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A 14-Day Recovery and Physical Activity Levels After an Ankle Sprain in Mice

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Context: Research is needed to find ways of improving physical activity after a lateral ankle sprain.

Objective: To investigate the effects of a prolonged rest period on lifelong activity after a surgically induced ankle sprain.

Design: Controlled laboratory study.

Setting: Research laboratory.

Patients or Other Participants: A total of 18 male CBA/J mice (age at surgery = 7 weeks).

Main Outcome Measure(s): We transected the anterior talofibular ligament and calcaneofibular ligament of the right hindlimb. Each mouse was placed in a separate cage and randomized into 1 of 3 groups of 6 mice each. A running wheel was placed in each cage at 3 days, 7 days, or 14 days after surgery. Physical activity was measured daily. Daily duration (ie, time spent on the wheel), distance, and running speed were examined using analysis of variance (group × age) with repeated measures at 15-week periods to approximate the first 3 quartiles of the lifespan.

Results: From weeks 3 to 15 after surgery, we observed no differences in duration, distance, or running speed among groups ($P > .05$). From weeks 16 to 30, distance ($F_{2,14} = 0.57$, $P = .041$) and running speed ($F_{2,14} = 0.93$, $P = .01$) were greater in the 14-day group than in the 3- and 7-day groups. From weeks 31 to 45, duration ($F_{2,14} = 0.74$, $P = .02$), distance ($F_{2,14} = 0.95$, $P = .009$), and running speed ($F_{2,14} = 1.05$, $P = .007$) were greater in the 14-day group than in the 3- and 7-day groups.

Conclusions: Our findings suggest that the longer recovery period of 14 days can increase activity levels throughout the lifespan after a severe ankle sprain. Rest after an ankle injury is critical to restoring physical activity levels across the lifespan. Rest and time away from exercise after an ankle sprain may be necessary to restore physical activity to normal, uninjured levels.

Key Words: ankle injuries, exercise, animal model

Key Points

- After a severe ankle injury, mice that rested for 14 days before engaging in voluntary physical activity via a running wheel had higher physical activity outcomes than mice given a running wheel 3 or 7 days after injury.
- Rest and time away from exercise after an ankle sprain may be critical for restoring physical activity levels to normal, uninjured levels.
- Resting and facilitating tissue healing after an ankle sprain may best restore function and minimize the negative long-term changes associated with injury.
- More research is needed to determine the optimal rest windows for different ankle-sprain severities in humans.

Researchers have continued to demonstrate not only the commonality of ankle sprain[1] but also the negative long-term implications.[2] The high percentage (up to 70%) of patients who develop chronic ankle instability (CAI) after an ankle sprain[3] and the potential effect of CAI on overall health and wellbeing are important public health concerns.[4–6] Therefore, we need to better understand the changes that occur after an ankle sprain so that programs and treatments can be developed to decrease the long-term consequences reported in the literature.

Investigators[4–6] have demonstrated changes in physical activity levels after an ankle sprain in both human and animal models. In one of the first animal studies, Hubbard-Turner et al[5] and Wikstrom et al[6] established that mice that had undergone surgical transection of the ankle ligaments (ie, a severe ankle sprain) were less physically active than mice that had undergone a sham surgical procedure. Physical activity was measured using voluntary wheel running. Most concerning in the aforementioned study[5] was that only a single ankle sprain was surgically induced, yet this decreased physical activity level continued across the entire lifespan of the mouse.

Whereas no lifelong physical activity data for humans are available, college-aged participants with CAI took fewer steps per week on average than healthy matched college-aged students.[4] Among participants with acute ankle sprains, decreased physical activity levels were evident after the initial ankle sprain compared with self-reported physical activity levels before the sprain.[7] This decrease in physical activity was reported to continue at 1 year after the initial sprain.[7] In both human and animal models, it appears that a single ankle sprain can lead to decreased physical activity levels.[4–7] This finding is concerning because physical inactivity is one of the leading risk factors for death worldwide and a key risk factor in developing cardiovascular disease, cancer, and type 2 diabetes.[8]

Researchers and clinicians should not be surprised that individuals would be less physically active after an ankle sprain. Changes in subjective and objective function were found in participants after an ankle sprain and with CAI.[9–13] If the ankle hurts or is unstable, why engage in an activity that increases those symptoms and potentially leads to further injury? One of the potential reasons for the subjective and objective changes reported after an ankle sprain and, thus, the decreased physical activity levels, may be that joint function was not restored after the initial injury. After a sprain, ligaments need 6 to 12 weeks for the scar tissue to mature to full tensile strength.[12] Whereas data on return-to-play times after an ankle sprain are limited, Nelson et al[14] reported that 50% of individuals with ankle sprains returned in less than 1 week. In a more recent study, Medina McKeon et al[15] described a 70% chance of returning to play within 3 days of the injury, and 90% of injured athletes returned to play within 1 week. This quick return to activity does not facilitate proper healing or restoration of ankle-joint function.

Based on the current literature, physical activity levels decrease after an ankle sprain. This decreased activity may be secondary to a quick return to activity, which does not facilitate tissue healing after injury. In previous animal studies, mice were given access to a running wheel 3 days after injury. We questioned whether a longer time away from activity would facilitate tissue healing and, thus, higher levels of physical activity. Therefore, the purpose of our study was to examine running-wheel restriction in mice after a surgically induced ankle sprain. We hypothesized that mice lacking access to a running wheel for a longer time (14 days) would have greater physical activity levels across the lifespan than mice that gained earlier access (3 or 7 days).

METHODS

Animals

Eighteen male CBA/J mice (age range, 5–6 weeks) were purchased from Jackson Laboratory (JAX, Bar Harbor, ME). The sample size necessary to detect a difference in physical activity (distance, duration, speed) was calculated using JMP statistical analysis software (SAS Institute, Cary, NC) based on research[5,6] from our laboratory in which physical activity in mice was examined. Based on 80% power and an α level of .05, a total of 6 mice per group were needed. The housing facilities, diet, and care of the mice have been described.[5,6] All study procedures were approved by the University of North Carolina at Charlotte Institutional Animal Care and Use Committee.

Surgical Procedures

The surgical procedures and postoperative care replicated those described for severe lateral ankle sprain.[5,6] The anterior talofibular ligament and calcaneofibular ligament of the right ankle were transected in each mouse. Although the mice received 12-mg carprofen (Rimadyl; Zoetis Petcare, Kalamazoo, MI) tablets for pain management upon return to their cages and ad libitum throughout the first 3 days after surgery, they consumed none of the tablets provided during this period.

Physical Activity Measurement

After surgery, each mouse was individually housed and randomized to 1 of 3 groups: 3-day (3D), 7-day (7D), or 14-day (14D) rest group. All mice were free to ambulate within their cages during their rest periods but were withheld from voluntary physical activity. After the 3D, 7D, or 14D rest period was completed, a solid-surface, 127-mm running wheel (Ware Manufacturing, Phoenix, AZ), magnetic sensor, and digital odometer (model BC600; Sigma Sport, Olney, IL) that recorded the number of running-wheel revolutions[16–18] were added to the mouse's cage. Daily running-wheel measurements of duration (in minutes) and distance (in kilometers) were recorded beginning 1 day after the running wheel was placed in the cage. Average daily speed (in m/min) was calculated for each week. For analysis, we averaged the data over 3 age quartiles. The age quartiles were divided into weeks 3 to 15, 16 to 30, and 31 to 45. We started analysis at week 3 because the last group was given access to running wheels at week 2. We did not continue data collection after week 45 because, based on previous research,[17,18] running-wheel activity does not change after that period.

Statistical Analysis

Two-way analyses of variance (group $\times$ age) with repeated measures were performed to compare changes in physical activity (duration, distance, speed). Post hoc comparisons of between-age means were performed using Tukey honestly significant difference tests. Hedges *g* measures of the effect sizes were calculated to determine the magnitude of the effect. The strength of the effect size was determined as *small* (0.02–0.49), *moderate* (0.50–0.79), or *large* ($\geq$0.80). We set the α level at .05. All statistical analyses were performed using the JMP statistical analysis software.

RESULTS

From weeks 3 to 15 after surgery, duration, distance, and running speed were not different among groups ($P > .05$), but all activity variables increased across time in this quartile ($F_{2,12}$ range = 31.1–76.3, $P < .05$; Figure 1). From weeks 16 to 30 after surgery, distance ($F_{2,14} = 0.57$, $P = .041$) and running speed ($F_{2,14} = 0.93$, $P = .01$) were greater in the 14D group than in the 3D and 7D groups, whereas duration was not different among the groups ($F_{2,14} = 0.27$, $P = .19$). We observed no differences across time in this quartile ($F_{2,14}$ range = 30.5–166.2, $P > .05$). From weeks 31 to 45 after surgery, duration ($F_{2,14} = 0.74$, $P = .02$), distance ($F_{2,14} = 0.95$, $P = .009$), and running speed ($F_{2,14} = 1.05$, $P = .007$) were greater in the 14D group than in the 3D and 7D groups. No differences across time were found from weeks 31 to 45 in this quartile ($F_{2,14}$ range = 4.0–306.0, $P > .05$).

Effect sizes and 95% confidence intervals are presented in Tables 1 and 2. Effect sizes for the variables of distance and speed that were different among the 3 groups were moderate to large (range = 0.60–3.00).

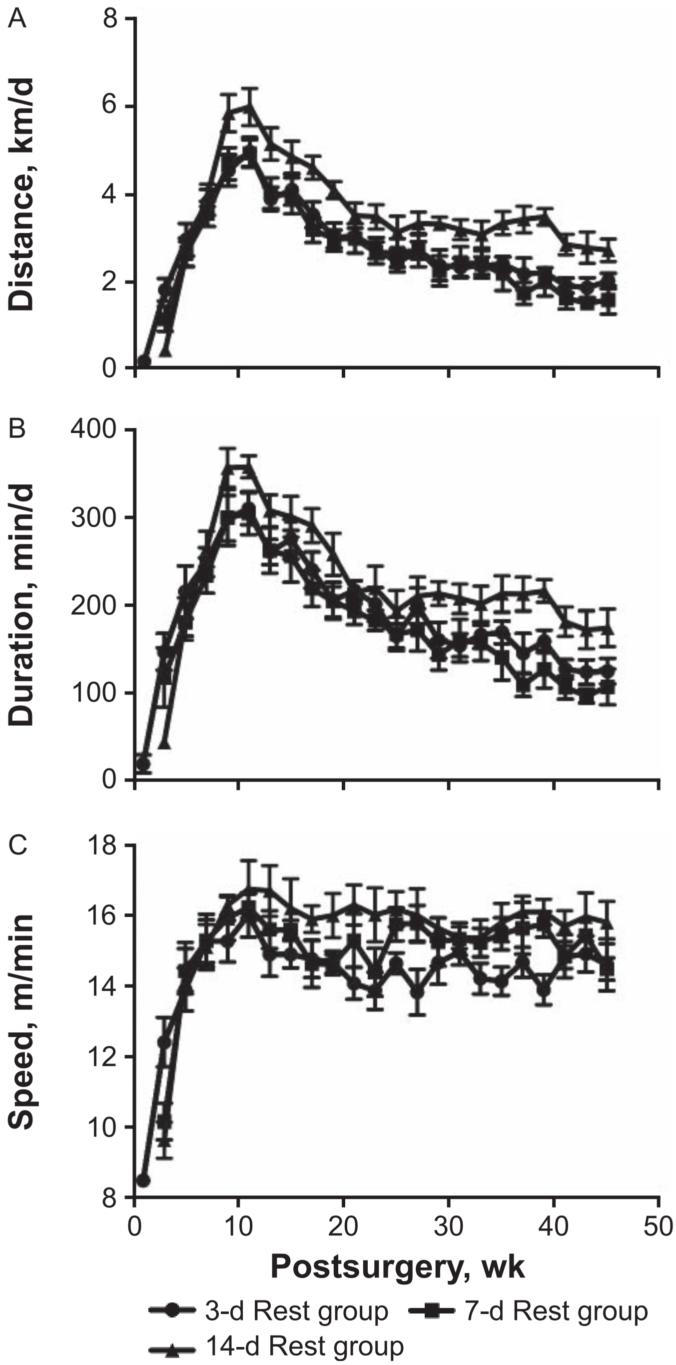

Figure 1. Mean ± standard deviation of A, the daily distance; B, the daily duration; and C, the average running speed for the 3 running-wheel groups across the lifespan.

Table 1. Effect Sizes for Distance and Speed of Running-Wheel Activity Between the 14- and 7-Day Rest Groups

	Effect Size (95% Confidence Interval)	
Time, wk	Distance	Speed
16	1.23 (0.54, 1.92)	1.20 (0.42, 1.98)
20	1.54 (1.16, 1.92)	2.66 (2.09, 3.23)
24	0.70 (0.10, 1.30)	0.78 (0.21, 1.95)
28	2.04 (1.65, 2.43)	0.71 (0.16, 1.53)
32	1.24 (0.81, 1.67)	0.64 (0.13, 1.15)
36	1.96 (1.46, 2.46)	0.60 (0.30, 0.90)
40	1.90 (1.48, 2.32)	0.79 (0.29, 1.29)

Table 2. Effect Sizes for Distance and Speed of Running-Wheel Activity Between the 14- and 3-Day Rest Groups

	Effect Size (95% Confidence Interval)	
Time, wk	Distance	Speed
16	1.17 (0.48, 1.86)	1.58 (0.79, 2.36)
20	1.66 (1.28, 2.04)	3.00 (2.43, 3.57)
24	0.80 (0.20, 1.40)	1.07 (0.42, 2.24)
28	1.90 (1.51, 2.29)	0.92 (0.24, 1.74)
32	1.21 (0.78, 1.64)	1.10 (0.59, 1.61)
36	1.97 (1.47, 2.47)	0.82 (0.52, 1.12)
40	1.59 (1.17, 2.01)	1.98 (1.48, 2.48)

DISCUSSION

Our findings suggested that the commonly observed decline in physical activity throughout the lifespan after an ankle sprain can be minimized by a longer rest period after injury. The mice in the 14D rest group had higher physical activity levels than the mice in the 3D and 7D rest groups across the lifespan. From a tissue-healing standpoint, this makes sense. By not having access to a running wheel, the amount of activity the mice could perform was minimized shortly after injury, which could have facilitated tissue healing. A new, updated model[19] has been developed to describe the immediate management of injury: protection, optimal loading, ice, compression, and elevation. It is based on the need to replace rest with optimal loading, so the joint is subject to balanced and incremental loading.[19] Not having access to a running wheel may have served as a period of unloading of the joint. For the mice that had no access to running-wheel activity for 14 days, the optimal loading may have facilitated healing. When given a wheel, the mice were able to return to normal physical activity levels. All effect sizes between the 14D and 3D rest groups were large for both speed and distance in the second and third quartiles of the lifespan. Between the 14D and 7D rest groups, the effect sizes were moderate to large. The large effect sizes demonstrated the magnitude of the difference between the 2 groups and further showed the benefit of a longer time away from exercise on physical activity in the mice.

In previous research from our laboratory,[5,6] mice underwent a sham surgery that included an incision and closure similar to that used in this study but no ligament transection. When we compared the data from the sham-surgery group with the data from our 14D rest group, no differences in activity levels were present (Figure 2). More specifically, from weeks 3 to 15 after surgery, duration and distance were not different between the sham-surgery group in the previous study and the 14D rest group in our current study, illustrating the effect of the ligament transection. However, no long-term differences were observed among groups for duration, distance, or running speed in the second and third quartiles of life (ie, from weeks 16 to 30 and from weeks 31 to 45 after surgery, respectively; $P > .05$). Duration, distance, and running speed also did not change within the individual quartiles. Based on the comparison between our study and the previous research,[5,6] the physical activity levels in the 14D rest group were not only greater than in the 3D and 7D rest groups but were also not different from a sham-surgery group. In other words, a 14D rest period restored physical activity to the levels of mice that did not have a surgically induced ankle sprain.

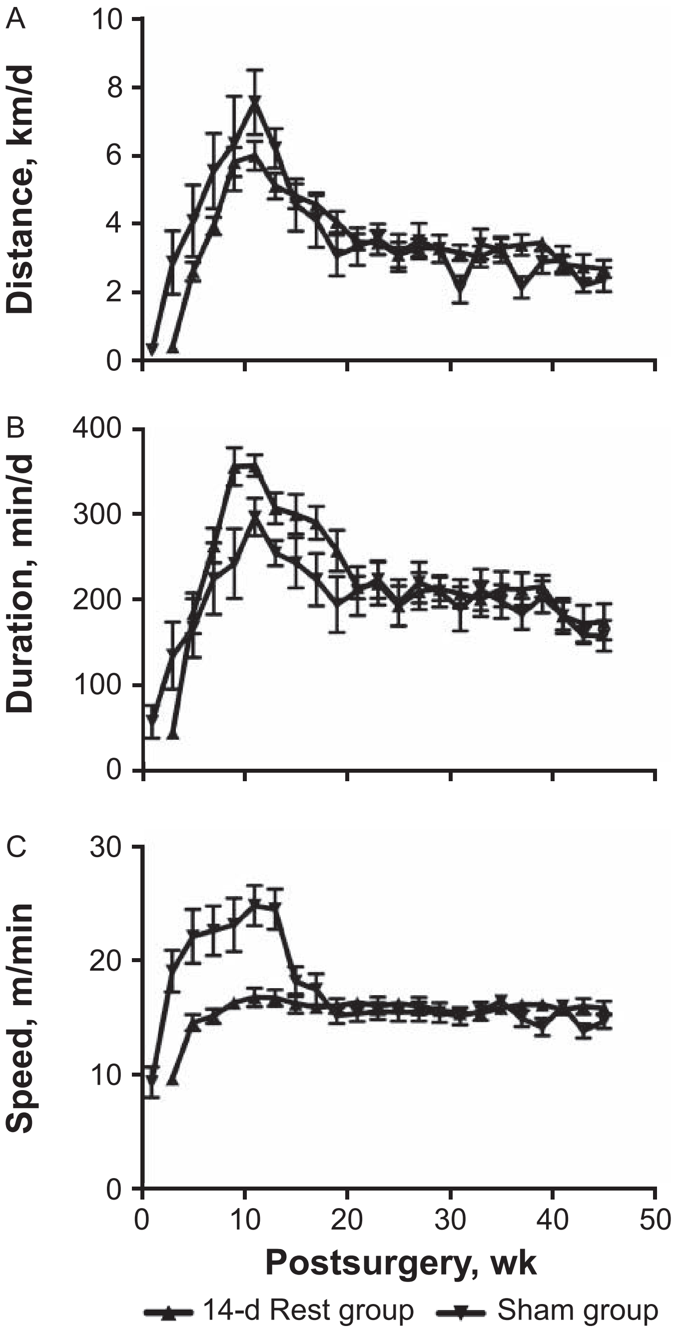

Figure 2. Mean ± standard deviation of A, the daily distance; B, the daily duration; and C, the average running speed for the 14-day no-wheel group and the sham (no-injury) group from previous research[5,6] in weeks across the lifespan.

The 14D rest group was likely able to have and maintain higher physical activity levels throughout the lifespan secondary to greater healing of the ankle-joint ligaments. Whereas we did not measure healing or ligamentous laxity, researchers[20] have reported that increased ligamentous laxity persists 8 weeks after a lateral ankle sprain. This lack of ligamentous healing most likely results in altered mechanics that, in turn, contribute to talar cartilage degeneration over time. The ankle sprains that Hubbard and Cordova[20] observed (grades 1 and 2) were less severe than the surgically induced sprains in our study. The human participants did not receive formal treatment and returned to exercise or sport a few days after injury.[20] Although we are speculating, the quick return to activity and lack of treatment probably led to the persistent ligamentous laxity present 8 weeks after the initial injury. Other investigators[21,22] have demonstrated increased laxity weeks to months after an initial sprain. Facilitating longer periods of rest from activity may allow better tissue remodeling and improved restoration of joint stability, subsequently contributing to long-term functional gains.

Based on the existing literature, we know that ankle sprains are the most common musculoskeletal injury[1] and a large percentage (up to 70%) of patients who sprain their ankles develop CAI.[3] We also know from both human and animal research[4–7] that physical activity levels decrease after an ankle sprain and in those with CAI. Physical inactivity can lead to the development of chronic diseases, such as cardiovascular disease, cancer, and type 2 diabetes.[8] Patients appear to return to activity with limited rest after an ankle sprain.[14,15,23] If the ankle ligaments are not allowed time to heal, ligamentous laxity may persist. This can lead to an unstable ankle joint and alter neuromuscular control at the ankle.[24] Changes in both mechanics and neuromuscular control can lead to repetitive injury and the chronic symptoms reported by patients with CAI, including pain, instability, and "giving way." Individuals are likely to be less physically active due to these symptoms.

The clinical implication of this study is that an ankle sprain is a severe injury that needs to be managed as such. Patients with ankle sprains should rest and have time away from exercise or sport to facilitate tissue healing and restoration of function. Those who return to activity too quickly are at greater risk for long-term changes in physical activity levels. Clinically, the emphasis should be on immediate care and management of the ankle sprain and then on returning to activity after joint function has been restored. Maintaining physical activity levels is important for overall health and wellbeing.

The primary limitation of our study was that the duration of rest and time since injury did not translate well to human models due to the difference in the lifespans (the breed of mice used in our study has a lifespan of 1.82 years). These preclinical data inform both researchers and clinicians about the effect that an ankle sprain can have on physical activity and how more time away from exercise may benefit long-term physical activity levels.

CONCLUSIONS

Mice that had severe ankle sprains and rested for 14 days before being permitted to engage in voluntary physical activity via a running wheel had higher physical activity outcomes than mice that were given a running wheel 3 or 7 days after injury. Rest and time away from exercise after an ankle sprain may be critical to restoring physical activity levels to normal, uninjured levels. Researchers continue to show the negative long-term consequences of ankle sprains; allowing rest and facilitating tissue healing may best restore function, minimizing those long-term changes. Further study is needed to determine the optimal rest windows for different severities of ankle sprains in humans.

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