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Prolonged Rest, Long-Term Dynamic Balance, and Gait in a Mouse Ankle-Sprain Model

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Context: Lateral ankle sprains (LASs) result in short- and long-term adaptations in the sensorimotor system that are thought to contribute to the development of chronic ankle instability and posttraumatic ankle osteoarthritis. Debate continues as to the appropriateness of rapid return to sport after LASs given the prevalence of long-term consequences.

Objective: To examine the short- and long-term effects of prolonged rest, as a model of immobilization, on dynamic balance and gait outcomes after a severe LAS in a mouse model.

Design: Controlled laboratory study.

Setting: Research laboratory.

Intervention(s): At 7 weeks of age, 18 male mice (CBA/J) had their right anterior talofibular and calcaneofibular ligaments transected. Mice were then randomized to 1 of 3 groups representing when access to a running wheel postsurgery was gained: at 3 days, 1 week, and 2 weeks.

Main Outcome Measure(s): Dynamic balance and spatial gait characteristics were quantified before surgery (baseline) and at 3 days and 1, 2, 4, 6, 12, 18, 24, 30, 36, 42, 48, and 54 weeks postinjury.

Results: Relative to prolonged rest, resting for only 3 days resulted in worse dynamic balance during the later assessment points (42–54 weeks postinjury, $P < .01$). Mice that underwent a prolonged rest period of 2 weeks crossed the balance beam faster than the group that rested for only 3 days when averaged across all time points ($P < .012$). Spatial gait characteristics did not differ among the groups ($P > .05$).

Conclusions: Relative to 3 days of rest, prolonged rest (1 and 2 weeks) after a severe LAS in mice positively affected balance. The apparent benefit of prolonged rest was noted on both dynamic-balance outcomes and performance. Stride length was not altered by the duration of rest after a surgically induced severe LAS in mice. Future research is needed to determine if these results translate to a human model.

Key Words: murine, ankle injury, postural control, immobilization

Key Points

- In a mouse model of a severe lateral ankle sprain, prolonged rest resulted in better dynamic balance outcomes and performance.
- The benefits of prolonged rest were most pronounced at the later assessment points (42 to 54 weeks postinjury).
- In a mouse model, the duration of rest after a severe lateral ankle sprain did not influence spatial gait characteristics.

Lateral ankle sprains (LASs) are the most common musculoskeletal injuries associated with interscholastic and intercollegiate athletes.[1,2] Among the general public, more than 900 000 patients with LASs were seen in US emergency departments in 2010 alone.[3] These events resulted in excess of $1.1 billion in health care charges.[3] The perception that LASs are minor injuries is likely due to the relatively rapid resolution of immediate symptoms[4–6] and high likelihood of rapid return to activity.[7] Unfortunately, LASs are not an innocuous injury, as prospective evidence[8] demonstrated that at least 40% of individuals who sustained a first-time ankle sprain developed residual symptoms, often defined as *chronic ankle instability* (CAI). Further, more than 50% of individuals who reported a history of an LAS indicated that the duration of their residual symptoms was >10 years.[9] Symptoms, both acute and in those with CAI, often include perceptual (eg, worse self-reported function), mechanical (eg, increased laxity), and sensorimotor (eg, static- and dynamic-balance deficits and gait alterations) deficits and alterations.[10]

Although the lack of treatment likely plays a role in the development of CAI,[11] the lack of effective interventions for LAS is also a major concern. A position statement on the conservative care of LAS in athletes[12] noted that only 3 of 12 treatment options reviewed had consistent patient-oriented evidence to support their use in improving short-term LAS outcomes. For example, functional rehabilitation relative to immobilization was more effective in managing mild to moderate LAS. Yet only inconsistent evidence[13–15] suggested that patients with severe sprains should be treated with a prolonged immobilization period (ie, at least 10 days) to improve patient-reported outcomes. However, no investigators have examined the effect of immobilization type or duration on sensorimotor function in those with acute LAS of any severity. Thus, we need to establish appropriate time frames for immobilization of these injuries and determine an optimal period of unloading to help restore sensorimotor function.

Mild and severe LAS mouse models have been developed. These models mimic the short-term[16] and residual[17–21] symptoms of mild and severe human LAS, respectively. More specifically, these models have demonstrated both acute and chronic sensorimotor dysfunction as evidenced by impaired dynamic balance and altered gait outcomes.[16,18,20] As a result, these models[16–21] may provide an opportunity to gather preclinical data regarding the effectiveness of LAS interventions, including the effectiveness of prolonged rest or immobilization (or both) on dynamic balance and gait outcomes after a severe LAS. However, the validity of these models in providing insights into the efficacy and effectiveness of therapeutic interventions has not yet been investigated. Therefore, the purpose of our research was to examine the short- and long-term effects of prolonged rest, as a model of immobilization, on dynamic-balance and gait outcomes after a severe LAS in mice. We chose rest instead of cast immobilization, as used in the literature on humans, because we felt that given an option, most humans would prefer to refrain from voluntary exercise rather than be immobilized in a cast. Based on the human literature,[13–15] we hypothesized that longer rest times would result in better short- and long-term dynamic-balance and gait outcomes.

METHODS

Animals

Eighteen male mice (CBA/J), 5 to 6 weeks old, were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in the university vivarium. The vivarium had 12-hour light-dark cycles with room temperature and relative humidity standardized to 18°C to 22°C and 20% to 40%, respectively.[16–22] Water and standard chow (Teklad 8604 Rodent Diet; Harlan, Madison, WI) were provided ad libitum.[16–22] The Institutional Animal Care and Use Committee at the University of North Carolina at Charlotte approved all study procedures as meeting the US Department of Agriculture and Animal Welfare Act guidelines for the appropriate treatment of animal subjects.

Surgical Procedures

Surgical procedures replicated those previously described for mice with severe LASs.[16–21] In brief, mice were anesthetized before having the right ankle prepared for surgery. Once anesthetized, mice were moved to a sterile surgical field under a warming lamp, where a small curvilinear incision was made behind the lateral malleolus using sterile equipment and a microscope. The anterior talofibular and calcaneofibular ligaments were then transected before the incision was closed with surgical adhesive. Postoperative care consisted of an injection of 5.0 mg/kg carprofen (Rimadyl; Zoetis Inc, Parsippany-Troy Hills, NJ) diluted with saline, time under a warming lamp, and visual monitoring at least once every 24 hours for 72 hours. A 12.5-mg carprofen (Rimadyl; Zoetis Petcare, Kalamazoo, MI) tablet was administered for pain management; no additional medication was needed.

Rest

After surgery, each mouse was individually housed in a cage that marked its assigned group: 3 days, 1 week, or 2 weeks of rest. Mice were randomized to groups using a random number generator before the surgical procedure. During the rest period, mice were free to ambulate within their cages, which we viewed as the equivalent of allowing a human to ambulate within the home after an ankle sprain. Upon completion of the rest period, a solid-surface running wheel (127 mm; Ware Manufacturing, Phoenix, AZ), magnetic sensor, and digital odometer (model BC600; Sigma Sport, Olney, IL) that recorded the number of running-wheel revolutions were added to each cage to allow voluntary exercise to begin.[16,17,19] Previous authors[23] reported high correlation and agreement between days of wheel running and wheel-running measurements (eg, distance and duration run), suggesting that this model of voluntary exercise is repeatable and stable in mice. Further, previous mouse LAS research[16] showed that if a running wheel was introduced 3 days postsurgery, mice voluntarily exercised, albeit at a lower volume than mice that had not undergone LAS surgery.

Outcome Measures

The main outcome measures replicated those previously used with the severe LAS mouse model.[16,18,20] Before the baseline assessments, all mice were trained, as previously described,[16,18,20] to perform the required tasks to ensure more consistent performance throughout the investigation. Dynamic balance was assessed by measuring the ability of the mice to cross an inclined (15°), narrow, round wooden beam, 1-m long with a 19-mm diameter. Mice were allowed up to 60 seconds to cross the beam. The 2-trial average of the time taken to cross the beam and the number of times the right hind foot slipped off the beam were recorded as dependent variables.[16,18,20] Interrater reliability of these outcomes ranges from good to excellent (intraclass correlation coefficient = 0.83–0.92).[18] Gait was assessed using the footprint test, which requires the mice to traverse a 50-cm-long, 10-cm-wide runway after having their feet painted with nontoxic paint. The dependent measure of interest was the right-limb stride length, quantified using the heel-to-heel measuring technique. Intrarater and interrater reliability of this outcome and paw print selection have been established as excellent (intraclass correlation coefficient = 0.96–0.99).[16] Data were scheduled to be collected before (baseline) and at 3 days and 1, 2, 4, 6, 12, 18, 24, 30, 36, 42, 48, and 54 weeks postinjury. No data were captured at 30 weeks postinjury because of unforeseen long-duration travel by the research team members responsible for testing. Gait data were also not recorded at 54 weeks postinjury because of limited availability of the testing suite within the university vivarium before study termination.

Statistical Analysis

Three 2-way repeated-measures analyses of variance were conducted to compare changes in balance (time, slips)

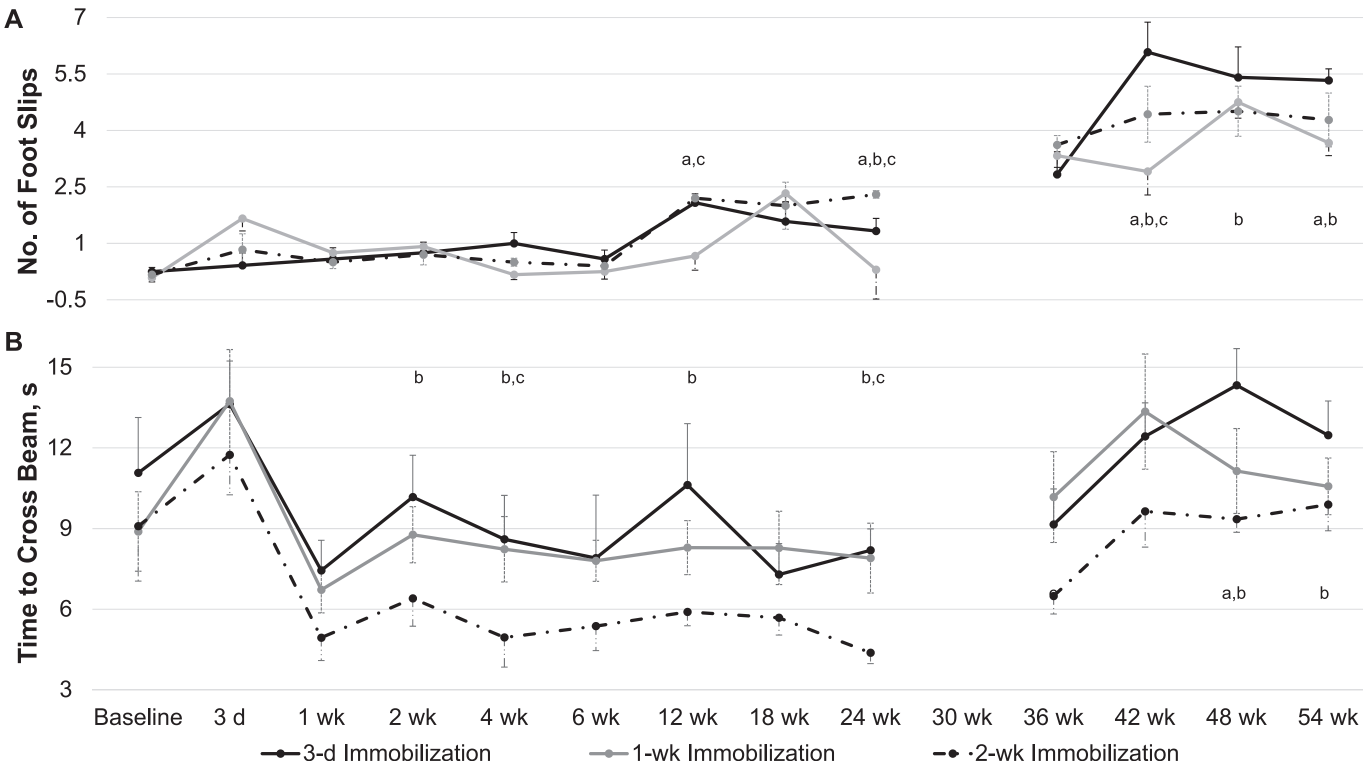

Figure 1. A, Number of foot slips, and B, time to cross the beam for each treatment group (mean ± standard deviation). [a] Indicates a difference between the 3-day and 1-week rest groups ($P < .05$). [b] Indicates a difference between the 3-day and 2-week rest groups ($P < .05$). [c] Indicates a difference between the 1-week and 2-week rest groups ($P < .05$).

and gait (right-limb stride length) variables due to group (3 days, 1 week, or 2 weeks of immobilization) and time. Missing data (8%) were replaced using a linear interpolation technique. The Wilk λ was used to interpret statistical differences, and Bonferroni post hoc comparisons were performed, when appropriate, to identify differences when main effects or interactions were observed. An α level of $P < .05$ was used to determine significant differences for all analyses. Because of the high number of comparisons, we chose to calculate between-groups Hedges *g* effect sizes and 90% confidence intervals (CIs)[24] where significant group main effects and interactions were noted in order to confirm our hypothesis testing. Effect sizes were interpreted as *weak* ($\leq$0.40), *moderate* (0.41–0.69), or *strong* ($\geq$0.70).[25]

RESULTS

Three days of rest resulted in more foot slips at the later assessment points (42–54 weeks postinjury; Figure 1). The effect sizes and 90% CIs associated with these differences are shown in Table 1. The number of foot slips ($P < .001$) increased over time (Table 2). The time needed to cross the balance beam ($P < .001$) initially decreased before increasing at the later assessments (ie, weeks 42 to 54; Table 2). Relative to the group that rested for only 3 days, the time to cross the beam ($P = .012$) was faster in mice that rested for 2 weeks (Table 3). This group difference was associated with a moderate effect size (–0.61) and a 90% CI (–1.58, 0.36) that crossed zero. The number of foot slips ($P = .068$) in the group that rested 1 week approached significance relative to the group that rested 3 days (Table 3). This trend was associated with a small effect size (–0.24) and a 90% CI (–1.19, 0.72) that crossed zero. Right-limb stride length was different over time ($P < .001$), increasing from baseline (Table 2). Stride length did not differ ($P > .05$) among the groups (Table 3) and no group × time interaction ($P > .05$) was noted (Figure 2).

DISCUSSION

The purpose of our investigation was to examine the short- and long-term effects of prolonged rest on balance and gait outcomes after a severe LAS in mice. Our main finding was that longer rest periods resulted in better short- and long-term dynamic postural control but not better spatial gait characteristics in mice after a severe LAS. More specifically, longer periods of rest resulted in a better dynamic-balance outcome (time to cross the beam) without compromising dynamic-balance performance (foot slips) relative to shorter rest periods. These results are consistent with some[13] but not all[14,15] of the published literature regarding the benefits of prolonged rest or immobilization on short-term patient-reported outcomes after severe LAS in humans. Further, the results support our a priori hypothesis.

The similarities between current and previous dynamic-balance data from mice[16,18,20] support the credibility of our results. Previous baseline dynamic-balance assessments indicated that mice averaged approximately 1 slip per crossing.[16,18,20] Although the current sample demonstrated a lower baseline mean, large impairments were observed both in the current study at 3 days (482%) and 4 weeks (333%) postinjury and by Hubbard-Turner et al[17] at 3 days (approximately 175%) and 4 weeks (209%). Previously reported foot slips at 48 weeks (5.75) and 54 weeks (5.85)

Table 1. Effect Sizes (90% Confidence Intervals) for Time Points When Between-Groups Differences Were Detected

Time Postinjury	Foot Slips			Time to Cross Beam		
	3 d to 1 wk	3 d to 2 wk	1 wk to 2 wk	3 d to 1 wk	3 d to 2 wk	1 wk to 2 wk
Baseline	NA	NA	NA	NA	NA	NA
3 d	NA	NA	NA	NA	NA	NA
1 wk	NA	NA	NA	NA	NA	NA
2 wk	NA	NA	NA	NA	−1.07 (−2.09, −0.06)	NA
4 wk	NA	NA	NA	NA	−0.98 (−1.99, 0.02)	−1.18 (−2.21, −0.15)
6 wk	NA	NA	NA	NA	NA	NA
12 wk	−2.89 (−4.25, −1.53)	NA	2.08 (0.90, 3.26)	NA	−1.07 (−2.09, −0.06)	NA
18 wk	NA	NA	NA	NA	NA	NA
24 wk	−1.58 (−2.67, −0.49)	0.61 (−0.36, 1.58)	1.35 (0.30, 2.41)	NA	−2.27 (−3.49, −1.05)	−1.38 (−2.43, −0.32)
36 wk	NA	NA	NA	NA	NA	−1.07 (−2.09, −0.06
42 wk	−1.53 (−2.61, −0.45)	−0.86 (−1.85, 0.13)	0.83 (−0.16, 1.82)	NA	NA	NA
48 wk	NA	−0.52 (−1.49, 0.44)	NA	−0.76 (−1.75, 0.22)	−1.76 (−2.87, −0.64)	NA
54 wk	−1.14 (−2.16, −0.12)	−1.22 (−2.25, −0.18)	NA	NA	−0.86 (−1.85, 0.14)	NA

Abbreviation: NA, not applicable.

postinjury[18,20] were comparable with those observed in the current investigation (Table 2). The time to cross the balance beam was even more consistent with previous data at baseline, 4 weeks, and 48 weeks postinjury. The current time of 9.7 seconds is comparable with the approximately 6 seconds reported earlier.[16,18,20] Percentage increases at 4 weeks (104% versus 121%)[18] and 48 weeks (121% versus 118%)[18,20] were also comparable between previous research and the current sample. Clearly, the LAS surgical procedure caused large acute and long-term (up to 1 year) impairments in dynamic postural control. These similarities support the model's appropriateness for investigating the effects of various therapeutic interventions.

The current data suggest there is a benefit to longer rest periods after a severe LAS in mice, as evidenced by a better dynamic-balance outcome (ie, time to cross the beam) averaged across all time points. However, directly comparing this evidence with that from human models is difficult. First, no authors have examined the recovery trajectory of dynamic-balance or gait outcomes after acute LAS immobilization. Second, only inconsistent patient-oriented evidence supports prolonged immobilization after severe LAS in humans. For example, Ardevol et al[15] noted that a functional treatment focused primarily on strapping (ie, taping) followed by 2 weeks of active rehabilitation resulted in greater improvements in pain, swelling, and subjective instability at 3 and 6 months postinjury relative to 21 days in a plaster cast followed by 2 weeks of active rehabilitation. At 12 months postinjury, all patient-reported outcomes were comparable between the groups, but the functional group did have less radiologic laxity. Beynnon et al[14] found that an Air-Stirrup ankle brace (Aircast, Inc,

Table 2. Balance and Gait Outcomes Over Time When Collapsed Across Groups (Mean ± SD)

Time Postinjury	Foot Slips, No.	Time Across Beam, s	Right-Limb Stride Length, cm
Baseline	0.17 ± 0.24[a,b,c,d,e,f]	9.69 ± 4.45	5.89 ± 0.55[a,b,c,d,e,g,h,i,j]
3 d	0.81 ± 0.90[b,c,d,e,f]	13.04 ± 3.98[b,g,h,j,k]	6.41 ± 0.67[b,c,d,i,j]
1 wk	0.61 ± 0.56[a,b,c,d,e,f]	6.37 ± 2.45[d,e,f,l]	6.46 ± 0.68[c,d,e,j]
2 wk	0.79 ± 0.57[c,d,e,f]	8.45 ± 3.27[l]	6.63 ± 1.11[b]
4 wk	0.56 ± 0.57[a,b,c,d,e,f]	7.27 ± 3.38[l]	7.13 ± 0.72[b,m]
6 wk	0.41 ± 0.46[a,b,c,d,e,f]	7.027 ± 3.69[e]	7.25 ± 0.72[l,m]
12 wk	1.65 ± 0.94[c,d,e,f,h,i,k,m]	8.27 ± 3.93	7.35 ± 0.62[a]
18 wk	1.97 ± 1.15[c,d,e,f,g,h,i,k,l,m]	7.09 ± 2.75[d,e,f,l]	8.04 ± 0.60[c,d,g,h,k,l,m]
24 wk	1.31 ± 1.41[c,d,e,f]	6.83 ± 2.76[d,e,f,l]	7.39 ± 0.61[k,l,m]
36 wk	3.26 ± 1.01[a,b,g,h,i,j,k,l,m]	8.61 ± 3.39	7.53 ± 0.49[k,l,m]
42 wk	4.48 ± 2.15[a,b,g,h,i,j,k,l,m]	11.48 ± 3.97[b,h,j,k]	7.22 ± 0.45[b,k,m]
48 wk	4.89 ± 1.56[a,b,g,h,i,j,k,l,m]	11.54 ± 3.50[b,i,j]	7.16 ± 0.64[b,k,m]
54 wk	4.43 ± 1.33[a,b,g,h,i,j,k,l,m]	10.97 ± 2.79[b,j,k]	NA

Abbreviation: NA, not available.

[a] Different from 12 weeks.
[b] Different from 18 weeks.
[c] Different from 36 weeks.
[d] Different from 42 weeks.
[e] Different from 48 weeks.
[f] Different from 54 weeks.
[g] Different from 2 weeks.
[h] Different from 4 weeks.
[i] Different from 6 weeks.
[j] Different from 24 weeks.
[k] Different from 1 week.
[l] Different from 3 days.
[m] Different from baseline.

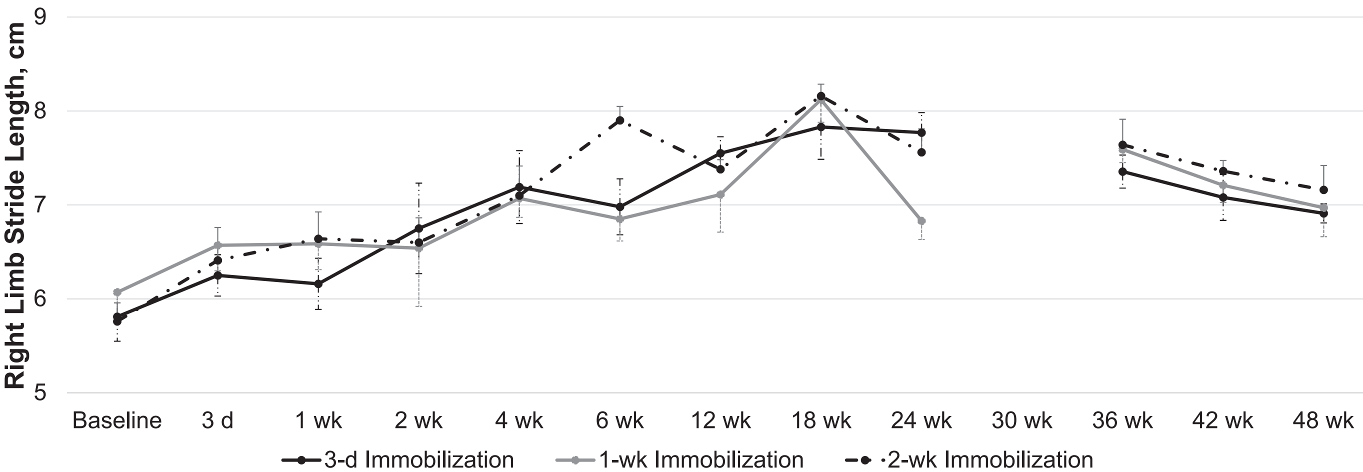

Figure 2. Right-limb stride length for each treatment group (mean ± standard deviation).

Summit, NJ) and a fiberglass walking cast worn for 10 days were equally effective with regard to the rate of return of function, clinical testing, activity levels, and patient satisfaction at 6 months postinjury. However, Lamb et al[13] observed that a below-knee cast for 10 days resulted in better pain and symptom resolution as well as functional activity at 3 months postinjury relative to a tubular compression bandage and a Bledsoe boot (Medical Technology Inc, Grand Prairie, TX). An Aircast ankle brace also had positive effects at 3 months postinjury, but the effects were not as broad as those from the below-knee cast. Yet at 9 months postinjury, all treatments demonstrated similar outcomes. Lastly, as a percentage of life span, duration of immobilization and time since injury in mice do not translate well to human models because of the drastic difference in life span (approximately 1.82 versus 79 years). For this reason, it is important to remember that the current results represent preclinical data that can inform researchers and clinicians about the potential benefits of prolonged rest until prospective human data are available.

Cumulatively, the human-based results suggest that patient-reported outcomes will resolve between 6 and 12 months after a severe LAS, regardless of the type and length of immobilization. Unfortunately, a large percentage of patients who sustain an LAS will develop CAI,[10] suggesting that patient-reported outcomes may not be the best indicator of complete symptom resolution. For example, previous research[26] on treating sensorimotor dysfunction in those with CAI has demonstrated a disconnect between patient-reported perceptions of improvement and actual improvements in sensorimotor function. The current results would suggest that acutely and through the first 24 weeks, prolonged immobilization is not any better or worse than the control condition (ie, 3 days of rest) when quantified using foot slips. However, the benefits of prolonged rest appear to mature over time, as significant and moderate to large foot-slip interaction effects were consistently observed between 42 and 54 weeks postinjury (Table 1). It is possible that our small sample size was not capable of detecting more subtle deficits that may have been present within the first 24 weeks. Nevertheless, we hypothesized that this response pattern was more likely due to the maturation of centrally mediated changes[27] after a cascade of events[28] postinjury that ultimately result in worse dynamic-balance performance (ie, foot slips) unless mitigated by prolonged rest.

Prolonged rest (ie, 1–2 weeks) also appears to have an immediate and sustained effect when quantifying the time needed to cross the balance beam (ie, dynamic-balance task outcome) relative to the control condition of 3 days of rest. It is important to note that between 6 months and 1 year after injury (ie, 24–52 weeks), differences in human-based patient-reported outcomes after various immobilization strategies resolve. Yet, during this same time window, times to cross the beam were consistently better in the group that underwent 2 weeks of rest relative to the control condition of 3 days of rest, as evidenced by large effect sizes and CIs that did not cross zero (Table 1). Despite these dynamic-balance differences, spatial characteristics of gait were unaltered among the groups. This is most likely because stride length is not sensitive enough to reflect differences. In humans, kinematic and kinetic differences during gait have been noted after LAS and in those with CAI but only when using research-oriented equipment.[6,29–32]

Previous mouse model data[16,18,20] supported the theoretical framework that peripheral joint injuries result in centrally mediated changes over time and not necessarily

Table 3. Balance and Gait Outcomes Among Groups When Collapsed Across All Time Points (Mean ± SD)

	Rest		
Outcome	3 d	1 wk	2 wk
Foot slips, No.	2.173 ± 2.228	1.638 ± 1.963[a]	2.035 ± 1.929
Time across beam, s	10.241 ± 4.845	9.454 ± 3.419	7.221 ± 4.321[b]
Right-limb stride length, cm	6.975 ± 0.982	6.963 ± 0.865	7.177 ± 0.901

[a] Trend toward a difference from the 3-day rest group ($P = .068$).
[b] Different from 3-day rest group ($P = .012$).

at the point of initial trauma.[27] The current data indicate that prolonged rest after severe LAS protected long-term dynamic-balance task outcomes (ie, time to cross the beam) and performance (ie, foot slips). These results may suggest that prolonged rest can mitigate centrally mediated changes in sensorimotor function. Further, free ambulation within the cage for 2 weeks before the resumption of voluntary exercise also appeared to support our understanding of the benefits of optimal loading[33] as a means to maximize the mechanical properties of the injured ligament(s). Previous researchers[34,35] have linked proper sensorimotor function with better mechanical stability of the lateral ligaments in those with CAI. Although this is speculative, better ligamentous healing may preserve mechanoreceptor function in the lateral ligaments or promote better muscle activation after return to participation (or both).

Although our results provide an innovative look into how prolonged rest affects dynamic balance after a severe LAS in mice, future research is needed to address a number of critical gaps in the literature and limitations of the current study. For example, clinical trials on the effects of prolonged immobilization on sensorimotor function in humans are needed. It is also noteworthy that the underlying neurophysiological mechanism for the observed benefits remains unknown. As a result, future authors using animal models should focus on more precise measures of muscle mechanics, nervous system function, and biochemical markers to elucidate the underlying mechanisms as to why prolonged rest appears protective against centrally mediated changes. Further research is also needed to determine if prolonged rest after severe LAS mitigates lifelong physical activity reductions, ankle posttraumatic osteoarthritis, and other consequences to body systems observed using this mouse LAS model[17,19,21] as well as to explore the contextual dependence among these consequences and measures of sensorimotor function. Additionally, investigators should determine the postinjury time points that represent key points within the cascade of events that therapeutic interventions may be able to mitigate.

CONCLUSIONS

Prolonged rest (1 and 2 weeks), as a model of immobilization, after severe LAS in mice positively affected dynamic balance relative to 3 days of rest, as evidenced by moderate to large effect sizes. The most robust benefits were observed after 2 weeks of rest, as shown by less time to cross the beam and better dynamic-balance performance (ie, fewer foot slips) between 42 and 54 weeks postinjury. Stride length was not altered by the duration of rest after a surgically induced severe LAS in mice.

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REFERENCES

1. Fernandez WG, Yard EE, Comstock RD. Epidemiology of lower extremity injuries among U.S. high school athletes. *Acad Emerg Med*. 2007;14(7):641–645.
2. Hootman JM, Dick R, Agel J. Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. *J Athl Train*. 2007;42(2):311–319.
3. Shah S, Thomas AC, Noone JM, Blanchette CM, Wikstrom EA. Incidence and cost of ankle sprains in United States emergency departments. *Sports Health*. 2016;8(6):547–552.
4. Bleakley CM, McDonough M, MacAuley DC. Some conservative strategies are effective when added to controlled mobilisation with external support after acute ankle sprain: a systematic review. *Aust J Physiother*. 2008;54(1):7–20.
5. Doherty C, Bleakley C, Hertel J, et al. Lower extremity coordination and symmetry patterns during a drop vertical jump task following acute ankle sprain. *Hum Mov Sci*. 2014;38:34–46.
6. Doherty C, Bleakley C, Hertel J, Caulfield B, Ryan J, Delahunt E. Lower extremity function during gait in participants with first time acute lateral ankle sprain compared to controls. *J Electromyogr Kinesiol*. 2015;25(1):182–192.
7. Medina McKeon JM, Bush HM, Reed A, Whittington A, Uhl TL, McKeon PO. Return-to-play probabilities following new versus recurrent ankle sprains in high school athletes. *J Sci Med Sport*. 2014;17(1):23–28.
8. Doherty C, Bleakley C, Hertel J, Caulfield B, Ryan L, Delahunt E. Recovery from a first-time lateral ankle sprain and the predictors of chronic ankle instability: a prospective cohort analysis. *Am J Sports Med*. 2016;44(4):995–1003.
9. Hiller CE, Nightingale EJ, Raymond J, et al. Prevalence and impact of chronic musculoskeletal ankle disorders in the community. *Arch Phys Med Rehabil*. 2012;93(10):1801–1807.
10. Gribble PA, Bleakley CM, Caulfield BM, et al. Evidence review for the 2016 International Ankle Consortium consensus statement on the prevalence, impact, and long-term consequences of lateral ankle sprains. *Br J Sports Med*. 2016;50(24):1496–1505.
11. Feger MA, Glaviano NR, Donovan L, et al. Current trends in the management of lateral ankle sprain in the United States. *Clin J Sport Med*. 2017;27(2):145–152.
12. De Ridder R, Willems T, Vanrenterghem J, Robinson MA, Palmans T, Roosen P. Multi-segment foot landing kinematics in subjects with chronic ankle instability. *Clin Biomech (Bristol, Avon)*. 2015;30(6):585–592.
13. Lamb SE, Marsh JL, Hutton JL, Nakash R, Cooke MW; Collaborative Ankle Support Trial (CAST Group). Mechanical supports for acute, severe ankle sprain: a pragmatic, multicentre, randomised controlled trial. *Lancet*. 2009;373(9663):575–581.
14. Beynnon BD, Renstrom PA, Haugh L, Uh BS, Barker H. A prospective, randomized clinical investigation of the treatment of first-time ankle sprains. *Am J Sports Med*. 2006;34(9):1401–1412.
15. Ardevol J, Bolibar I, Belda V, Argilaga S. Treatment of complete rupture of the lateral ligaments of the ankle: a randomized clinical trial comparing cast immobilization with functional treatment. *Knee Surg Sports Traumatol Arthrosc*. 2002;10(6):371–377.
16. Hubbard-Turner T, Wikstrom EA, Guderian S, Turner MJ. Acute ankle sprain in a mouse model. *Med Sci Sports Exerc*. 2013;45(8):1623–1628.
17. Hubbard-Turner T, Wikstrom EA, Guderian S, Turner MJ. An acute lateral ankle sprain significantly decreases physical activity across the lifespan. *J Sports Sci Med*. 2015;14(3):556–561.
18. Wikstrom EA, Hubbard-Turner T, Woods S, Guderian S, Turner MJ. Developing a mouse model of chronic ankle instability. *Med Sci Sports Exerc*. 2015;47(4):866–872.
19. Turner MJ, Guderian S, Wikstrom EA, et al. Altered left ventricular performance in aging physically active mice with an ankle sprain injury. *Age (Dordr)*. 2016;38(1):15.
20. Wikstrom EA, Guderian S, Cline JH, et al. Life-long sensorimotor dysfunction following a lateral ankle sprain. *J Athl Train*. 2018;53(3):249–254.

21. Hubbard-Turner T, Wikstrom EA, Guderian S, Turner MJ. Acute ankle sprain in a mouse model: changes in knee-joint space. *J Athl Train*. 2017;52(7):629–635.
22. Turner MJ, Chavis MN, Turner TH. Enhanced diastolic filling performance with lifelong physical activity in aging mice. *Med Sci Sports Exerc*. 2013;45(10):1933–1940.
23. Knab AM, Bowen RS, Moore-Harrison T, Hamilton AT, Turner MJ, Lightfoot JT. Repeatability of exercise behaviors in mice. *Physiol Behav*. 2009;98(4):433–440.
24. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3–13.
25. Fritz CO, Morris PE, Richler JJ. Effect size estimates: current use, calculations, and interpretation. *J Exp Psychol Gen*. 2012;141(1):2–18.
26. Wikstrom EA, McKeon PO. Predicting balance improvements following STARS treatments in chronic ankle instability participants. *J Sci Med Sport*. 2017;20(4):356–361.
27. Needle AR, Lepley AS, Grooms DR. Central nervous system adaptation after ligamentous injury: a summary of theories, evidence, and clinical interpretation. *Sports Med*. 2017;47(7):1271–1288.
28. Wikstrom EA, Hubbard-Turner T, McKeon PO. Understanding and treating lateral ankle sprains and their consequences: a constraints-based approach. *Sports Med*. 2013;43(6):385–393.
29. Doherty C, Bleakley C, Hertel J, Caulfield B, Ryan J, Delahunt E. Locomotive biomechanics in persons with chronic ankle instability and lateral ankle sprain copers. *J Sci Med Sport*. 2016;19(7):524–530.
30. Doherty C, Bleakley C, Hertel J, et al. Gait biomechanics in participants, six months after first-time lateral ankle sprain. *Int J Sports Med*. 2016;37(7):577–583.
31. Terada M, Bowker S, Thomas AC, et al. Alterations in stride-to-stride variability during walking in individuals with chronic ankle instability. *Hum Mov Sci*. 2015;40:154–162.
32. Crosbie J, Green T, Refshauge K. Effects of reduced ankle dorsiflexion following lateral ligament sprain on temporal and spatial gait parameters. *Gait Posture*. 1999;9(3):167–172.
33. Bleakley CM, Glasgow P, MacAuley DC. PRICE needs updating, should we call the POLICE? [editorial]. *Br J Sports Med*. 2012;46:220–221.
34. Hubbard TJ, Kramer LC, Denegar CR, Hertel J. Contributing factors to chronic ankle instability. *Foot Ankle Int*. 2007;28(3):343–354.
35. Hubbard TJ, Kramer LC, Denegar CR, Hertel J. Correlations among multiple measures of functional and mechanical instability in subjects with chronic ankle instability. *J Athl Train*. 2007;42(3):361–366.

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