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ORIGINAL ARTICLE

CROSS-PLATFORM VALIDATION OF TREADMILL-BASED AND FREE-MOVEMENT GAIT ANALYSIS FOLLOWING MURINE ACHILLES TENDON HEMI-TRANSECTION

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LEVEL OF EVIDENCE: 5

ABSTRACT

Background: Gait analysis is widely used as a non-terminal method to assess functional recovery following Achilles tendon injury; however, differences between treadmill-based and free-movement systems limit cross-study comparability.
Purpose: To compare gait parameters obtained from treadmill-based and free-movement systems following murine Achilles tendon injury and to evaluate a velocity-matched framework for cross-platform comparison.
Methods: Twenty adult C57BL/6 mice underwent unilateral Achilles tendon hemi-transection (n = 10) or served as non-operative controls (n = 10). Gait was assessed at baseline and postoperative day (POD) 3, 14, and 21 using DigiGait and BlackBox. Velocity-matched windows (± 10%) were analyzed. Primary outcomes included duty factor, stance duration, swing duration, and peak paw area. Functional asymmetry was quantified using a Functional Deficit Index (FDI).
Results: BlackBox detected significant stance and swing duration alterations in the injured limb through POD 14, whereas DigiGait reached significance only at POD 3 for swing duration and peak paw area. BlackBox demonstrated greater sensitivity, with significantly larger FDI magnitudes for duty factor and swing duration at POD 3 ($p < 0.05$). Peak paw area demonstrated divergent platform behavior.
Conclusions: Velocity-matched comparisons demonstrate that treadmill-based and free-movement systems capture functionally meaningful gait alterations after Achilles tendon injury, although free-movement systems appear more sensitive to early changes. These findings support cross-platform gait comparisons and highlight methodological considerations for preclinical tendon research.

KEYWORDS

Gait analysis, treadmill-based locomotion, Achilles tendon injury, murine model, biomechanics, free-movement locomotion, tendon healing

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INTRODUCTION

Achilles tendon injuries are among the most frequently encountered musculoskeletal injuries and are associated with persistent functional impairment despite appropriate treatment. Even following recovery, deficits in strength, load transmission, and gait mechanics are commonly observed, reflecting the limited regenerative capacity of tendon tissue[1].

Preclinical models play a critical role in understanding the mechanisms of tendon healing and in evaluating novel therapeutic strategies[2,3]. In this context, gait analysis has emerged as a valuable, non-terminal method for assessing functional recovery over time. Unlike mechanical testing, which requires sacrifice of the animal, gait analysis allows for longitudinal assessment within the same subject, thereby improving reproducibility and reducing the number of animals required[4,5].

Commercially available gait analysis systems can be broadly categorized into treadmill-based and free-movement platforms. Treadmill-based systems offer standardized experimental conditions and facilitate acquisition of continuous, uniform gait cycles. In contrast, free-movement systems allow animals to ambulate voluntarily, thereby more closely approximating natural locomotion and minimizing stress-related artifacts. Despite these advantages, fundamental biomechanical differences between these systems complicate direct comparisons of outcomes across studies[6].

Prior work by Crosio et al. identified specific gait parameters associated with Achilles tendon injury using a free-movement system and demonstrated that paired comparisons between the injured and contralateral limb provide the most reliable and reproducible assessment of functional deficits[7]. However, the applicability of these findings to treadmill-based systems remains unclear. In contrast, Herbin et al. demonstrated that although absolute gait parameters differ between treadmill and overground locomotion, the relationships between key temporal variables such as stance and swing time remain conserved across conditions[6].

Taken together, these findings suggest that cross-platform comparisons are feasible when locomotion is evaluated under comparable velocity conditions[8]. The purpose of the present study was therefore to directly compare treadmill-based and free-movement gait analysis following Achilles tendon injury using a velocity-matching approach. We hypothesized that both systems would detect similar directional functional deficits, but that free-movement analysis would demonstrate greater sensitivity to early changes following injury.

MATERIALS AND METHODS

Study design and animal model

A controlled laboratory study was performed using a murine model of Achilles tendon injury. Twenty adult C57BL/6 mice aged 10-13 weeks were included. Animals underwent unilateral Achilles tendon hemi-transection of the left hindlimb (n = 10) or served as non-operative controls without anesthesia or surgical intervention (n = 10). The hemi-transection model, as opposed to complete transection, was selected to produce a less severe injury and allow evaluation of early functional recovery within a shortened experimental timeline. The present investigation was designed as a pilot, proof-of-concept study with the primary methodological aim of evaluating cross-platform comparability of treadmill-based and free-movement gait analysis under a velocity-matched framework. Because each surgical animal served as its own internal control through paired comparison of the injured left and contralateral right hindlimbs, the principal experimental requirement was the reliable induction of a quantifiable, unilateral functional deficit rather than maximization of injury severity. The hemi-transection model was therefore selected over complete transection in order to produce a less severe, more rapidly healing injury that permitted evaluation of acute and subacute gait alterations within a compressed observation window. By contrast, prior work by Crosio et al. utilized a complete transection model with a 56-day postoperative observation period to capture the full healing trajectory[7]. The 21-day endpoint of the present study was therefore aligned with the reduced severity of the hemi-transection model and was sufficient to detect injury-related gait alterations and their early recovery for the purposes of cross-platform validation. Future studies aimed at characterizing the full healing trajectory would benefit from incorporation of a complete transection model with extended postoperative observation, consistent with prior literature[7].

All animals underwent gait analysis at baseline (preoperative) and at POD 3, POD 14, and POD 21. These timepoints were adapted from previously established Achilles tendon injury models while accounting for the reduced severity of the hemi-transection injury[7]. POD 3 was selected as the earliest postoperative assessment timepoint to maintain consistency with prior Achilles tendon injury protocols[7], to avoid imposing forced locomotion on a freshly injured limb in accordance with animal welfare considerations, and to ensure that gait was assessed after elimination of perioperative buprenorphine analgesia, which could otherwise have introduced a pharmacological confounder to locomotor behavior.

All animal procedures were reviewed and approved by the Chancellor's Animal Research Committee (Animal Research Committee; Institutional Animal Care and Use Committee) of the University of California, Los Angeles (Protocol No. ARC-2022-098) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines.

Gait analysis platforms and protocol

Gait was assessed using two complementary platforms

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(Fig. 1). The first was a free-movement plantar pressure system (BlackBox; BlackBox Inc., Dallas, TX, USA), in which mice were allowed to ambulate voluntarily within a 30 × 30 cm arena *(Fig. 1B)*. The second was a treadmill-based ventral imaging system (DigiGait; Mouse Specifics, Inc., USA), operated at a fixed incline of 0° and controlled belt speeds of 12.5 cm/s and 17.5 cm/s *(Fig. 1A)*. Prior to data collection, all animals underwent a standardized habituation protocol consisting of 10 minutes of exposure to each system to minimize stress and variability in gait behavior.

Velocity matching and data acquisition

Dynamic gait analysis was performed using a velocity-matching approach. Locomotion bouts from the BlackBox system were segmented into continuous windows of 3-6 seconds and included for analysis if the average velocity fell within ± 10% of the corresponding DigiGait belt speed (12.5 cm/s or 17.5 cm/s). Only windows with continuous forward movement and without turning or acceleration artifacts were included. A minimum of three qualifying windows per animal was required at each timepoint. For each mouse and each treadmill-matched speed, the three runs with the smallest absolute deviation from the target velocity were selected for analysis.

Outcome measures and functional deficit index

Primary gait parameters extracted from both systems included duty factor, stance duration, swing duration, and peak paw area. Swing speed was included as an additional validation parameter in paired hindlimb analyses. Internal symmetry was assessed by comparing the injured left hindlimb (LH) and contralateral uninjured right hindlimb (RH) within the surgical cohort.

Functional asymmetry was quantified using an FDI. For parameters in which injury-related deficit was represented by an increase in the injured limb, FDI was calculated as [(LH - RH) / (0.5 × (LH + RH))] × 100. For parameters in which injury-related deficit was represented by a decrease in the injured limb, the numerator was reversed as [(RH - LH) / (0.5 × (LH + RH))] × 100 to maintain a positive deficit convention. Values approaching zero indicated symmetry. To verify that observed asymmetries reflected the surgical intervention rather than system bias, the same calculations were performed on the non-operative control cohort, in which no consistent difference between left and right hindlimbs was anticipated.

Statistical analysis

All gait parameters were subjected to ROUT outlier tests (Q = 1%) and Shapiro-Wilk normality tests before analysis. Longitudinal differences across timepoints (baseline, POD 3, POD 14, and POD 21) were evaluated using one-way analysis of variance or mixed-effects models, as appropriate. When significant main effects were detected, Sidak or Dunnett post hoc testing with appropriate corrections was performed to compare postoperative timepoints with baseline. For cross-platform comparisons, the FDI was computed for each metric, and the sensitivity differential between the BlackBox and DigiGait systems was evaluated. Statistical significance was defined as $p < 0.05$. All data processing and statistical computations were performed using Python (v3.12) with the pandas, SciPy, and NumPy libraries.

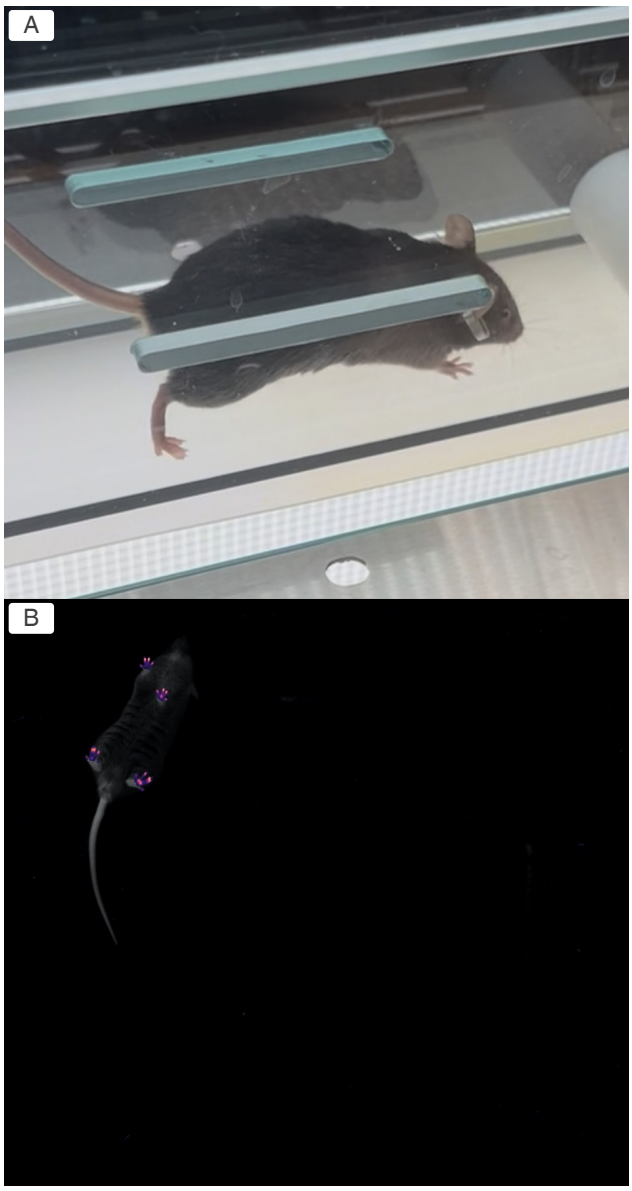


Figure 1. *Experimental gait analysis platforms. A) Treadmill-based ventral imaging system (DigiGait, Mouse Specifics, Inc.), in which mice ambulate on a transparent motorized belt with paw placement captured from below. B) Free-movement plantar pressure system (BlackBox), in which mice ambulate voluntarily within an enclosed arena and paw contact is captured by light refraction at the walking surface, producing illuminated paw prints on a dark background.*

RESULTS

All animals successfully completed baseline and early postoperative data collection. Two animals in the surgical cohort were lost before completion of the study; data from

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these animals were included through POD 3 and excluded from subsequent timepoints.

Longitudinal changes in injured limb gait parameters

Following unilateral Achilles tendon hemi-transection, both systems tracked the progression of functional recovery, although detection of acute deficits and the duration over which deficits remained detectable varied by platform *(Fig. 2)*. For the injured limb, the BlackBox system detected a significant increase in stance duration that persisted from POD 3 through POD 14 ($p < 0.001$ at both timepoints) and a significant increase in swing duration over the same window ($p < 0.05$), reflecting prolonged gait alteration and compensatory guarding behavior. Peak paw area showed a significant transient increase at POD 3 ($p < 0.01$) before returning toward baseline.

In contrast, the DigiGait system did not reach statistical significance for stance duration at any timepoint and detected significant changes in swing duration and peak paw area only at POD 3 ($p < 0.05$). Duty factor did not reach significance in either system. By POD 21, all temporal parameters across both systems approached baseline values, suggesting a return to near-normal gait.

Gait asymmetry and functional deficit index

Analysis of the FDI revealed significant gait asymmetry acutely after injury *(Fig. 3)*. The BlackBox system demonstrated a robust positive FDI for duty factor and stance duration at POD 3 ($p < 0.05$), highlighting significant functional asymmetry of the injured limb. The FDI for swing duration was also significantly positive in BlackBox data ($p < 0.05$), indicating compensatory alteration of the swing phase. In contrast, the DigiGait system reported FDI values closer to zero across most timepoints, indicating a higher degree of perceived symmetry despite injury. Notably, the BlackBox system detected a negative FDI for peak paw area during the recovery phase, potentially suggesting

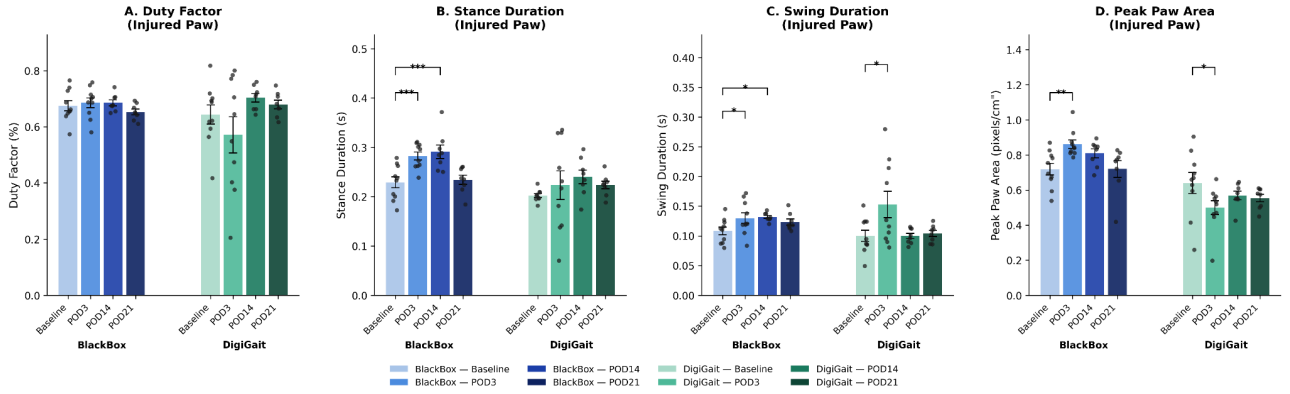


Figure 2. *Longitudinal gait parameters in the injured hindlimb following Achilles tendon hemi-transection. Time course of duty factor (A), stance duration (B), swing duration (C), and peak paw area (D) in the injured hindlimb at baseline, POD 3, POD 14, and POD 21, captured by the BlackBox free-movement and DigiGait treadmill-based systems. The BlackBox system detected significant changes in stance duration through POD 14 and swing duration through POD 14, whereas DigiGait detected significant changes only at POD 3 for swing duration and peak paw area and did not reach significance for stance duration at any timepoint. Bars represent mean ± standard error of the mean with individual data points overlaid. n = 8-10 mice per group; *p <0.05, **p <0.01, ***p <0.001 versus baseline.*

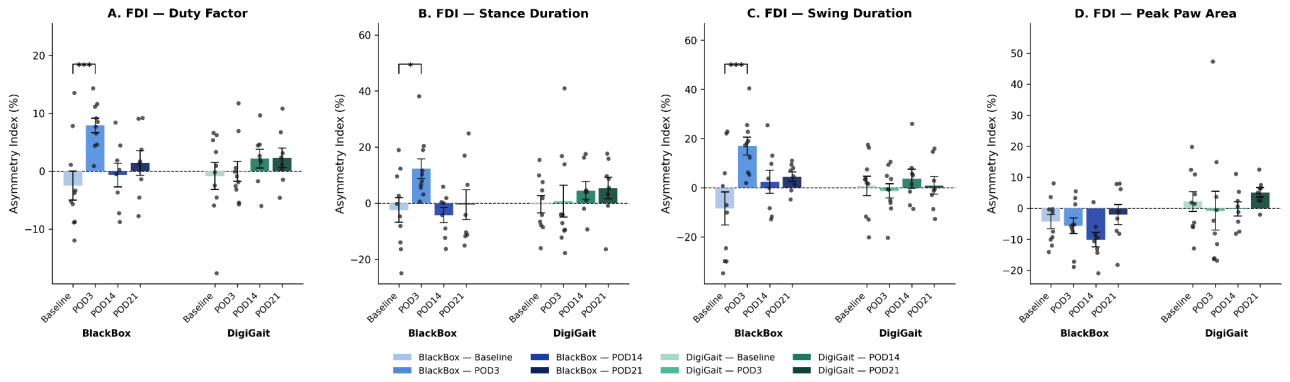


Figure 3. *Functional Deficit Index across timepoints. Within-animal asymmetry between the injured left hindlimb and contralateral right hindlimb for duty factor (A), stance duration (B), swing duration (C), and peak paw area (D), captured by the BlackBox free-movement and DigiGait treadmill-based systems at baseline, POD 3, POD 14, and POD 21. Positive values indicate functional deficit of the injured limb. The BlackBox system detected significant FDI elevations at POD 3 for duty factor, stance duration, and swing duration, whereas DigiGait FDI values remained near zero at all timepoints. Bars represent mean ± standard error of the mean with individual data points overlaid. n = 8-10 mice per group; *p <0.05, ***p <0.001 versus baseline.*

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compensatory splaying of the injured paw, although this did not reach statistical significance.

These findings were corroborated by within-animal paired comparisons between the injured and contralateral hindlimbs in the surgical cohort, with significant differences detected by BlackBox for duty factor, stance duration, and swing speed at POD 3 and for peak paw area at POD 14 *(Fig. 4)*. To assess validity, identical paired comparisons were performed in the non-operative control group, which showed only sporadic significant differences between limbs at later timepoints with no discernible pattern *(Fig. 5)*, supporting that the asymmetries observed in the surgical cohort reflect injury-related functional change rather than systemic platform bias.

Cross-platform sensitivity comparison

At POD 3, the BlackBox system demonstrated a statistically significant advantage in detecting functional deficits for duty factor and swing duration, as indicated by a significant positive sensitivity differential ($p < 0.05$; *Fig. 6*). This finding indicates that BlackBox exhibits heightened sensitivity in capturing changes in these gait metrics during the acute postoperative phase relative to DigiGait. Stance duration and peak paw area showed no significant cross-platform sensitivity differential at any timepoint.

DISCUSSION

The principal finding of this study was that treadmill-based and free-movement gait analysis identified functionally meaningful gait alterations after Achilles tendon hemi-transection, although the apparent magnitude and temporal persistence of impairment differed between platforms. The BlackBox free-movement system demonstrated greater sensitivity for detecting early functional asymmetry, particularly for duty factor and swing duration at POD 3, and detected changes in temporal parameters that persisted through POD 14. Prior work in murine tendon models supports gait analysis as a sensitive, non-terminal measure of functional recovery, and recent free-movement data in Achilles-injured mice similarly identified temporal and loading-related parameters as reproducible indicators of tendon dysfunction[7].

Although both platforms detected early impairment, the free-movement system appeared more sensitive to both the magnitude and temporal extent of functional loss. Notably, the BlackBox system identified significant deviations in stance and swing duration that persisted through POD 14, whereas the DigiGait system did not reach significance for stance duration at any timepoint and detected swing duration changes only at POD 3. This extended detection window reinforces the sensitivity

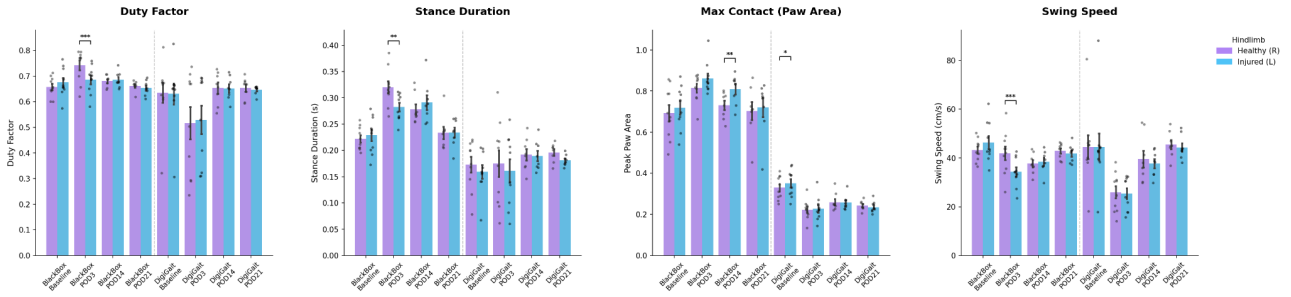


Figure 4. *Gait validation suite: paired hindlimb comparisons in the surgical cohort. Within-animal paired comparisons of the healthy right hindlimb and injured left hindlimb across the BlackBox and DigiGait systems for duty factor, stance duration, peak paw area, and swing speed at baseline, POD 3, POD 14, and POD 21. The BlackBox system detected significant differences between limbs at POD 3 for duty factor, stance duration, and swing speed, and at POD 14 for peak paw area; the DigiGait system detected significant differences only at POD 3 for peak paw area. Bars represent mean ± standard error of the mean with individual data points overlaid. n = 8-10 mice per group; *p <0.05, **p <0.01, ***p <0.001.*

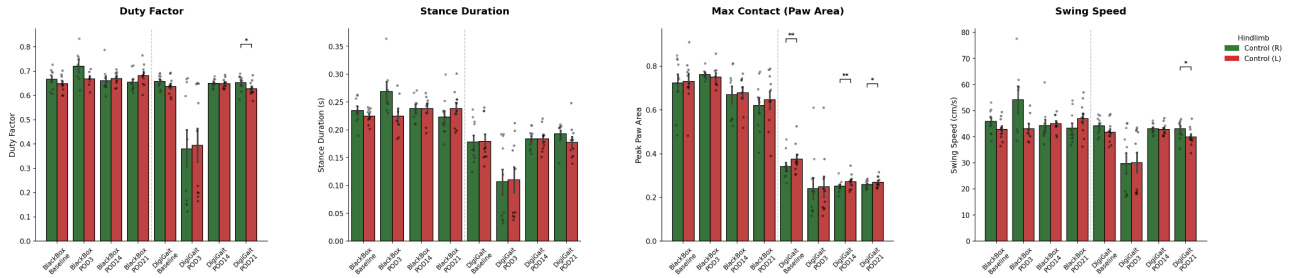


Figure 5. *Internal symmetry validation: paired hindlimb comparisons in the non-operative control cohort. Within-animal paired comparisons of right and left hindlimbs across the BlackBox and DigiGait systems for duty factor, stance duration, peak paw area, and swing speed at baseline, POD 3, POD 14, and POD 21. Only sporadic differences between limbs were observed at later timepoints with no discernible pattern, supporting that the asymmetries observed in the surgical cohort reflect injury-related functional change rather than systemic platform bias. Bars represent mean ± standard error of the mean with individual data points overlaid. n = 10 mice per group; *p <0.05, **p <0.01.*

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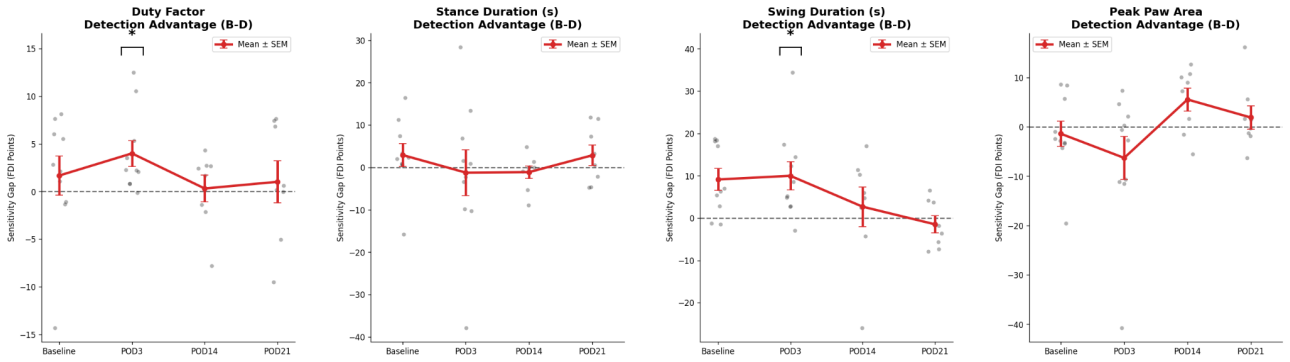


Figure 6. *BlackBox sensitivity advantage relative to DigiGait in the surgical cohort across baseline through POD 21. Sensitivity differential is expressed as the FDI gap (BlackBox FDI - DigiGait FDI, in FDI points) for duty factor (A), stance duration (B), swing duration (C), and peak paw area (D). Positive values indicate greater sensitivity of the BlackBox system in detecting injury-related asymmetry. Red lines represent mean ± standard error of the mean with individual data points overlaid. n = 8-10 mice per group; *p <0.05.*

advantage of free-movement analysis, particularly for temporal parameters tied to plantarflexor function. A likely explanation is that treadmill-based systems impose a fixed speed and constrain locomotor behavior, whereas overground systems allow animals to self-select movement strategies. Prior locomotion studies have demonstrated that mice exhibit altered stride characteristics during treadmill locomotion compared with overground locomotion, even when fundamental temporal relationships remain conserved[6]. Reviews of rodent gait methodology similarly emphasize that different platforms capture distinct aspects of locomotor adaptation and weight-bearing behavior[9].

Parameter selection in the present study was informed by prior tendon and gait literature. Duty factor, stance duration, swing duration, and related loading measures have been repeatedly identified as biologically meaningful markers of limb dysfunction in rodent gait studies[9]. Prior Achilles tendon injury work has also demonstrated that intensity-based and duty cycle-related parameters are particularly informative following Achilles tendon injury and that paired comparisons between injured and contralateral limbs provide a sensitive and reproducible method of detecting functional asymmetry[7].

A major strength of this study is the use of velocity matching to compare two fundamentally different locomotion systems. Prior treadmill and overground locomotion work has demonstrated that while these modalities differ in absolute stride parameters, relationships between stance time, swing time, and stride frequency remain largely preserved across conditions[6]. Our findings extend this concept by demonstrating that, when evaluated at comparable velocities, both platforms capture injury-related gait alterations, suggesting that differences between systems are driven primarily by magnitude and platform-specific locomotor constraints rather than by the absence of an underlying biological signal.

An additional distinction between platforms is the ability to manipulate mechanical demand. Treadmill-based systems such as DigiGait allow for controlled variation in incline, which can alter locomotor biomechanics and increase the mechanical demands placed on the plantarflexor complex. Experimental studies have shown that treadmill conditions modify stride characteristics and loading patterns compared with overground locomotion, reflecting differences in sensorimotor input and mechanical environment[6]. In contrast, free-movement systems do not permit incline modulation but instead capture unconstrained locomotion, which may better reflect natural compensatory strategies. Although incline was not evaluated in the present study, this capability represents an important difference in experimental flexibility and may provide a means of probing functional deficits under increased mechanical demand in future investigations.

Peak paw area demonstrated a distinct pattern compared with other parameters and should be interpreted cautiously. Prior work using CatWalk systems has shown that peak paw area and intensity-related measures reflect limb loading and weight-bearing behavior[10]. Variability in this parameter within the free-movement system may therefore reflect compensatory loading strategies that are less constrained than in treadmill-based conditions. As a result, platform-specific differences in peak paw area are not unexpected and highlight the importance of interpreting individual parameters within the context of the testing modality.

Longitudinally, functional deficits improved over time, with the greatest impairments observed at early postoperative timepoints and partial recovery thereafter. This recovery trajectory was reproducibly observed across animals in the surgical cohort, with the largest measured deficits consistently occurring at the earliest postoperative gait assessment (POD 3)—which captures the descending limb of the recovery trajectory rather than the true biologic peak deficit, expected to occur acutely after surgery—followed by partial improvement by POD 14 and a return toward baseline values by POD 21 across all primary gait parameters captured by both platforms. This trajectory

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is consistent with prior tendon injury models, in which early inflammatory and mechanical deficits gradually improve during the healing process[11]. The relatively rapid normalization observed in the present study likely reflects the reduced severity of the hemi-transection model compared with full transection injuries, which have been shown to produce more persistent functional and structural deficits[12]. Mechanistically, the use of a hemi-transection model preserves a portion of the intact tendon substance and avoids the formation of the persistent fibrous gap that is characteristic of complete transection injuries, which is consistent with the more rapid functional recovery observed within the present experimental window.

Several limitations should be acknowledged. Rodent gait outcomes are influenced by multiple factors including speed, body weight, sex, and testing conditions, all of which can introduce variability if not carefully controlled[13]. Free-movement systems in particular require attention to habituation and variability in locomotor behavior. A further consideration is the nature of the control group; non-operative controls were used in lieu of sham-operated animals, allowing direct evaluation of injury-specific gait changes but precluding isolation of effects attributable to anesthesia, skin incision, or wound healing alone. Future studies incorporating sham-operated controls may help further refine attribution of acute gait changes to the tendon injury itself. Additionally, this study focused on functional outcomes without direct correlation to structural or mechanical tendon properties, which may provide further insight into recovery. Finally, while velocity matching improves comparability between platforms, it does not fully account for inherent biomechanical differences between treadmill and free-movement locomotion[6].

An additional consideration is that the present study was designed as a pilot, proof-of-concept investigation utilizing a hemi-transection injury model with a 21-day postoperative observation window. While this design was appropriate for the methodological aim of cross-platform validation, it does not capture the full healing trajectory associated with more severe injury models. Future investigations utilizing complete Achilles tendon transection with extended postoperative observation, as previously described[7], would provide a more comprehensive assessment of cross-platform sensitivity across the entire healing timeline.

Clinically and methodologically, these findings suggest that investigators should exercise caution when comparing absolute gait values across platforms. Free-movement systems may be preferable when the primary objective is to detect subtle early compensatory behavior after tendon injury, whereas treadmill-based systems may be advantageous when controlled speed, continuous gait acquisition, or manipulation of mechanical demand is required. For preclinical tendon studies, velocity matching provides a practical framework for improving comparability across studies that use different gait analysis systems.

CONCLUSIONS

Velocity-matched gait analysis demonstrates that treadmill-based and free-movement systems capture functionally meaningful gait alterations following Achilles tendon injury; however, free-movement systems exhibit greater sensitivity to early changes. The use of a velocity-matched framework enables meaningful cross-platform comparison and provides a foundation for integrating findings across studies utilizing different gait methodologies. The ability of treadmill systems to modulate mechanical demand through variables such as incline represents an additional experimental advantage that may be leveraged in future studies to further interrogate tendon function under increased loading conditions. These findings may inform selection of gait analysis methodologies in future preclinical tendon studies and improve the interpretability of cross-study comparisons.

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