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Acute Ankle Sprain in a Mouse Model: Changes in Knee-Joint Space

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Context: Ankle sprains remain the most common orthopaedic injury. Conducting long-term studies in humans is difficult and costly, so the long-term consequences of an ankle sprain are not entirely known.

Objective: To measure knee-joint space after a single surgically induced ankle sprain in mice.

Design: Randomized controlled trial.

Setting: University research laboratory.

Patients or Other Participants: Thirty male mice (CBA/2J) were randomly placed into 1 of 3 surgical groups: the transected calcaneofibular ligament (CFL) group, the transected anterior talofibular ligament/CFL group, or a sham treatment group. The right ankle was operated on in all mice.

Main Outcome Measure(s): Three days after surgery, all of the mice were individually housed in cages containing a solid-surface running wheel, and daily running-wheel measurements were recorded. Before surgery and every 6 weeks after surgery, a diagnostic ultrasound was used to measure medial and lateral knee-joint space in both hind limbs.

Results: Right medial ($P = .003$), right lateral ($P = .002$), left medial ($P = .03$), and left lateral ($P = .002$) knee-joint spaces decreased across the life span. The mice in the anterior talofibular ligament/CFL group had decreased right medial ($P = .004$) joint space compared with the sham and CFL groups starting at 24 weeks of age and continuing throughout the life span. No differences occurred in contralateral knee-joint degeneration among any of the groups.

Conclusions: Based on current data, mice that sustained a surgically induced severe ankle sprain developed greater joint degeneration in the ipsilateral knee. Knee degeneration could result from accommodation to the laxity of the ankle or biomechanical alterations secondary to ankle instability. A single surgically induced ankle sprain could significantly affect knee-joint function.

Key Words: ankle injuries, physical activity, proximal changes, joint degeneration

Key Points

- A surgically induced ankle sprain may lead to joint degeneration in the ipsilateral knee in mice.
- Clinicians should focus on proper rehabilitation of ankle sprains to potentially avoid deleterious changes at the knee.
- After ankle injury, knee mechanics should be assessed for changes.

Lateral ankle sprains are one of the most common orthopaedic injuries.[1] Although the initial ankle sprain is concerning, 70% of patients go on to resprain their ankles and develop long-term impairments in function.[2] Previous researchers have examined the multiple insufficiencies that likely lead to the development of chronic ankle instability (CAI). A combination of mechanical instability[3–6] and neuromuscular impairment[7–9] is the probable primary cause of CAI. The bigger concern with the development of CAI is decreased physical activity secondary to the associated symptoms.[10] This decreased physical activity could lead to a host of diseases: physical inactivity is currently classified as 1 of the 3 highest risk behaviors in the development of cardiovascular disease, cancer, and other chronic diseases such as diabetes and obesity.[11] Continued research is needed to understand not only the changes at the ankle after an ankle sprain but also the changes at proximal joints so that clinicians can provide appropriate rehabilitation and treatment to prevent long-term impairment.

Several groups[12–16] have reported alterations in knee-joint kinematics, neuromuscular control, and knee torque production in those with CAI. Most recently, Terada et al[12] demonstrated altered feed-forward patterns of the vastus medialis oblique (VMO) muscle in those with CAI. The authors also found that participants with CAI had altered postlanding knee sagittal-plane kinematics. More specifically, the CAI group demonstrated a decreased knee-flexion angle after landing during a stop-jump task.[12] A decreased knee-flexion angle negatively influences the energy-dissipation capability of the knee joint. Increased activation of the VMO may be a compensatory mechanism to allow greater energy dissipation at the knee during landing to protect the ankle joint. However, if the increased activation of the VMO is robust enough to decrease knee-joint stress due to the altered landing pattern is unknown.

One of the primary concerns with altered knee kinematics and neuromuscular control is its influence on knee-joint injury and, in particular, the development of knee osteoarthritis (OA).[16–18] Altered joint loading at the knee

has been suggested to contribute to knee OA. Biomechanical changes such as those described earlier may modify the regions where tibiofemoral joint contact occurs. These changes in load may lead to damaged cartilage and potentially the start of knee OA. We know that recurrent ankle sprains are the second leading cause of posttraumatic ankle OA, and, as at the knee,[19,20] alterations in mechanical stability and neuromuscular control may contribute.[21] Tallroth et al[22] reported that 30 of 104 patients (29%) with knee OA also had ankle OA. They also observed that the greater the tilt at the ankle, the more degenerative changes in the knee, as damage to the ankle negatively affected the load on the knee, leading to greater amounts of knee-joint degeneration.

Although ankle sprains are the most common orthopaedic injury, have high recurrence rates, and negatively affect physical activity and subjective function, they are typically not regarded as significant injuries. Part of the problem is the difficulty in conducting long-term research to understand the effect of an ankle sprain on proximal joint changes. The mouse is an ideal model for prospectively measuring proximal joint changes and lifelong physical activity levels. Recent investigators[23] have reported that the anatomical and histologic features of human and mouse ankle joints are comparable. We do not currently know if an ankle sprain can lead to knee-joint degeneration and the development of knee OA. If so, it extends the effect of an ankle sprain and the need for research examining interventions to prevent changes not only at the ankle but at the knee joint as well. Therefore, the purpose of our study was to examine the effects of surgically transecting the lateral ligaments of a mouse hind limb (thereby inducing mechanical ankle instability) and measure knee-joint space.

METHODS

Animals

Thirty male mice (CBA/J), 5 to 6 weeks old, were purchased from Jackson Laboratory (Bar Harbor, ME). Baseline testing was performed when the mice were 7 weeks of age; this is equivalent to postpuberty age in humans (midteens). All mice were housed in the university vivarium (an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility) with 12-hour light-dark cycles and room temperatures and relative humidity standardized to 18°C to 22°C and 20% to 40%, respectively. All mice were provided with water and standard chow (8604 Rodent Diet; Harlan Teklad, Madison, WI) ad libitum. Each mouse was monitored daily for health. The staff that provided daily care to the mice was blinded to group assignment. All study procedures were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Charlotte.

Joint-Space Width Measurements

Knee joint-space measurements replicated methods reported in the literature.[17,24] All measurements were taken by 1 investigator who was blinded to group assignment. Intertester and intratester reliability were previously established[17] for the investigator obtaining the measurements. Intratester reliability (intraclass correlation coefficient [2,1]) for right medial joint-space measurements was 0.92 and intertester reliability (intraclass correlation coefficient [2,1]) was 0.86. The standard errors of measurement were 0.003 and 0.0004 mm, respectively. Throughout the study, all knee joint-space measurements were taken around the same time (between 10 and 11 AM) and always immediately after administration of anesthesia to account for possible changes in cartilage thickness. Baseline measurements of knee-joint space were taken on all mice before they were randomly allocated to a surgery condition (anterior talofibular ligament [ATFL]/calcaneofibular ligament [CFL], CFL only, or sham treatment [SHAM]). Medial and lateral knee-joint spaces (mm) in both hind limbs were measured as a marker for the development of OA in all 30 mice before surgery and every 4 weeks thereafter for the duration of their life spans. To measure joint space, we anesthetized each mouse with 4% isoflurane gas and supplemental oxygen. The mouse hind limbs were then shaved, and ultrasound gel was applied to the medial and lateral aspect of both hind limbs. Diagnostic ultrasound (model SONOS 5500; Agilent Technologies, Andover, MD) with a SONOS 15-6_L ultrasound probe was used to image both hind limbs. Once the medial and lateral joint lines were located, all joint-space measures were made 1 mm from the medial or lateral epicondyle. From this location, the distance from the medial or lateral condyle of the femur to the medial or lateral condyle of the tibia was measured to identify joint space for the respective hind limb.[24] Diminished knee-joint space is a clinical indicator of knee OA development.

Surgery Procedures

The surgical methods replicated methods previously reported in the literature.[25] Each mouse was anesthetized with 4% isoflurane gas and supplemental oxygen. The right ankle was then shaved and cleaned with alcohol, followed by a chlorhexidine scrub. After the site was prepared, the mouse remained anesthetized and was moved to a sterile surgical field under a microscope.[25] To help guide our transections, we used the techniques of Kim et al.[26] For the CFL-only group, a small incision was made under the microscope using sterile equipment. The skin was retracted, the CFL was transected, and the skin was closed using 2 drops of formulated cyanoacrylate surgical adhesive. For the ATFL/CFL group, after the skin was retracted, both the ATFL and CFL were transected, and the skin was closed using 2 drops of formulated cyanoacrylate surgical adhesive. For the SHAM group, a small incision was made in the same place as for the CFL-only and ATFL/CFL groups; however, no ligaments were damaged, and the skin was closed using 2 drops of formulated cyanoacrylate surgical adhesive. After the surgery was completed, the anesthesia was stopped and the mouse taken to a recovery area. Each mouse received a subcutaneous injection of 5.0 mg/kg carprofen (Rimadyl; Zoetis, Parsippany, NJ) diluted with saline and was allowed to recover under a warming lamp until freely mobile. Mice were monitored every 24 hours after surgery and were given 12.5-mg carprofen (Rimadyl) tablets ad libitum for pain management throughout the first 3 days after surgery.

Table 1. Right Medial Joint Space, mm (Mean ± SD)

Age, wk	Group: Anterior Talofibular/ Calcaneofibular Ligament Transection	Group: Calcaneofibular Ligament Transection	Group: Sham
6 (Baseline)	0.42 ± 0.09	0.44 ± 0.10	0.42 ± 0.08
12	0.41 ± 0.09	0.42 ± 0.07	0.42 ± 0.10
24	0.36 ± 0.07[a]	0.39 ± 0.05	0.41 ± 0.02
32	0.32 ± 0.09[a]	0.35 ± 0.07	0.39 ± 0.004
40	0.29 ± 0.10[a]	0.33 ± 0.008	0.38 ± 0.08
48	0.26 ± 0.05[a]	0.29 ± 0.04	0.33 ± 0.06
56	0.20 ± 0.08[b]	0.22 ± 0.01[c]	0.29 ± 0.07
64	0.15 ± 0.04[b]	0.17 ± 0.08[c]	0.24 ± 0.09
72	0.12 ± 0.03[b]	0.14 ± 0.07[c]	0.20 ± 0.09
80	0.12 ± 0.05[b]	0.13 ± 0.02[c]	0.18 ± 0.05

[a] Differences among all groups ($P < .05$).
[b] Difference between the anterior talofibular/calcaneofibular ligament transection and sham groups ($P < .05$).
[c] Difference between the calcaneofibular ligament transection and sham groups ($P < .05$).

Table 2. Right Lateral Joint Space, mm (Mean ± SD)[a]

Age, wk	Group: Anterior Talofibular/ Calcaneofibular Ligament Transection	Group: Calcaneofibular Ligament Transection	Group: Sham
6 (Baseline)	0.41 ± 0.06	0.42 ± 0.006	0.42 ± 0.04
12	0.42 ± 0.002	0.42 ± 0.10	0.41 ± 0.05
24	0.42 ± 0.06	0.41 ± 0.03	0.41 ± 0.30
32	0.41 ± 0.20	0.39 ± 0.02	0.39 ± 0.06
40	0.39 ± 0.03	0.37 ± 0.11	0.37 ± 0.05
48	0.37 ± 0.006	0.36 ± 0.003	0.36 ± 0.10
56	0.34 ± 0.02	0.35 ± 0.06	0.34 ± 0.02
64	0.30 ± 0.10	0.29 ± 0.09	0.30 ± 0.002
72	0.25 ± 0.09	0.23 ± 0.06	0.24 ± 0.04
80	0.19 ± 0.01	0.20 ± 0.08	0.19 ± 0.006

[a] No significant differences were demonstrated.

Statistical Analysis

Separate multivariate analyses of variance (group × time) with repeated measures were performed to compare changes in knee-joint space (right medial, right lateral, left medial, and left lateral) dependent variables. Our last measurement time was at 80 weeks of age. We did not test past 80 weeks of age because the decrease in joint space was beyond measurement error. At 80 weeks, the numbers of mice that had died because of age-related health problems in each group were ATFL/CFL group, 2; CFL-only group, 1; and SHAM group, 0. Data were not censored after an animal died. Post hoc comparisons of between-times means were performed using Tukey honestly significant difference tests. An α level of $P < .05$ was used to determine significant effects for each analysis. All statistical analyses were performed using JMP Statistical Analysis software (SAS Institute, Cary, NC).

RESULTS

Mice

The average life span for each group of mice was ATFL/CFL group = 92.4 ± 1.9 weeks; CFL-only group = 88.2 ± 9.4 weeks; and SHAM group = 87.9 ± 16.1 weeks. No differences ($P = .07$) were present in body weight or food consumption among the groups across the life span.

Knee-Joint Space

Right medial ($P = .003$), right lateral ($P = .002$), left medial ($P = .03$), and left lateral ($P = .002$) knee-joint spaces decreased across the life span. The mice in the ATFL/CFL group had decreased right medial ($P = .004$) joint space compared with the CFL-only and SHAM groups starting at 24 weeks of age (Table 1). There was a difference between the CFL-only and SHAM groups beginning at 56 weeks of age ($P = .012$). Mice in the CFL-only group had a smaller joint space compared with the SHAM group. No differences were found in contralateral knee-joint degeneration among any of the groups ($P > .05$) or in the right lateral joint space (Table 2). Effect sizes (95% confidence intervals) between groups are presented in Table 3.

DISCUSSION

This study represents the first use of a mouse ankle-sprain model to investigate the effects of an ankle sprain on knee-joint space. After inducing an ankle sprain, we followed knee-joint space across the life span. As seen in a human model, knee-joint space declined across the life span in all groups. The mice with the more severe ankle sprains had diminished knee-joint space compared with the CFL-only and SHAM groups. It is important to note the CFL-only group had diminished knee-joint space later in the life span than the SHAM group. Based on the results of this study, inducing an ankle sprain in a mouse model leads to less knee-joint space across the life span relative to a SHAM condition.

McKinley et al[27] demonstrated in human cadaveric ankles that joint instability increased contact stress and resulted in articular surface incongruity. However, it would be reasonable to assume that contact stress and surface incongruity changes occurred at the ankle after sectioning 1 (CFL) or 2 (ATFL and CFL) ankle ligaments and that such sectioning could also change the contact stress, distribution, and directional gradients at the knee joint. The mechanism responsible for degeneration at the knee joint may be changes in the articular cartilage. The major components of articular cartilage are water, extracellular matrix (ECM), and chondrocytes.[18] Several researchers[28–31] have hypothesized that abnormal loading secondary to joint instability could disrupt the ECM. This disruption in the ECM may lead to the release of glycosylated aminoglycans and collagen molecules. Changes in gene expression and cartilage metabolism may then occur that could set up a cascade of events leading to degradation of the articular cartilage.[28–31] The mechanical instability from the surgically induced ankle sprains in the mice is hypothesized to cause abnormal loading at the knee. This may have led to the diminished joint space we measured secondary to degradation of the articular cartilage, but future research is needed to confirm this hypothesis. It is also important to note that loading may affect not only the articular cartilage but could also lead to bone changes, which were not measured in the current study.

Table 3. Right Medial Joint-Space Effect Sizes (95% Confidence Intervals)

	Group Comparison		
Age, wk	Anterior Talofibular/CF Ligament Transection With CF Ligament Transection	CF Ligament Transection With Sham	Anterior Talofibular/CF Ligament Transection With Sham
6 (Baseline)	0.21 (−0.17, 0.67)	0.28 (−0.10, 1.04)	0 (−0.04, 0.27)
12	0.12 (−0.24, 0.37)	0 (−0.10, 0.27)	0.10 (−0.12, 0.34)
24	0.49 (0.11, 0.98)	0.40 (0.12, 0.99)	0.97 (0.41, 1.84)
32	0.37 (0.01, 0.87)	0.57 (−0.02, 1.35)	1.00 (0.61, 2.14)
40	0.56 (−0.10, 1.14)	0.63 (0.09, 1.54)	0.99 (0.61, 2.01)
48	0.66 (0.12, 1.27)	0.66 (0.08, 1.39)	1.26 (0.74, 2.31)
56	0.35 (−0.23, 0.99)	0.70 (0.27, 1.74)	1.19 (0.60, 1.99)
64	0.32 (0.10, 0.78)	0.87 (0.17, 1.89)	1.29 (0.71, 2.04)
72	0.40 (0.01, 1.01)	0.85 (0.10, 1.82)	1.19 (0.57, 2.11)
80	0.26 (−0.27, 0.72)	1.0 (0.29, 1.99)	1.20 (0.62, 2.15)

Abbreviation: CF, calcaneofibular.

Several previous groups[16,22,32] have examined the association of knee and ankle OA. All reported an increased risk of ankle OA ipsilateral to knee OA.[16,22,32] In a more recent study, Kraus et al[33] noted a high prevalence of radiographic features of ankle OA in their knee OA cohort. Interestingly, 22% of patients in the study had a history of ankle injury.[33] However, with the retrospective design, it is impossible to identify the role the ankle injury could have had on the ankle or knee OA. Although the focus of this study was patients with knee OA, the results show a relationship between knee- and ankle-joint degeneration.

The development of decreased knee-joint space in the current study may also be related to the physical activity of the mice.[34] This work was part of a larger study in which we also measured lifelong physical activity levels. We reported previously[34] that the mice in this study with the more severe injuries had lower levels of physical activity throughout the life span compared with the other groups.[34] Several authors[35,36] have described the positive effect of physical activity on the health of the knee joint. Previously, Hubbard-Turner et al[17] found that increased physical activity protected the knee joint from degeneration in otherwise healthy mice prone to the development of knee OA (C57Bl/6J). The mice used in the current study are not genetically predisposed to knee OA, and thus, the injury to the ankle or the subsequent decreased physical activity (or both) may have caused the observed knee-joint degeneration. In another study[37] of rats, gentle treadmill walking suppressed proinflammatory gene networks and upregulated matrix synthesis to prevent progression of cartilage damage in induced arthritis. In the rats that had knee OA induced, walking on a treadmill (12 m/min for 45 min/d) prevented further cartilage destruction.[37]

There are some limitations in the current study. We used diagnostic ultrasound to measure knee-joint space in the mice. Other methods to more directly evaluate joint health with endpoint analysis techniques (Safranin-O staining) could be used in future research. This study was part of a larger study in which the purpose was to measure and evaluate physical activity across the life span and thus we did not want to use endpoint analysis. Ultrasound imaging allowed us to measure joint space and maintain all mice throughout their natural life spans and did not interfere with the additional data being collected for the other outcomes. Another limitation is that we surgically transected the lateral ligaments, which does not replicate human injury. We surgically transected the ligaments to standardize the amount of damage across animals. This may cause more injury than an ankle sprain in a human model because of damage to the joint capsule; therefore, greater neuromuscular dysfunction may occur, which could affect physical activity and knee joint-space measurements.

Numerous researchers have reported the mechanical,[3–6] sensory,[7–9] and proximal changes[12–15] that occur after an ankle sprain and in those with CAI. Any of these negative alterations could lead to decreased physical activity. Many could also contribute to the changes in joint function leading to knee-joint degeneration and potentially knee OA after an ankle sprain. Injury to the ankle joint is not an innocuous injury, and examination of treatment considerations to prevent these negative changes needs to occur more frequently. Initial management may be critical to restoring mechanical stability and normal joint mechanics. Further treatment should address sensory and motor changes as well as the restoration of joint function, not only at the ankle but at the proximal joints. With more research into the treatment of ankle sprains, perhaps degenerative changes in the lower extremity can be prevented and physical activity after an ankle sprain can continue.

CONCLUSIONS

Sectioning of 1 or both of the lateral ankle ligaments may result in increased knee-joint degeneration across the life span. We currently do not know the exact mechanism that leads to the changes at the knee. Decreased physical activity and knee-joint changes may be related. Future authors should aim to elucidate the mechanisms that lead to the changes in knee-joint health across the life span and seek ways to improve physical activity in those with musculoskeletal injuries.

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