Lateral Ankle Sprain in a Mouse Model: Lifelong Sensorimotor Dysfunction

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**Context:** Ankle sprains are the most common orthopaedic injury that occurs during sport and physical activity. Many individuals who sprain their ankles develop chronic ankle instability (CAI), a condition characterized by recurrent injury, decreased physical activity, and decreased quality of life. These residual impairments are believed to persist for the remainder of the patient's life, in part due to the link between CAI and posttraumatic ankle osteoarthritis. However, this belief remains speculative due to the lack of long-term prospective investigations.

**Objective:** To use a mouse model of mild (MILD) and severe (SEVERE) ankle sprains to quantify balance and locomotor adaptations across the lifespan.

**Design:** Cohort study.

**Setting:** University research laboratory.

**Patients or Other Participants:** Fifty male mice (CBA/J) were randomly placed into a control (SHAM), MILD, or SEVERE group and housed individually.

**Intervention(s):** The MILD group underwent surgical transection of a single right hind-limb lateral ankle ligament, and the SEVERE group had 2 of the lateral ligaments transected. The SHAM group underwent a sham surgery during which no lateral ligaments were transected.

**Main Outcome Measure(s):** After surgically inducing the ankle sprain, we measured balance and gait using a balance beam and footprint test before and every 6 weeks for 78 weeks.

**Results:** Age-related declines in balance but not stride length were exacerbated by an ankle sprain ($P < .001$). Balance and stride lengths changed with age ($P < .001$). Foot slips were worse in the SEVERE ($4.32 \pm 0.98$) and MILD ($3.53 \pm 0.98$) groups than in the SHAM group ($2.16 \pm 0.99$; $P < .001$). Right-limb stride length was shorter in the SEVERE group ($6.45 \pm 0.41$ cm) than in the SHAM group ($6.87 \pm 0.40$ cm; $P = .04$).

**Conclusions:** Transecting the lateral ligaments of a mouse hind foot resulted in lifelong sensorimotor dysfunction. Declines starting at 42 weeks postinjury may have represented the onset of posttraumatic osteoarthritis.

**Key Words:** murine, balance, locomotion, chronic ankle instability

**Key Points**

- As the mice aged, balance declined and stride lengths decreased in the injured limb.
- The decline in sensorimotor function beginning at 42 weeks postinjury may have coincided with a transition from chronic ankle instability to posttraumatic ankle osteoarthritis.
- The mouse ankle-sprain model may be appropriate for studying the acute and long-term effects of a lateral ankle sprain and for quantifying the proposed cascade of events believed to result in chronic ankle instability.

Lateral ankle sprains (LASs) are the most common injuries associated with athletic participation. This injury is also extremely frequent among the general public in the United States, with more than 1 million ankle sprains seen in emergency departments each year, accounting for more than $1 billion in health care charges in 2010 alone. Lateral ankle sprains accounted for approximately 92% of all health care visits for ankle sprains and approximately 90% of all health care charges for ankle sprains. Unfortunately, LASs are not 1-time injuries, as commonly thought; at least 30% of those who sustain a first-time LAS develop chronic ankle instability (CAI), a condition characterized by recurrent injury, decreased physical activity, and decreased quality of life. These residual impairments are believed to persist for the remainder of the patient's life, in part due to the link between CAI and posttraumatic ankle osteoarthritis. Indeed, patients with posttraumatic ankle osteoarthritis have balance and locomotive impairments. However, a lack of lifelong prospective studies, for obvious logistical reasons, limits our understanding of how balance and locomotor adaptations change over the lifespan after an LAS and the development of CAI.

The recent development of an LAS mouse model has the potential to allow us to address these critical gaps in the literature. First, the model can leverage genetically identical mice that sustain the same injury and undergo identical treatment plans to minimize the heterogeneity of injury symptoms and treatments so that critical long-term prospective data on the effects of LAS and the development of CAI can be captured. Hubbard-Turner et al demonstrated that the symptoms resulting from transection of 1
lateral ligament represented a mild LAS (MILD) in humans and transection of 2 lateral ligaments represented a moderate to severe LAS (SEVERE) in humans. Subsequently, Wikstrom et al. reported that at 1-year postinjury, these same mice demonstrated sensorimotor adaptations and physical activity reductions that were similar to those in human patients with CAI.

Given the relatively brief lifespan of mice, the model can also prospectively capture the ramifications of an ankle sprain. Studying the same cohort of mice, Hubbard-Turner et al. and Turner et al. quantified lifelong declines in physical activity and cardiovascular function, respectively. However, the lifelong effects of an LAS on sensorimotor functions, such as postural control and locomotion, remain unknown. We wanted to expand on previous reports of sensorimotor dysfunction in this same cohort of mice. Therefore, the purpose of our study was to examine the lifelong effects of surgically induced MILD and SEVERE on postural control and locomotor outcomes relative to a control treatment (SHAM) in mice. On the basis of the human CAI and ankle osteoarthritis literature and the work by Hubbard-Turner et al. and Turner et al., we hypothesized that the SEVERE would have a more profound effect on postural-control impairments over the entire lifespan than the MILD or SHAM. Similarly, we hypothesized that the SEVERE would have a more profound effect on locomotor outcomes than the MILD or SHAM.

METHODS

Animals

Fifty male mice (CBA/J) were purchased from Jackson Laboratory (Bar Harbor, ME) at 5 to 6 weeks old. These mice were housed in the university vivarium with 12-hour light-dark cycles and standardized room temperatures (range = 18°C–22°C) and relative humidity (range = 20%–40%). As previously reported, all mice were provided with water and standard chow (8604 Rodent Diet; Harland Teklad, Madison, WI) ad libitum. All study procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Charlotte.

Surgical Procedures

All surgical procedures occurred when the mice were 7 weeks of age and have been described previously. After the mice were anesthetized, their right hind limbs were shaved and prepared with alcohol and a chlorhexidine scrub. We moved each mouse to a sterile surgical field under a surgical microscope and made a small curvilinear incision behind the lateral malleolus. The SHAM group did not have any ligaments transected; the MILD group had the calcaneofibular ligament transected, and the SEVERE group had the calcaneofibular and anterior talofibular ligaments transected. Subsequently, the skin was closed using a surgical adhesive (cyanoacrylate), and the mice were observed in a recovery area. Each mouse received a subcutaneous injection of 5.0 mg/kg of carprofen (Rimadyl; Zoetis, Parsippany, NJ) diluted in saline and was allowed to move freely under a warming lamp. After recovery, a 12.5-mg tablet of carprofen was given. Additional carprofen was available at 24-hour checks if the initial 12.5-mg tablet was consumed but no additional analgesia was needed beyond the initial 12.5 mg of carprofen.

Balance and Locomotion Assessments

Balance and locomotion were assessed as previously reported. In brief, balance was assessed by measuring the ability of the mice to cross an inclined (15°), narrow, round, wooden beam that had a length of 1 m and a diameter of 19 mm and was connected to an enclosed 20-cm² box into which they could escape. Sensorimotor function, such as postural control and locomotion, was tested at baseline (presurgery) and every 6 weeks for 3 consecutive attempts. Intrarater and interrater reliability of calculating these gait outcomes has been established as excellent. The dependent measures were left- and right-limb stride length. For each outcome, the maximum number of values was obtained from each test trial while excluding footprints made as the mouse was initiating and terminating gait. The mean value of each set of outcomes was used for statistical analysis. Stride length was calculated using a heel-to-toe measuring technique. Intrarater and interrater reliability of calculating these gait outcomes has been established as excellent. Before surgery, all mice were trained to help promote a consistent performance. Training was considered complete when a mouse traversed the beam in less than 20 seconds for 3 consecutive attempts. Sensorimotor function was tested at baseline (presurgery) and every 6 weeks for 78 weeks postsurgery.

Statistical Analysis

A between-groups 2-way repeated-measures analysis of variance (group × time) was performed to compare changes in each primary outcome measure (time to cross the beam, slips, left-limb stride length, right-limb stride length) due to the independent variables. Bonferroni post hoc comparisons were performed, when appropriate, to determine the location of differences when main effects or interactions were observed. Hedges g effect sizes (ESs) were calculated and interpreted as small (g < 0.3), moderate (0.31–0.7), or large (g > 0.71). We set the α level at .05 and used SPSS (version 21; IBM Corp, Armonk, NY) for all analyses.

RESULTS

Balance declined with age, as both the number of slips (F13,611 = 23.78, P < .001) and time to cross the beam (F13,611 = 8.93, P < .001) worsened over the life span. The largest ESs between times were between baseline and week 78 for the number of slips (Hedges g = −2.11; 95% CI = −2.60, −1.62) and the time to cross the beam (Hedges g = −0.82; 95% CI = −1.22, −0.41). Balance, as measured by foot slips, was also worse in both ankle-sprain groups than in the SHAM group (P < .001). Large ESs with 95% CIs
that did not cross zero were noted between the SEVERE and SHAM groups (Hedges $g = -1.49$; 95% CI = -2.33, -0.64) and between the MILD and SHAM groups (Hedges $g = -0.89$; 95% CI = -1.54, -0.24). However, we did not observe differences between the MILD and SEVERE groups ($P = .08$). Time to cross the beam did not differ among the groups ($F_{2,47} = 3.09, P = .16$). Most important, sustaining an LAS exacerbated the effect of aging on balance, as evidenced by group × time interactions for time to cross the beam ($F_{26,611} = 2.30, P < .001$) and the number of foot slips ($F_{26,611} = 3.50, P < .001$). Means and standard deviations for the number of foot slips and time to cross the beam are presented in Figure 1A and B, respectively.

Figure 1. Means ± standard deviations. A, Number of foot slips, and B, time to cross the beam for each ankle-sprain group across the lifespan. * Indicates the severe ankle-sprain (SEVERE) group was different from the sham treatment (SHAM) group. b Indicates different from baseline. c Indicates the mild ankle-sprain (MILD) group was different from the SHAM group. d Indicates different from week 6. e Indicates the SEVERE group was different from the MILD group. f Indicates different from week 18. g Indicates different from week 24. h Indicates different from week 30. i Indicates different from week 36. j Indicates different from week 66. k Indicates different from week 12. l Indicates different from week 42. m Indicates fewer foot slips for the SHAM than both the MILD and SEVERE groups. n Indicates different from week 48. o Indicates different from week 54. p Indicates different from week 60.

Locomotion also changed with age, as the left-limb ($F_{13,611} = 5.21, P < .001$) and right-limb ($F_{13,611} = 3.34, P < .001$) stride length increased from baseline over the lifespan. The largest differences observed between time points occurred between baseline and week 36 for the left (Hedges $g = -1.20$; 95% CI = -1.63, -0.78) and right (Hedges $g = -0.87$; 95% CI = -1.28, -0.46) limbs. Left-limb stride length did not differ among groups ($F_{2,47} = 1.30, P = .39$), and having an LAS did not influence the effect of aging on left-limb stride length, as no group × time interaction was noted ($F_{26,611} = 1.44, P = .07$). Right-limb stride length differed among groups ($F_{2,47} = 3.51, P = .04$) but did not influence the effect of aging on right-limb stride length.
length, as no group × time interaction was noted ($F_{26,611} = 1.01, P = .46$). More specifically, the SEVERE and SHAM groups differed, with a moderate ES and a 95% CI that crossed zero (Hedges $g = 0.66; 95\% \text{ CI} = -0.12, 1.44$). Means and standard deviations for right- and left-limb stride lengths are presented in Figure 2A and B, respectively.

**DISCUSSION**

This study is an extension of the short- and long-term work previously published using this model. More specifically, we quantified the lifelong consequences of a single LAS on sensorimotor function in mice. To date, the longest prospective tracking of sensorimotor function in humans has been 12 months. Our results illustrated that consequences persist throughout the lifespan and, when combined with previous reports, demonstrated a broad effect, given that physical activity levels and the cardiovascular system can be negatively affected. These findings partially supported our hypotheses because sustaining a single LAS negatively affected sensorimotor function over the lifespan relative to the SHAM group. However, we observed minimal differences between the MILD and SEVERE groups, contrary to our a priori hypotheses.

Humans with CAI have been shown to have static and dynamic balance deficits across a range of postural-control tasks and outcome measures. These findings were consistent with the mouse model in this study, acutely postinjury, in a CAI model (approximately 1 year postinjury), and now across the lifespan. Similarly,
humans with CAI have been shown to have locomotive adaptations acutely\textsuperscript{26} and after developing CAI.\textsuperscript{27–29} However, only in the acute phase postinjury have spatial variables, such as stride length, been altered. In those with CAI, only kinematic variables obtained using motion analysis have shown alterations. As Wikstrom et al.\textsuperscript{19} suggested, these findings coincide with clinical observations associated with acute LASs and CAI in humans; specifically, an obvious limp disappears during the natural course of healing from an LAS, and such a limp does not reappear with the development of CAI. Contrary to traditional clinical observations, our data demonstrated reduced stride lengths of the injured limb of the SEVERE group relative to the SHAM group when the data were collapsed across the entire lifespan (ie, group main effect). A possible explanation for the contrary results can be seen in the inverted U shape of the right-limb stride length, whereby clear reductions in stride length relative to the SHAM group were present acutely after the injury and later in life. Indeed, declines in both the MILD and SEVERE groups started at roughly 42 to 48 weeks postinjury, which preceded rapid increases in (worsening of) the number of foot slips in the SEVERE group. Although this proposal is speculative, we hypothesize that this decline in sensorimotor function may coincide with a transition from CAI to posttraumatic ankle osteoarthritis.

Researchers\textsuperscript{11,12} have suggested that up to 78% of those who develop CAI will go on to develop posttraumatic ankle osteoarthritis. Investigators\textsuperscript{30} have demonstrated that early signs of ankle-joint degeneration are present immediately after an acute LAS and progress until the onset of ankle osteoarthritis, which occurs, on average, at 58 years of age. Therefore, the relative age of the study mice at 42 to 48 weeks (approximately 50%–60% of the lifespan) is roughly consistent with the relative age of humans at the average onset of ankle osteoarthritis. Not surprisingly, humans with posttraumatic ankle osteoarthritis have balance impairments\textsuperscript{33–35} and altered locomotive patterns. More specifically, researchers\textsuperscript{16,17} have identified altered spatio-temporal gait variables, such as step length and gait velocity, which are consistent with our results.

Although we are speculating, we hypothesize that the acute trauma caused somatosensory dysfunction and altered ankle contact strains.\textsuperscript{31} The combination would accelerate the natural degeneration of articular cartilage associated with aging. This early degenerative progression could lead to further degenerative progression, particularly when the ankle was submitted to high volumes of static (stance) and cyclical (gait) loading over the lifespan. This speculative negative-feedback loop fits within both the continuum of disability\textsuperscript{32} and the theorized cascade of events\textsuperscript{33} that highlight the progression to CAI and appear to be visible within our data. For example, the acute constraints recovered but led to postural-control deficits at 30 weeks. These declines could be responsible for the declines in right-limb stride length at 54 weeks and time to complete the balance task at 60 weeks.

Ankle-joint osteoarthritis was not confirmed by our data set, but the evidence provides further support for the appropriateness of a mouse ankle-sprain model to study both the acute and long-term consequences of an LAS and to quantify the proposed cascade of events thought to lead to CAI development.\textsuperscript{27,33,34} To date, the mouse ankle-sprain model we used has resulted in adaptations consistent with human models of an acute LAS, CAI, and posttraumatic ankle osteoarthritis. Furthermore, the cumulative findings from this model demonstrate that, despite the cultural perspective that an LAS is an innocuous injury, lifelong sequelae persist and manifest as sensorimotor dysfunction, decreased physical activity,\textsuperscript{20} and impaired cardiovascular function.\textsuperscript{21} However, future research is needed to establish the consistency of the model across different mouse strains because each strain has unique genetic characteristics that could alter the results. For example, the CBA/J strain that we studied has a retinal-degeneration allele that should be considered a limitation of our model on sensorimotor function. We chose this strain despite this limitation due to its higher activity levels and because it is not predisposed to developing osteoarthritis. Quantifying physical activity and monitoring the development of osteoarthritis were major components of a larger study.\textsuperscript{18–21} Whereas we acknowledge that retinal degeneration may have affected our results over time, the only difference between the groups was the presence and severity of a surgically induced ankle sprain. We do not believe that studying the CBA/J strain compromised our results, but using different strains in future research will confirm or refute the validity of the mouse ankle-sprain model. The open technique we used to induce the ankle sprain likely resulted in inflammatory events and obvious disruption of additional tissues relative to a closed human ankle sprain. This should also be considered a limitation of this model. Several researchers\textsuperscript{35–37} have induced a manual (closed) ankle sprain in rats, but our preliminary work has suggested that the smaller hind limb of a mouse could not handle the stress of a closed ankle-sprain model and fibular fractures resulted.

CONCLUSIONS

The transection of the lateral ankle ligaments of a mouse hind limb resulted in lifelong dysfunction of the sensorimotor system as evidenced by worse balance and decreased stride lengths on the injured side. This model fits within the framework of contemporary theoretical models of CAI and highlights the need to use this model to gather preclinical data regarding the effectiveness of intervention.

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