Quantitative and temporal differential recovery of articular and muscular limitations of knee joint contractures; results in a rat model

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Trudel G, Laneuville O, Coletta E, Goudreau L, Uhthoff HK. Quantitative and temporal differential recovery of articular and muscular limitations of knee joint contractures; results in a rat model. J Appl Physiol 117: 730–737, 2014. First published August 14, 2014; doi:10.1152/japplphysiol.00409.2014.—Joint contractures alter the mechanical properties of articular and muscular structures. Reversibility of a contracture depends on the restoration of the elasticity of both structures. We determined the differential contribution of articular and muscular structures to knee flexion contractures during spontaneous recovery. Rats (250, divided into 24 groups) had one knee joint surgically fixed in flexion for six different durations, from 1 to 32 wk, creating joint contractures of various severities. After the fixation was removed, the animals were left to spontaneously recover for 1 to 48 wk. After the recovery periods, animals were killed and the knee extension was measured before and after division of the transarticular posterior muscles using a motorized arthrometer. No articular limitation had developed in contracture of recent onset (≤2 wk of fixation, P > 0.05); muscular limitations were responsible for the majority of the contracture (34 ± 8° and 38 ± 6°, respectively; both P < 0.05). Recovery for 1 and 8 wk reversed the muscular limitation of contractures of recent onset (1 and 2 wk of fixation, respectively). Long-lasting contractures (≥4 wk of fixation) presented articular limitations, irreversible in all 12 durations of recovery compared with controls (all 12 P < 0.05). Knee flexion contractures of recent onset were primarily due to muscular structures, and they were reversible during spontaneous recovery. Long-lasting contractures were primarily due to articular structures and were irreversible. Comprehensive temporal and quantitative data on the differential reversibility of mechanically significant alterations in articular and muscular structures represent novel evidence on which to base clinical practice.

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Knee joint contractures of various severities were induced in a rat model to study the effect of spontaneous recovery. Two hundred fifty male Sprague-Dawley rats had one knee surgically fixed for one of six durations: 1, 2, 4, 8, 16, or 32 wk. The internal fixation procedure has been described previously and induces knee flexion contractures of various severities (49). Briefly, under general anesthesia, two separate 1-cm incisions were made to attach a Delrin plate to the proximal femur and distal tibia with screws to fix the knee at a 45° angle. The plate followed an internal but extra-articular submuscular course, preserving the knee joint integrity. We alternated the side of surgery. Postoperatively, rats were allowed unrestricted movement in their cages. After completion of the assigned fixation period, the plate and any covering fibrous tissue were removed. Animals with surgical failures or requiring euthanasia were replaced.

For each duration of fixation, four groups of rats were left to spontaneously recover (Tables 1 and 2). For the groups internally fixed for 1, 2, 4, and 8 wk, groups were harvested immediately after the plate was removed (baseline) and at one, two, and four times the internal fixation duration. For the groups internally fixed for 16 and 32 wk, groups were harvested immediately after plate removal, at a time equal to half the fixation duration and at one and two times the fixation duration, except for the group fixed for 32 wk, which was harvested after 48 wk of recovery. Groups are defined as week-week, where the first is the duration of fixation and the second is the duration of recovery.

At the predetermined end point of spontaneous recovery, the rats were killed by carbon dioxide inhalation and the angle of knee extension was measured. The experimental leg mounted on a previously described operator-independent motorized arthrometer (G. Trudel, unpublished observations). Knee extension was defined as the angle between knee flexion fixation (45°) and full knee extension (180°). The femur was clamped into a metal groove with the lateral condyle positioned over the center of rotation of the arthrometer. The leg rested on a movable arm that pushed the leg into extension at 0.69 rad/s. The speed gradually slowed to reach a torque of 12.5 N-cm and then stopped, at which point a camera took a photograph. This torque was compared using a univariate analysis. Finally, we calculated the articular and muscular limitations as a proportion of the total contracture (Fig. 1) (35, 51).

**Statistical analysis.** All data were analyzed with SPSS version 20 (IBM, Armonk, NY). We assumed a smaller angle of knee extension in the experimental group and used a paired, one-tailed t-test to compare experimental and control knees after myotomy. The effect of increasing durations of recovery on knee extension after myotomy was compared using a univariate analysis. Finally, we compared the gain in knee extension from myotomy between experimental and control using two-tailed t-tests. Values of $P \leq 0.05$ were considered statistically significant.

**RESULTS**

Thirteen rats required local wound care, of which 12 received antibiotics; all 13 were treated and included. At endpoint, data for 12 animals were not analyzed because of

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### Table 1. Articular contribution to knee flexion contracture

<table>
<thead>
<tr>
<th>Immobilization, wk</th>
<th>No Recovery</th>
<th>Recovery Duration 1</th>
<th>Recovery Duration 2</th>
<th>Recovery Duration 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Extension angle, °</td>
<td>Proportion of the contracture, %</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>7/40</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>13/44</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>28/50</td>
<td>56</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>53/59</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>11</td>
<td>68/70</td>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>10</td>
<td>71/70</td>
<td>100</td>
<td>11</td>
</tr>
</tbody>
</table>

Articular limitation larger than the total contracture indicated that myotomy produced less gain of extension in experimental compared with control (Figs. 1 and 2). Data above 100% were expressed as 100%.
persistent fibrous adhesions, leg fracture during testing, extension angle over 195°, or images not recorded (Table 1). Both knees of 238 rats were analyzed.

**Articular limitation.** Immediately after fixation removal, myotomy restored knee extension to control levels in recent-onset contractures (fixation for 1 and 2 wk), indicating that no significant articular limitation persisted after removal of the fixation device (Fig. 2, Table 1). At baseline, just after fixation removal, myotomy did not restore knee extension to control levels in long-lasting contractures; fixation for 4, 8, 16, and 32 wk progressively reduced the angle of extension, indicating significant articular limitations ($P < 0.001$; Fig. 2, Table 1). Spontaneous recovery for 4 and 8 wk allowed contractured knees to achieve angles of extension after myotomy comparable to those of controls in recent-onset contractures (fixation for 1 and 2 wk, respectively; Fig. 2). In the other 16 durations of recovery, a significant articular limitation to knee extension persisted ($P < 0.05$; Fig. 2). Spontaneous recovery permitted partial improvements of the articular limitation in groups 4–8 and 16–8 (Fig. 2 and Table 1).

**Muscular limitation.** At baseline, division of transarticular muscles caused large gains in knee extension in contractures with onsets of 1, 2, and 4 wk compared with control knees (46 ± 7°, 51 ± 6°, and 38 ± 4°, vs. 13 ± 4°, 12 ± 4°, and 16 ± 3°, $P = 0.007, 0.000, and 0.002$, respectively; Fig. 2), indicating a significant muscular limitation contributing to the flexion contracture (Fig. 3). At baseline, myotomy in long-lasting contractures (fixation of 8, 16, and 32 wk) did not lead to significant gain in extension (Fig. 3, Table 2). Spontaneous recovery for 1 wk after 1-wk-onset contracture reversed the muscular limitation ($P > 0.05$; Fig. 3). Recovery for 2 and 4 wk following a 2-wk-onset contracture did not reverse the muscular limitation compared with control ($P = 0.042$ and 0.047, respectively; Fig. 3) but 8 wk of recovery did. Interestingly, myotomy performed in long-lasting contractures produced less gain in knee extension compared with control

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**Fig. 2.** Angles of knee extension of rat knee joint fixed in flexion and spontaneously recovering for various durations and of controls with articular and muscular structures present and with muscular structures divided. All data were rounded half to even, which explains differences of ±1° between figures, text, and tables. *$P < 0.05$ knee extension after myotomy compared with control; †$P < 0.05$ knee extension after myotomy compared with previous time point.
knees for groups 8–8, 16–16, 32–16, and 32–48 (all P < 0.05; Fig. 3).

Proportion of knee contracture. The contribution of articular limitation to the original knee joint contracture at baseline increased from recent-onset to long-lasting contractures (from 18% after 1 wk internal fixation to 100% after 32 wk, P < 0.001; Fig. 4, Table 1). Spontaneous recovery identified the calculated articular contribution to the joint contracture as predominant (Fig. 4, Table 1).

DISCUSSION

The articular and muscular limitations to knee range of motion during spontaneous recovery of contractures have not been systematically characterized. We collected quantitative and temporal knee extension data to assess both the articular and muscular limitations. The articular contribution was measured directly by sectioning posterior muscles, whereas the muscular contribution was calculated from data measured on knee joints including both articular and muscular structures. Results evaluated the extent of contracture reversibility—or lack thereof—in the rat immobilization-induced knee flexion contracture model.

Baseline assessment of contracture severity immediately after fixation showed that knees with recent-onset contractures (1 or 2 wk of fixation) had no significant articular limitation. However, knees with long-lasting contractures developed articular limitation intensifying with the duration of fixation. In contrast, muscular limitation arose rapidly in response to immobilization and limited the knee extension of recent-onset contractures (1 and 2 wk of fixation).

After characterizing the contractures without recovery, we measured the temporal evolution of the articular and muscular limitations to knee extension of rat knees left to spontaneously recover.

Articular limitation. Recovery for extended durations did not reverse the articular limitation in knee extension angle of Fig. 3. In recent-onset contractures, myotomy produced larger gains of knee extension in experimental compared with controls (muscular limitation). Recovery of long-lasting contractures produced the reverse effect, lesser gains of knee extension in experimental compared with control joints, possibly explained by the muscle lengthening from a shortened position to an angle nearer to the articular limitation angle compared with controls. *P < 0.05 gain in knee extension statistically smaller than control for groups 8–8 (P = 0.047), 16–16 (P = 0.043), 32–16 (P = 0.028), and 32–48 (P = 0.031).
long-lasting contractures (4 or more wk of fixation). This incomplete mechanical reversibility of the posterior articular alterations confirmed our first hypothesis.

Of all articular structures, which may be responsible for the irreversible articular limitation? Bone and cartilage undergo microscopic alterations but have not been reported to limit range of joint motion (26, 47). Intra- and extra-articular ligaments from immobilized knees demonstrated weaker biomechanical characteristics in a rabbit model (36, 46). Yasuda and Hayashi (61) reviewed 37 studies on joint disuse and confirmed our first hypothesis.

Muscular limitation below 0° indicated that myotomy produced less gain of extension in experimental than in control knees (Figs. 1–3). Data below 0% were expressed as 0%.

In the rat model used in the current study, the posterior capsule adopts a folded configuration when the knee joint is flexed. Upon extension of the knee the posterior capsule unfolds to a full stretch (Fig. 5). Opposing synovial intima folds slide on each other possibly owing to constant movement, contact inhibition, and synovial fluid (50). Internal fixation in flexion alters this homeostasis: opposing synovial folds and rearranged subsynovial collagenic matrix cannot spontaneously reestablish a linear, foldable, and extensible posterior knee capsule; attempts at stretching such a capsule may end in a tear (Fig. 5). In this study the irreversibility of articular limitation may be attributable to 1) the large extent of posterior synovial intima length had decreased from a normal value of 8.6 mm to 1.4 mm after 16 wk of immobilization, a sizeable shortening of 84% (48) (Fig. 5). The synovial layer also has fewer proliferating synoviocytes and produces less synovial fluid (25, 43, 48). In addition, the capsule undergoes subsynovial changes characterized by disordered alignment of collagen fibers, increased type I collagen, and advanced glycation end-products (29).

This structural reorganization of the synovial and subsynovial tissues forming the posterior capsule resists elongation after myotomy and can explain the articular limitation to knee extension in long-lasting contractures. Adhered and fused capsular folds and rearranged subsynovial collagenic matrix cannot spontaneously reestablish a linear, foldable, and extensible posterior knee capsule; attempts at stretching such a capsule may end in a tear (Fig. 5). In this study the reversibility of articular limitation may be attributable to 1) the large extent of posterior synovial intima.

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the articular structural alterations and 2) the poor potential of these structures for reversibility.

Muscular limitation. In a purely myogenic contracture, dividing the transarticular muscles restores full range of knee motion in the absence of arthrogenic limitation (35, 51, 57, 58). This was the case in the current study in which spontaneous recovery reversed the muscular limitation of recent-onset contractures, confirming our second hypothesis. In long-lasting contractures, the potential for reversibility of posterior knee muscles was blunted by articular limitations. Interestingly, recovery of contractures caused by 8 or more wk of internal fixation created situations in which posterior myotomy resulted in less gain of knee extension than control knees (shown as negative values in Table 2 and Fig. 3). This finding was unexpected and, to our knowledge, is reported here for the first time. This demonstrated that spontaneous recovery reversed the muscular limitation to an angle nearer to the fixed articular limitation angle than in controls.

The potential of muscular structures to adaptively shorten or lengthen has been precisely documented (5, 15, 20, 33, 42, 53, 54, 57, 59, 60). Loss of sarcomeres characterizes muscles immobilized in a shortened position after 4, 7, and 14 days (20, 52, 54, 59), and was accompanied by reduced protein synthesis and downregulated pathways of energy metabolism, mitochondrial function, and cell cycle regulation (8, 40). Muscle adaptive shortening happened longitudinally, as opposed to the folding of the posterior capsule. In addition to the depolymerization of thick and thin myofilaments, changes in muscle connective tissues contribute to myogenic contractures. Järvinen et al. (23) showed increased endomysial and perimysial connective tissue with disturbed crimp angle, whereas Okita et al. (38) demonstrated increased circumferential rearrangement of endomysium fibrils with immobilization. Muscle connective tissue increases were less in a shortened position compared with a lengthened position (24, 45) and with slow compared with a fast shortening (57), as in the current study. Lastly, atrophy of muscles around the immobilized knee can participate in the myogenic component of joint contractures. However, atrophy characterized by decrease in protein synthesis and increase in protein degradation resulting in a decreased protein content, smaller fiber diameter, force production, fatigue resistance, and muscle volume was shown to decrease the muscle passive extensibility (4, 13, 14, 22, 56). This mechanical effect of muscle atrophy is opposite to the measures we obtained in this model of knee joint contractures. In the current experiment, the increased muscular limitation may be explained by a rapid loss of sarcomeres from adaptive shortening of knee flexor muscles in the posterior thigh and leg and by muscle connective tissue alterations.

Reversibility of adaptively shortened muscles can proceed with addition of sarcomeres, via the elastic properties of connective tissue through stretching and active muscle contraction (5, 54, 57). Quantitatively, Grieve et al. (17) measured shortening of the human gastrocnemius muscle of 10% with the knee flexed 150° from full extension. Visser et al. (55) measured shortening of human gastrocnemius and biceps femoris at 7% and 4%, respectively, between extension and the knee flexed 100° from full extension (Fig. 5). During bone distraction in humans, muscles lengthen 20% to 30% during limb lengthening (28, 31). The high plasticity of muscle to elongation is reflected in a case report in which the tibia was elongated by 22 cm in 371 days (30). The reversibility of the muscular limitation in recent-onset contractures may be attributable to 1) the small extent of the muscular structural alterations and 2) their favorable potential for reversibility.

Quantitative and temporal data in the current study correlate with the pathophysiology of articular and muscular structures. Spontaneous recovery can reverse early onset knee flexion contractures if posterior knee muscles regain up to 10% of their original longitudinal length through adaptive lengthening in combination with posterior capsule unfolding not mechanically limiting knee extension; myotomy leading to complete knee extension shows that the capsule was not involved in the contracture. In contrast, spontaneous recovery cannot reverse long-lasting knee flexion contractures if structural alterations in the posterior capsule mechanically prevent regaining 84% of its original length and prevent exploiting the muscle lengthening potential; myotomy failing to improve knee extension shows that adhesions between the capsule folds may have blocked contracture reversibility.

Clinical relevance. Articular limitations herein temporally and quantitatively characterized may explain the high prevalence of irreversible chronic joint contractures identified in the literature (2, 62). The current study design corresponds to the clinical practice in which most knee joint contractures presenting to clinicians have formed over some duration of immobility and may have undergone some duration of spontaneous recovery. The posterior knee capsule had been identified as the primary cause of extension loss in posttraumatic and postsurgical contractures (7). The lack of reversibility has led many surgeons to perform posterior or posteroomedial knee capsulotomy with positive results in contractures secondary to hemophilia, ligament repair, fracture, or infection (7, 18, 27, 32, 34, 39, 41). However, many avoid this procedure because of the high risk of neurovascular damage. Chen and Dragoo (7) summarized the procedure as “Posterior capsular release, although technically demanding, is effective for treating flexion contractures secondary to scarring and contracture of the posterior capsule.” Importantly, dividing the posterior capsule without the need to lengthen muscles suggested spontaneous reversibility of the muscular limitation (18, 27, 32, 39, 41). Our finding that the articular structures, including the capsule but not the muscular structures, prevent reversibility of extension in long-lasting knee flexion contractures, supports the clinical recommendation for posterior capsulotomy.

Limitations. The rat habitual knee flexion position may be more resistant to flexion contracture; we used the contralateral knees also in habitual flexion to isolate the change in knee range of motion and attribute the contracture to the intervention. Mechanical testing carried out before myotomy may have weakened articular structures, underestimating the articular limitation. Oki et al. (37) directly assessed muscular limitation in immobilized rat ankles by dividing articular structures first. They found muscular limitations superior or equal to articular limitations after 1, 2, and 4 wk of immobilization, which is comparable to our results. We found no data on the direct measurement of muscular limitation after long periods of knee fixation or on their evolution over time after spontaneous recovery.

Our findings may not be generalized to all diarthrodial joints. Immobilized rat shoulders demonstrated similar structural changes of capsular folding, adhesion, decreased synovial
length, and lack of improvement with myotomy (25). Clinically, shoulder capsulotomy increased range of motion without intervention on shoulder muscles (3, 11, 16).

Future research can examine parsing the role of J) loss of sarcomere in length, loss of myofibrils in parallel (atrophy), and connective tissue accumulation in the myogenic contracture; and 2) capsular folding, intimal length, synoviocytes, and collagen fibers in the arthrogenic contracture.

Conclusion. Knee flexion contractures of recent onset were primarily due to muscular structures, and they were reversible during spontaneous recovery. Long-lasting contractures were primarily due to articular structures and were irreversible. Posterior capsule structural alterations are the candidate culprit given their unlikely return to anatomical integrity and function. Comprehensive temporal and quantitative data on the differential reversibility of mechanically significant alterations in articular and muscular structures represent novel evidence on which to base clinical practice.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.T., O.L., L.G., and H.K.U. conception and design of research; E.C. prepared figures; G.T. drafted of research; E.C. performed experiments; E.C. analyzed data; G.T., O.L., and

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