Neuromuscular Disorders 20 (2010) 16–20

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Kinematics of gait in Golden Retriever Muscular Dystrophy

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ARTICLE INFO

Article history:
Received 24 August 2009
Received in revised form 13 October 2009
Accepted 18 October 2009

Keywords:
Muscular dystrophy
Locomotion
Biomechanics
Canine
Physical function

ABSTRACT

The goal of this study was to quantify the two-dimensional kinematics of pathologic gait during over-ground walking at a self-selected speed at the stifle (knee) and hock (ankle) joints in six Golden Retriever Muscular Dystrophy (GRMD) dogs and six carrier littermates (controls). We found that GRMD dogs walked significantly slower than controls ($p < 0.01$). At the stifle joint, both groups displayed similar ROM (range of motion), but compared to controls, GRMD dogs walked with the stifle joint relatively more extended. At the hock joint, GRMD dogs displayed less ROM (range of motion) and walked with the joint relatively less flexed compared to controls. We controlled for gait speed in all analyses, so the differences we observed in joint kinematics between groups cannot be attributed solely to the slower walking speed of the GRMD dogs. This is the first kinematic study of gait in the GRMD dog, an important step in using this model in pre-clinical trials.

1. Introduction

Duchenne muscular dystrophy (DMD) affects approximately 1 in 3500 males born each year worldwide [1], resulting in devastating weakness, contractures, and early death. Despite over a century of research, there is no cure for this X-linked inherited disease, stemming, in part, from the lack of animal models that reflect both the genotype and phenotype of DMD patients. However, a canine model of DMD, termed Golden Retriever Muscular Dystrophy (GRMD), reflects both the genotype and phenotype of DMD. Affected dogs suffer rapidly progressive, fatal decline strikingly similar to the human condition [2–7]. Unlike the dystrophin-deficient *mdx* mouse, which exhibits a relatively mild phenotype, GRMD dogs display a severe phenotype, particularly in limb, respiratory, and heart muscles, such that by 8 months of age the majority of affected dogs manifest significant gait abnormalities.

Gait analysis in dogs has received an increasing amount of attention as a way to quantify both musculoskeletal diseases and also to assess the efficacy of clinical interventions [8]. Kinematic and kinetic analyses in dogs have been conducted using over-ground [9–15] and treadmill walking [16–20]. However, we are not aware of any published studies that have conducted a kinematic evaluation of gait in the GRMD model. Characterizing the functional phenotype of GRMD is essential if it is to be used as a clinically relevant endpoint to examine the efficacy of novel treatments of DMD, such as stem cell or gene therapy, alone or in combination with physical exercise. The goal of the present investigation was to quantify pathologic gait during over-ground walking in GRMD dogs using a two-dimensional kinematic analysis focused on the stifle and hock angles of the pelvic limb. We studied two groups: GRMD dogs and their age-matched carrier littermates heterozygous for the disease. We tested the hypothesis that GRMD dogs walk significantly slower than their carrier littermates. We also tested the hypothesis that compared to age-matched controls, stifle and hock joints demonstrate less range-of-motion (ROM) in GRMD dogs.

2. Methods

2.1. Dogs

Golden retriever cross-bred dogs (6 males, 6 females) from a GRMD colony were studied. Dogs were used and cared for according to principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals Newborn GRMD dogs were identified based on elevation of serum creatine kinase and subsequently developed characteristic clinical signs. Genotype was confirmed by polymerase chain reaction (PCR) methodology as described previously [21]. Prior to data collection, all dogs had a complete physical, orthopedic, and neurologic assessment to ensure that there were no underlying conditions that would influence gait.

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doi:10.1016/j.nmd.2009.10.007

2.2. Video collection

Prior to filming, retro-reflective markers (3 M Scotchlight, 3 M, St Louis, MO) were placed on anatomical landmarks of the right and left pelvic limbs by one of the authors (JNK). The landmarks were the greater trochanter of the femur (hip joint), a point equidistant between the lateral epicondyle of the femur and the fibular head (stifle joint), the lateral malleolus of the distal tibia (tarsal joint), and the distolateral aspect of the fifth metatarsus (metatarsophalangeal joint). Manual flexion and extension of the joints after application of the markers was used to verify that the marker positions were as close as possible to the joint centers. To facilitate marker placement, hair over the hindquarters was clipped and glue was used to fix the markers (Vetbond Tissue Adhesive, 3 M, St. Paul, MN). Following marker application, each dog was familiarized to the filming environment.

Digital video (DCR-TRV 18, Sony, Japan) of sagittal plane motion was collected at 30 Hz during over-ground walking at the dog's self-selected pace (Fig. 1) with a single camera. Gait speed was measured by timing the dog using a stopwatch over a 2.4 m distance in the sagittal plane within the camera's field of view. The filming location incorporated 3 m before and 3 m after the filming region to allow the dogs to accelerate to a constant speed with a consistent gait pattern prior to data collection and to slow down well past the filming field of view. We aligned the walking path of the dogs to the wall that created the backdrop to the filming environment. Next, tape was placed on the floor to define the distance that was used to time the gait speed and the plane of the path of progression. If the dog deviated from this plane the trial was not used for analysis. We used a bubble level to level the camera so that the axis of the lens was horizontal. The camera was positioned 8 m from the plane of progression with the axis of the lens perpendicular to the plane, and zoomed to capture the entire walking field (2.4 m) within the camera field of view.

During filming the dogs were led on a leash by the same experienced handler. Data were collected on both sides of each dog. A record was maintained regarding the quality of each gait pass ranked by three observers (APM, JMK, MKC). Due to the clinical signs of GRMD (weakness, lethargy, shortness of breath), affected dogs were encouraged with praise, an occasional leash tug, and a food reward to coax them through the filming area at their self-selected pace. Because brief periods of activity were challenging for GRMD dogs, rest was provided as needed. This procedure was repeated for age-matched carrier controls.

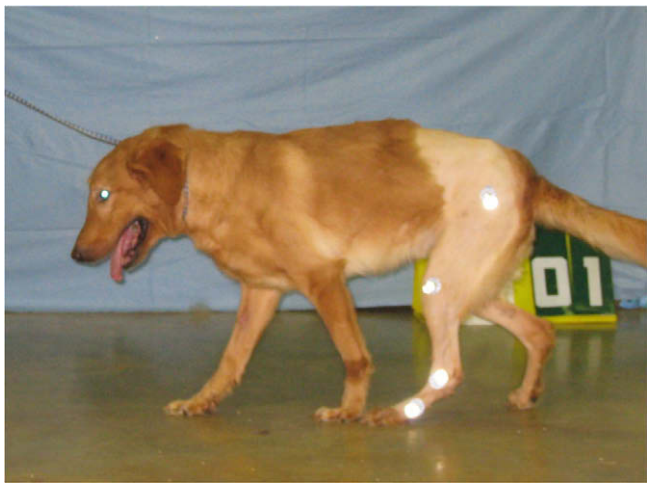

Fig. 1. Filming environment showing joint marker placement on a GRMD dog.

2.3. Video processing

Trials that represented a consistent, self-paced gait within the calibrated plane as identified by three of the investigators were selected for digitizing. Trials where dogs increased or decreased speed, stopped, or walked out of the calibrated sagittal plane were excluded from analysis. Three to four complete gait cycles were visible on each pass. A complete gait cycle was defined as starting at initial pelvic limb ground contact and ending with ipsilateral pelvic limb ground contact. Two trials for the right and left side of each dog were identified for further analysis.

Digitizing software (VideoExpert, Motion Analysis Corp., Santa Rosa, CA) and MATLAB (The MathWorks, Natick, MA) programs were used to calculate the joint angles of the stifle (knee) and hock (ankle). The three points used to operationally define the stifle joint were the centers of the reflective markers, which were placed on the three following landmarks: the greater trochanter of the femur (hip joint), a point equidistant between the lateral epicondyle of the femur and the fibular head (stifle joint), and the lateral malleolus of the distal tibia (tarsal joint). The three points used to operationally define the hock joint were the centers of the reflective markers placed at a point equidistant between the lateral epicondyle of the femur and the fibular head (stifle joint), the lateral malleolus of the distal tibia (tarsal joint), and the distolateral aspect of the fifth metatarsus (metatarsophalangeal joint). Raw x–y co-ordinate data were filtered using a Butterworth filter with a cutoff frequency of 4 Hz. Each gait cycle was normalized in time (100% of gait cycle) with complete cycles averaged together for each side of the body. Maxima and minima for each complete cycle were recorded and joint range-of-motion (ROM) calculated for each joint.

2.4. Statistical analysis

Statistical software (SPSS 16; SPSS Inc., Chicago, IL) was used for all analyses. Given the exploratory nature of this study, $p < 0.1$ was considered significant. Descriptive statistics (mean ± SD) were calculated for all variables. A three way repeated measures analysis of variance (RMANOVA) with one between factor (group: GRMD, carrier) and two within group factors (trial: 1, 2; side: right, left) was used to examine gait speed data. Fisher Least Significant Difference post hoc testing was used to determine if significant differences existed between trials or between the right and left sides. A three factor analysis of covariance (ANCOVA), using gait speed as the covariate, was used to examine the minimum and maximum angles, and ROM, at the stifle and hock joints.

3. Results

3.1. Subject demographics

The average age, shoulder height and body mass of dogs was 25.9 ± 8.8 months, 47.3 ± 3.5 cm; and 18.7 ± 3.1 kg, respectively (Table 1).

3.2. Gait speed

GRMD dogs walked significantly ($p < 0.01$) slower than their carrier littermates (1.18 ± 0.34 and 1.80 ± 0.49 m/s, respectively). There were no significant 3-way or 2-way interactions (3-way RMANOVA). There were no detectable differences with respect to trial or side. That is, gait speeds of trials from both the right and left sides were not different. The mean within dog coefficient of variation (CV) for gait speed was 5.1% and 5.5% for the affected and unaffected dogs, respectively. Therefore, gait speed was averaged and subsequently used in the ANCOVA analyses presented below.