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Ground reaction forces are more sensitive gait measures than temporal parameters in rodents following rotator cuff injury

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ABSTRACT

Gait analysis is a quantitative, non-invasive technique that can be used to investigate functional changes in animal models of musculoskeletal disease. Changes in ground reaction forces following injury have been observed that coincide with differences in tissue mechanical and histological properties during healing. However, measurement of these kinetic gait parameters can be laborious compared to the simpler and less time-consuming analysis of temporal gait parameters alone. We compared the sensitivity of temporal and kinetic gait parameters in detecting functional changes following rotator cuff injury in rats. Although these parameters were strongly correlated, temporal measures were unable to detect greater than 50% of the functional gait differences between injured and uninjured animals identified simultaneously by ground reaction forces. Regression analysis was used to predict ground reaction forces from temporal parameters. This model improved the ability of temporal parameters to identify known functional changes, but only when these differences were large in magnitude (i.e., between injured vs. uninjured animals, but not between different post-operative treatments). The results of this study suggest that ground reaction forces are more sensitive measures of limb/joint function than temporal parameters following rotator cuff injury in rats. Therefore, although gait analysis systems without force plates are typically efficient and easy to use, they may be most appropriate for use when major functional changes are expected.

1. Introduction

Animal studies provide valuable insight into the pathophysiology and treatment modalities of musculoskeletal injuries and diseases due to their ability to study tissue-level properties not possible with human studies. Interpretation of the clinical relevance of tissue-level animal studies can be aided with quantitative measures of limb/joint function (Fu et al., 2015; Reuther et al., 2014b). Gait analysis is a highly sensitive, non-invasive technique used to study the pathophysiology of various orthopaedic diseases (Clarke et al., 1997; Krizsan-Agbas et al., 2014; Mora-Macias et al., 2015). For example, abnormalities in kinetic gait parameters during locomotion have been observed in rat models of osteoarthritis, spinal cord injury recovery, and rotator cuff disease (Johnson et al., 2012; Reuther et al., 2015; Roemhildt et al., 2010).These disturbances in gait properties can be used to validate dysfunction in small animal disease models or to evaluate recovery of function following injury.

While previous systems used ink and paper methods, the primary types of rodent gait analysis systems currently utilize high-speed cameras that are coupled with or without force plate instrumentation. Coupled systems can simultaneously collect spatial (e.g., step width, length), temporal (e.g., duration of stance and swing phases), and kinetic (i.e., ground reaction forces) parameters to comprehensively quantify gait patterns. However, the use of such systems can be time consuming, as it requires isolation of the limb of interest on a force plate during locomotion and also involves laborious manual data processing (Muir and Whishaw, 1999). Small animal gait analysis systems without force plates are typically more time efficient as they are capable of continuous data collection during locomotion and often feature automated data processing, but lack the ability to calculate kinetic parameters.

Spatial parameters are less sensitive and consistent than both temporal and kinetic variables; however, it remains unknown whether temporal and kinetic parameters are equally sensitive measures compared to each other when analyzing gait compensations due to musculoskeletal injury (Allen et al., 2011; Reed et al., 2013). Previous human studies have reported the minimum detectable change (with 95% confidence) in temporal and kinetic gait parameters to be as low as 1% and 4%, respectively, compared

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to as high as 46% for spatial gait parameters (Owings and Grabiner, 2004; Paterson et al., 2008; Reed et al., 2013; Stolze et al., 1997). Importantly, accounting for velocity (which is strongly correlated to stride length and width) as a covariate did not improve the sensitivity of these spatial properties when used to analyze rat gait (Allen et al., 2010, 2009, 2011; Shamji et al., 2009). Alternatively, temporal parameters are believed to correspond with force generation, and similar gait patterns have been obtained from both temporal records of paw placement and force plate analysis (Hampton et al., 2011; Kale et al., 2004). Indeed, decreases in temporal parameters (e.g., stance time) may coincide with decreases in kinetic gait parameters (e.g., vertical ground reaction force) observed following injury (Hsu et al., 2011; Sarver et al., 2010; Shih et al., 1993). Nevertheless, the relationship between temporal properties and ground reaction forces during locomotion has not been thoroughly investigated. This information is critical given the relative ease of measuring temporal parameters compared to the more complicated and challenging process of quantifying kinetic parameters during small animal locomotion.

Therefore, the objective of this study was to compare the sensitivity of temporal versus kinetic parameters in detecting functional changes in rodent models of rotator cuff injury using a validated gait analysis system (Sarver et al., 2010). We hypothesized that temporal and kinetic parameters would correlate significantly, and that corresponding pairs of temporal and kinetic parameters would simultaneously denote changes in rodent gait following injury.

2. Methods

2.1. Study design

The data used in this study were obtained from our previous studies using rat rotator cuff injury models (Table 1). In study #1 (Reuther et al., 2014a), animals underwent unilateral detachment of the supraspinatus only (SO) or supraspinatus and infraspinatus (SI) rotator cuff tendons, and ambulatory measurements were collected up to 8 weeks post-operatively. To create a more general model, data from the SO and SI groups were combined and used for the correlation and regression analysis in the current study as significant differences between these groups were observed in shoulder function (as measured by kinetic gait parameters), as well as tendon and cartilage mechanical and histological properties. Data from two other studies were used to test the regression model created from the SO and SI data. In study #2 (Thomas et al., 2014), animals underwent unilateral detachment of the supraspinatus, infraspinatus, and biceps (SIB) tendons and ambulatory measures were collected identically to the SO and SI groups. In study #3 (Caro et al., 2014), animals underwent unilateral detachment and repair of the supraspinatus tendon and repair with (RW) or without (RWO) post-operative analgesics. Ambulatory measurements were collected up to 4 weeks following surgery.

2.2. Instrumented walkway

Forelimb gait was quantified using a stationary walkway as described previously (Sarver et al., 2010). Briefly, the system used features a clear acrylic walkway with two 6-degree-of-freedom miniature force/torque cells (nano17, ATI Industrial Automation, Apex North Carolina). Angled mirrors on both sides of the walkway capture ventral and sagittal views of the animal during locomotion using a 60 frames-per-second camera (A601fc, Basler Vision Technolgies, Ahrensburg Germany). A darkened box was placed at the end of the walkway to encourage the animal to walk. Only trials where the animal isolated a single limb on the force plate were selected for analysis, and data were averaged from a minimum of two walks per animal at each time point.

2.3. Gait parameters

Temporal and kinetic parameters were used to characterize rat locomotion (Fig. 1). Braking and propulsion times were calculated by determining when the anterior–posterior ground reaction force curve crossed zero during a single stride. Stance time was calculated as the total time the foot remained in contact with the ground. Peak vertical ($+z$ direction), braking ($-y$ direction), and propulsion ($+y$ direction) forces were determined for each stride, normalized to the animal's weight, and reported as a percentage of body weight (%BW).

2.4. Correlation analysis

Pearson's correlation coefficients were calculated between temporal parameters and kinetic parameters from the SO and SI pooled data set. Average values for SI or SO at each time point were used rather than data from individual animals in order to reduce variability and allow for a more representative analysis.

2.5. Kinetic and temporal parameter sensitivity

The total number of significant differences in kinetic parameters alone between SO & SI, SIB & baseline, and RW & RWO were compared to the subset of significant differences identified simultaneously in corresponding temporal–kinetic pairs. An illustration of this analysis is provided for example data where significant differences in vertical force between two groups were identified at 3, 7, and 14 days post-operatively (Fig. 2, left panel), but stance time was only different at the 3 day time point (Fig. 2, right panel). This analysis was repeated for each temporal–kinetic pair (i.e., vertical force and stance time, braking force and braking time, propulsion force and propulsion time) and totaled for each set of experimental groups. Similar comparisons between kinetic and temporal parameters were also performed for uninjured and injured (SO, SI, SIB pooled) animals after 3, 7, and 14 days of healing.

2.6. Regression modeling

Step-wise backward elimination linear regression analysis was performed on the combined SO and SI pooled data set to select the best temporal variables for predicting kinetic gait parameters. Similar to the correlation analysis, average values for a given parameter and group at each time point were used to reduce variability. The resulting regression coefficients were then used to predict vertical,

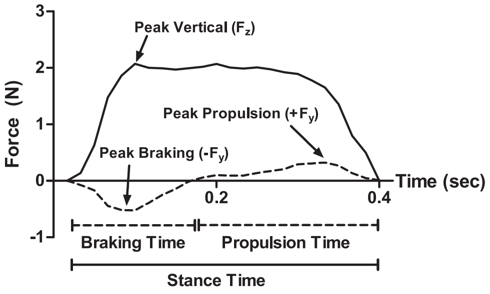

Fig. 1. *Model gait signals during stance phase*. Typical vertical and anterior–posterior (braking/propulsion) ground reaction forces are shown for a single step. Peak forces in each direction are highlighted. Braking and propulsion phases are separated by $F_y = 0$ in order to calculate their respective durations, whereas stance time is defined as the entire interval in which the foot remains in contact with the ground.

Table 1
Summary of data sets used for correlation and regression analysis of gait parameters.

	Study #1 (Reuther et al., 2014a)		Study #2 (Thomas et al., 2014)	Study #3 (Caro et al., 2014)	
Group (sample size)	SO ($n=14$)	SI ($n=14$)	SIB ($n=18$)	RW ($n=10$)	RWO ($n=10$)
Injury	Supraspinatus tendon tear	Supraspinatus and infraspinatus tendon tears	Supraspinatus, infraspinatus, and biceps tendon tears	Supraspinatus tendon tear and repair *with* post-op analgesia	Supraspinatus tendon tear and repair *without* post-op analgesia
Gait analysis time points (days post-op)	−1, 3, 7, 14, 28, 42, 56	−1, 3, 7, 14, 28, 42, 56	−1, 3, 7, 14, 28, 42, 56	2, 4, 6, 14, 28	2, 4, 6, 14, 28

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