1) untreated after spinal cord injury (S); (2) high-torque and long-duration static stretched (HL); (3) high-torque and intermittent stretched (HI); (4) high-torque and short-duration static stretched (HS); (5) low-torque and long-duration static stretched (LL); (6) low-torque and intermittent stretched (LI); and (7) low-torque and short-duration static stretched (LS). Four rats served as a control (C) group and had no interventions. The right and left knees served as different samples. The subgroup sample sizes were calculated by a power analysis based on pilot results for detecting a 10° difference in ROM 19 of 20 times [26].

Treatment Protocol

Each animal in the treatment groups was anesthetized, and after the first postoperative day both hind limbs were stretched daily for 10 days. The direction of the stretch was set toward extension with traction applied to the whole lower extremity for consistent torque as established by Usuba et al. [39]. The high-torque condition was an applied force of 0.045 Nm; this results in maximal stretching in the rat knee. The low-torque condition was an applied force of 0.02 Nm; this results in moderate stretching [39]. The static stretching time for one treatment was set at 30 minutes for long duration and 15 minutes for short duration. The intermittent stretch condition was one 30-minute set, in which the joint was stretched and held for 60 seconds with 30-second intervals of rest between each stretching.

Table 1. Intervention and sample size in each experimental group

Group	Magnitude	Duration	Number*		Intervention
			Animals	Legs	
C	—	—	4	8	No intervention (control)
S	—	—	4	8	No treatment after spinal cord injury
HL	High torque	Long	4	8	High-torque and long-duration static stretching after spinal cord injury
HI		Intermittent	4	8	High-torque and intermittent stretching after spinal cord injury
HS		Short	4	8	High-torque and short-duration static stretching after spinal cord injury
LL	Low torque	Long	4	8	Low-torque and long-duration static stretching after spinal cord injury
LI		Intermittent	4	8	Low-torque and intermittent stretching after spinal cord injury
LS		Short	4	8	Low-torque and short-duration static stretching after spinal cord injury

* The right and left knees served as different samples; C = control; S = untreated after spinal cord injury; HL = high-torque and long-duration static stretched; HI = high-torque and intermittent stretched; HS = high-torque and short-duration static stretched; LL = low-torque and long-duration static stretched; LI = low-torque and intermittent stretched; LS = low-torque and short-duration static stretched.

ROM Measurements and Determination of Muscular/Articular Components of Contracture

On the day after the last treatment session, while the animal was under general anesthesia, knee motion was measured for extension with a mechanical goniometer applying a standardized torque (0.06 Nm) by a slight modification of the method according to Trudel et al. [35, 38] (Fig. 1). The goniometer showed that reproducibility of repeated measurements was within $\pm 3.8^\circ$ and accuracy was an error less than 3.4° . Two examiners conducted the ROM measurements and repeated them three times for each leg. The examiners were blinded to each other's scores. Reported values are the mean of the six measurements, the combined measurements taken by both examiners.

The animals were euthanized by exsanguination while they were under anesthesia. Myotomy of the transarticular muscles then was performed and ROM was measured again. Muscular contracture was defined as contractures caused by the muscles including tendon and fascia and articular contracture as contractures caused by the articular structures (bone, cartilage, synovium/subsynovium, capsule, and ligaments); these ROM measurements were calculated following the modified method of Trudel and Uhthoff [37]. In brief, the formulas allow isolation of the muscular and articular factors of contractures and are as follows:

$$\begin{aligned} \text{muscular contracture} &= \text{ROM no myotomy} \\ &\quad - \text{ROM after myotomy (within each treatment group);} \\ \text{articular contracture} &= \text{ROM after myotomy of} \\ &\quad \text{each treatment group} - \text{ROM after myotomy} \\ &\quad \text{of the control (C) group} \end{aligned}$$

Fig. 1 The knee extension angle was measured with a mechanical goniometer. The position of the goniometer and hand are shown.

Tissue Preparation

The femur and tibia were dissected free of soft tissues and disarticulated at the hip and ankle. We prepared undecalcified frozen sections following the protocols established by Kawamoto [17]. Each sample was sectioned at a thickness of 5 μm using a disposable tungsten carbide blade at the medial midcondylar level in the sagittal plane.

Quantitative Histology

Synovial Intima Length

We measured shortening of the posterior synovial intima length responsible for a knee flexion contracture after a spinal cord injury [24]. The length of the superior and inferior subdivisions of the synovial intima in the posterior capsule were measured separately as described by Trudel et al. [33, 34], and also summed to provide a total synovial intima length. The synovial lining contour was traced on the histologic sections stained with aldehyde fuchsin-Masson Goldner and its length was measured with Image Tool software (Image J 1.43; National Institutes of Health, Bethesda, MD, USA).

Disorganization of Collagen Fibers

Collagen fiber disorganization of the posterior capsule was assessed because it is a measure of a reduction of plasticity and/or expansion leading to loss in the extension ROM [24]. Disorganization of collagen fibers was quantified by the protocols described by Lee et al. [18]. The angle of 10 fibers (which were chosen at random) per image from the section stained with picrosirius red was measured with Image Tool software. Each angle was measured and classified into six grades: 90°–105°, 105°–120°, 120°–135°, 135°–150°, 150°–165°, and 165°–180°. The number of fibers classified in each grade was compared among all groups.

Cartilage Thickness

Articular cartilage thickness was measured on sections stained with toluidine blue as described previously [21–23]. Changes in femoral and tibial cartilage were determined in the anterior femoral, posterior femoral, anterior tibial, and posterior tibial regions.

Immunohistochemical Analysis

Immunohistochemistry was assessed to characterize the localization of histologic changes in the joint capsule and articular cartilage in detail and was conducted following protocols established in our laboratory [21]. Briefly, frozen sections were incubated with mouse monoclonal anti-CD31 (diluted 1:250, MCA1334GA; AbD Serotec, Oxford, UK) or antipentosidine (diluted 1:50, KH012; Trans Genic, Kumamoto, Japan) antibodies for the joint capsule and rabbit polyclonal antivascular endothelial growth factor (VEGF;

diluted 1:400, ABS82; Merck Millipore, Billerica, MA, USA), mouse monoclonal anticollagen Type II (diluted 1:1000, F-57; Daiichi Fine Chemical, Toyama, Japan), or goat polyclonal antimatrix metalloproteinase 13 (MMP-13; diluted 1:2000, AB8120; Merck Millipore) antibodies for the articular cartilage. A subsequent reaction was made by the streptavidin-biotin-peroxidase complex technique using an Elite ABC kit (diluted 1:50, PK-6100; Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was observed with 3,3'-diaminobenzidine tetrahydrochloride (K3466; Dako Japan, Tokyo, Japan) followed by counterstaining with methylene green.

Statistical Analyses

All values are presented as mean $\pm$ SD. Analyses were performed with SPSS 16.0 J for Windows (SPSS Japan, Tokyo, Japan). Normality of distribution was assessed using the Shapiro-Wilk test and homogeneity of variance by the Levene test. Results for ROM, synovial intima length, collagen fiber disorganization, and cartilage thickness were compared among all groups with the ANOVA test followed by the Tukey's test. An alpha less than 0.05 was chosen as the significance level for these statistical analyses.

We identified the muscular and articular factors in the contractures after spinal cord injuries in rats that were significantly responsible for the difference among all groups when a length of the 95% CI did not overlap zero [26, 37].

Results

Knee extension ROM improved in all treated groups except for the LS group when compared with the S group ($p < 0.001$; Fig. 2). However, extension ROM in all treated groups did not recover to the same range as that in the C group ($p < 0.001$). Knee motion of the HL and HI ($p = 0.16$), HI and HS ($p = 0.99$), and LL and LI ($p = 1.00$) groups were not different. For the same treatment duration, ROM of a high-torque group was larger than of a low-torque group (HL versus LL, $p < 0.001$; HI versus LI, $p = 0.002$; HS versus LS, $p < 0.001$).

Stretching accelerated recovery of the articular contracture relative to the muscular contracture after spinal cord injury (Table 2). For the muscular factor, significant differences were found between the LS group and the HL, HI, HS, or LI group. For the articular factor, the HL group was significantly higher than all other groups. In contrast, the LS group was significantly smaller than all other groups

and the LI group was significantly smaller than the HL, HI, HS, and LL groups (Table 2).

There were no findings of trauma such as inflammation in the synovium and cartilage. In addition, formation of a new blood vessel (CD31-immunoreactive) in the synovium linked to synovitis [14, 41] was not observed in any group. These results imply that the improvement in contractures result from the effects of the stretching intervention. Stretching prevented some changes to the joint capsule and affected biochemical composition but did not change morphologic features of the cartilage. High-torque

Fig. 2 Knee extension ROM improved in the treated groups when compared with the untreated spinal cord injury group (S). Error bars = ± 1 SD. * There were significant differences between each treatment group and the untreated spinal cord injury group ($p < 0.001$). C = control; S = untreated after spinal cord injury; HL = high-torque and long-duration static stretched; HI = high-torque and intermittent stretched; HS = high-torque and short-duration static stretched; LL = low-torque and long-duration static stretched; LI = low-torque and intermittent stretched; LS = low-torque and short-duration static stretched.

stretching was effective against shortening of the total combined length of the posterosuperior and posteroinferior synovial intima (HL versus S, $p < 0.001$; HI versus S, $p = 0.03$; HS versus S, $p = 0.01$) (Fig. 3). Similarly, the lengths of the posterosuperior synovial intima of the HL and HS groups were longer than that of the S group (HL versus S, $p = 0.005$; HS versus S, $p = 0.01$), although the posteroinferior length did not change ($p = 0.56$). No statistical difference in the direction of collagen fibers was found among the groups among any of the six grades (90° – 105° , $p = 0.74$; 105° – 120° , $p = 0.30$; 120° – 135° , $p = 0.93$; 135° – 150° , $p = 0.12$; 150° – 165° , $p = 0.42$; 165° – 180° , $p = 0.28$). Histologically, adhesions between the posterosuperior synovial fold and the synovial membrane were observed after spinal cord injury, and the posterior femoral cartilage and synovial membrane were bridged by loose connective tissues to diminish the residual joint space. These observations were improved by stretching treatments, especially the appearance in the high-torque groups compared with that of the C group (Fig. 4). No pentosidine immunoreactivity was observed in the joint capsule of any of the groups. Total cartilage thickness (including uncalcified and calcified layers) did not differ among the groups in any region of the femur or tibia (anterior femur, $p = 0.34$; posterior femur, $p = 0.11$; anterior tibia, $p = 0.67$; posterior tibia, $p = 0.10$). However, only the uncalcified layer in the posterior femoral cartilage region was significantly thinner after spinal cord injury (C, $211.47 \pm 37.08 \mu\text{m}$; S, $123.80 \pm 32.54 \mu\text{m}$; HL, $119.93 \pm 8.53 \mu\text{m}$; HI, $108.54 \pm 4.68 \mu\text{m}$; HS, $121.61 \pm 12.76 \mu\text{m}$; LL, $97.28 \pm 20.81 \mu\text{m}$; LI, $98.50 \pm 15.56 \mu\text{m}$; LS, $118.58 \pm 13.75 \mu\text{m}$; $p < 0.001$), although the total cartilage thickness did not differ (C, $241.38 \pm 65.37 \mu\text{m}$; S, $200.09 \pm 43.88 \mu\text{m}$; HL, $224.25 \pm 21.62 \mu\text{m}$;

Table 2. ROM before and after myotomy and improvements in the muscular and articular factors after the stretching treatments

Group	ROM (mean ± 1 SD°)		Muscular factor		Articular factor	
	No myotomy	After myotomy	ROM (mean ± 1 SD°)	Rate (%)*	ROM (mean ± 1 SD°)	Rate (%)*
C	9.5 $\pm$ 0.9	9.2 $\pm$ 0.9	—	—	—	—
S	28.0 $\pm$ 0.7	19.0 $\pm$ 1.2	9.0 $\pm$ 1.2	—	9.8 $\pm$ 1.1	—
HL	18.3 $\pm$ 0.6	11.5 $\pm$ 0.6	6.8 $\pm$ 1.0 [†]	25.0	2.3 $\pm$ 0.8 ^{†‡§}	76.3
HI	20.3 $\pm$ 1.1	13.2 $\pm$ 0.6	7.1 $\pm$ 1.0 [†]	21.7	4.0 $\pm$ 0.9 ^{†‡}	59.3
HS	20.8 $\pm$ 0.9	14.3 $\pm$ 1.6	6.5 $\pm$ 1.8 [†]	28.1	5.2 $\pm$ 1.3 ^{†‡}	47.5
LL	23.3 $\pm$ 3.0	14.6 $\pm$ 1.4	8.8 $\pm$ 3.1	3.2	5.4 $\pm$ 1.1 ^{†‡}	45.0
LI	23.4 $\pm$ 1.0	16.3 $\pm$ 0.9	7.1 $\pm$ 0.8 [†]	21.4	7.2 $\pm$ 0.9 [†]	27.2
LS	27.1 $\pm$ 0.5	17.2 $\pm$ 1.6	9.9 $\pm$ 1.9	−9.4	8.0 $\pm$ 1.3	18.6

* Improvement rates of muscular and articular factors in each treatment group compared with S group; S = untreated after spinal cord injury; HL = high-torque and long-duration static stretched; HI = high-torque and intermittent stretched; HS = high-torque and short-duration static stretched; LL = low-torque and long-duration static stretched; LI = low-torque and intermittent stretched; LS = low-torque and short-duration static stretched; C = control; [†]significantly different from the LS group; [‡]significantly different from the LI group; [§]significantly different from all other groups.

Fig. 3A–C The (A) total and (B) posterosuperior synovial intima lengths in the treated groups were significantly longer than that of the untreated spinal cord injury group (S), but not the (C) posteroinferior length. Error bars = ± 1 SD. * There were significant differences between each treatment group and the untreated spinal cord injury group, $p < 0.05$. C = control; S = untreated after spinal cord injury; HL = high-torque and long-duration static stretched; HI = high-torque and intermittent stretched; HS = high-torque and short-duration static stretched; LL = low-torque and long-duration static stretched; LI = low-torque and intermittent stretched; LS = low-torque and short-duration static stretched.

Fig. 4A–C Adhesions in the posterosuperior regions after spinal cord injury were improved by stretch interventions in the (A) control group, (B) untreated spinal cord injury group (arrowheads), and (C) high-torque and long-duration static stretched group (arrows). Bar = 500 μ m; (Stain, aldehyde fuchsin-Masson Goldner staining; original magnification, $\times 40$).

Fig. 5A–C The uncalcified layer of the posterior femoral cartilage region was thinner after spinal cord injury and stretching had no significant effect on cartilage thickness in the (A) control group, (B) untreated spinal cord injury group, and (C) low-torque and long-duration static stretched group. UL = uncalcified layer; CL = calcified layer; Bar = 200 μ m; (Stain, toluidine blue staining; original magnification, $\times 100$).

HI, $138.07 \pm 61.51 \mu\text{m}$; HS, $217.68 \pm 15.64 \mu\text{m}$; LL, $163.07 \pm 56.83 \mu\text{m}$; LI, $189.20 \pm 7.57 \mu\text{m}$; LS, $186.23 \pm 72.63 \mu\text{m}$; $p = 0.11$) (Fig. 5). Stretch interventions had no

Fig. 6A–C VEGF immunostaining was seen strongly in the chondrocytes of the spinal cord injury groups and was most increased by the long- and short-duration static stretching with a high torque in the (A) control group, (B) untreated spinal cord injury group, and (C) high-torque and short-duration static stretched group. Bar = 100 μ m; (Stain, anti-VEGF antibodies; original magnification, $\times 200$).

significant effect on these changes in cartilage thickness (Fig. 5). VEGF immunostaining of the S group was stronger than that of the C group. The staining intensity and pattern

Fig. 7A–C Immunolabeling for collagen Type II was increased by stretch interventions. These findings were pronounced in the high-torque groups, and immunolabeling of the uncalcified layer was seen clearly in the (A) control group, (B) untreated spinal cord injury group, and (C) high-torque and short-duration static stretched group. Bar = 200 μ m; (Stain, anti-collagen Type II antibodies; original magnification, $\times 100$).

were most marked in the HL and HS groups followed by the LL, LI, and LS groups (Fig. 6). Immunostaining for collagen Type II was weakly noted after spinal cord injury and

immunolabeling was increased by stretching, especially in the uncalcified layer of the posterior femoral cartilage region. The staining intensity and pattern of antibody binding were most marked in the high-torque groups followed by the low-torque groups (Fig. 7). MMP-13 immunostaining showed intense expression in pericellular regions from the middle to deep cartilage layers after spinal cord injury. No apparent differences were observed between the treated groups and the untreated group.

Discussion

Contractures are a common complication of many neurologic conditions. Stretching has been advocated as an effective means of preventing and treating contractures [9, 10, 12, 15, 16]. However, the magnitude and duration of the stretching are guided mainly by subjective clinical impressions in practice. The goal of our study was to define an effective stretching protocol for a knee contracture after a spinal cord injury in a rat model. A high-torque and long-duration static stretching protocol led to greater restoration of the ROM than the other torque and duration treatment groups. Stretching was more effective in improving articular components of contractures than muscular components. Stretching in this rat model prevented shortening and adhesions of the joint capsule and affected biochemical composition but did not change morphologic features of the cartilage.

Our study has several limitations. Stretching did not completely prevent and treat the limitation in ROM after spinal cord injury and therefore future studies should assess the effect of stretch interventions with various modalities such as diathermy to improve to normal ROM. In addition, we evaluated the effectiveness of different protocols in the treatment of a knee contracture early after a T8 spinal cord injury in a rat model, with a focus on determining the efficacy of the stretching protocols to prevent contractures. In prior studies, we found that knee contractures progressed for the first 12 weeks after spinal cord injury in the rat [25, 26]. Thus, the availability and compliance with treatment in contractures occurring long after the onset of central paralysis warrant further investigation. Whether our results can be applied to humans with central nervous system injuries is a matter of investigation. Although high torque was calculated in the same way when the therapist applied maximal stretch and low torque applied mild to moderate stretch, the stretching time may not be applicable. Further, the differences between the awake human and the anesthetized rat remain unclear. Finally, we used the right and the left knees as different samples. The use of both joints has the advantages of minimizing the number of experimental animals needed and providing equivalency of

sample size for statistical purposes. However, its use cannot preclude chance findings attributable to intraindividual and interindividual variation.

Contractures are characterized by limitations in passive ROM of the affected joints [20, 34]. The limitation in ROM after spinal cord injury improved in all treatment groups except for the LS group, although ROM did not improve to normal knee extension within the 10-day intervention. For the same torque level, the long-duration static stretching protocol was the most effective followed by the intermittent stretching and short-duration static stretching protocols. Each of the total stretching times used in these cases were 30, 20, and 15 minutes; this suggests that the effectiveness of stretching is proportional to the stretching time. In line with these results, clinical strategies that position soft tissues in stretched positions for prolonged periods may be most effective [12]. Additionally, we found that a high torque contributes to an increase in ROM more than a low torque at the same duration of stretch. For comparison, in the case of contractures not attributable to central nervous system injuries, low-torque and long-duration stretching leads to greater restoration of contracture produced experimentally by immobilizing rat knees when compared with high-torque and short-duration stretching [39]. Together, the findings suggest that stretching with a torque applied to maximally stretch the affected knee and a longer duration may be an effective protocol for preventing and treating a contracture after a T8 spinal cord injury in a rat.

Identifying the relationship between the muscular and articular factors is key to investigating the pathophysiology of contractures after a spinal cord injury [26]. Stretch interventions are intended mainly to recruit the extensibility of skeletal muscle [3]. However, in our study, stretching accelerated the recovery of the articular contracture better than that of the muscular contracture. It therefore seems that concentrated effort should be directed at preventing and treating degeneration in the articular structures.

Stretching prevented shortening and adhesion of the joint capsule after spinal cord injury. We found that the synovial intima length was shortened only at the posterosuperior capsule after spinal cord injury. The adhesion and shortening of the posterosuperior synovial intima are directly responsible for contracture development after a spinal cord injury [24, 36]. High-torque stretching rectified, quantitatively, shortening of the posterosuperior synovial intima and, histologically, adhesion between the posterosuperior synovial fold and the synovial membrane. However, neither arrangement of the collagen fibers nor localization of pentosidine was significantly different among the treatment groups. Previously, we reported that disorganization of the collagen structures in the capsule tissue was observed 4 weeks after spinal cord injury [24]. Pentosidine appears to

be an indicator of chronic stiffness [18]. Therefore, the current results can be explained by differences between early and chronic stages of contractures.

Stretching affected biochemical composition but did not change morphologic features of the cartilage. When loading of articular cartilage is reduced and it is deprived of mechanical stimuli, there is rapid deterioration of biochemical and biomechanical properties [21, 23, 30]. The lower limb joints are unloaded and restricted in movement in animals and in humans with spinal cord injuries [23]. Previous studies have shown thinning of the rat knee cartilage 4 weeks after a spinal cord injury [22, 23]. However, in the current study, cartilage thickness remained unchanged and no effect of stretching was detected. This may be attributable to the differences in the experimental period and cartilage response to stretching. We found that VEGF was expressed in chondrocytes after spinal cord injury and was further increased by stretch interventions. Mechanical overload induces VEGF [29] and therefore mechanical stress by stretching may stimulate VEGF expression. However, we previously reported vascular invasions of the subchondral bone penetrating the cartilage 4 weeks after a spinal cord injury [22, 23]. VEGF encourages vascular tissue formation from bone marrow into the articular cartilage [29, 31]. Arguably, VEGF overexpression precedes neovascularization. Nevertheless, we cannot conclude from the current study whether stretching is harmful to the cartilage but this warrants future examination. Collagen Type II is responsible for stability and cell biological functions of healthy articular cartilage [27, 28]. Conversely, MMP-13 is capable of degrading collagen Type II at a much higher rate than other collagenases [2]. Interestingly, stretch interventions increased the collagen expression in the uncalcified layer, although stretching had no effect on the MMP-13 expression.

Our results indicate that stretching with a torque applied to maximally stretch the affected knee and a longer duration are more effective than a lower applied torque for a shorter duration in the improvement in contractures after a spinal cord injury in the rat. In addition, stretching appeared to be more effective in improving articular factors rather than muscular factors contributing to contractures. Our results showed that stretch interventions may prevent degeneration of the joint capsule leading to contractures and may affect biochemical composition rather than the morphologic features of the cartilage, although we cannot conclude from this study whether stretching is harmful to the cartilage. Several clinical studies in patients with spinal cord injuries suggest a substantial positive effect on soft tissues in stretched positions for prolonged periods [10, 11, 13]. The treatments evaluated in our study, using this particular animal model, should be considered to be geared toward treatment of contractures that might form very early after spinal cord injury,

not chronic contractures of long duration. We believe it is reasonable to consider preliminary clinical studies to determine whether stretching with tension applied to maximally stretch and a longer duration are clinically applicable to contractures after spinal cord injuries.

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