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Measuring Tibiotarsal and Pelvic Joint Angles as Surrogate Biomarkers for Contractures and Postural Instability in GRMD

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Author	Joe Kornegay College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX USA
Working group members	Robert Grange (Virginia Tech, Department of Human Nutrition, Foods and Exercise, Blacksburg, VA, USA) George Carlson (Department of Physiology, A.T. Still University, Kirksville, USA) Stephane Blot (Veterinary School of Medicine, Maison Alfort, France) Margot Coville (INSERM, Nantes)
SOP responsible	Joe Kornegay
Official reviewer	Stephane Blot

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1. OBJECTIVE

Most DMD natural history studies have included measurements of muscle strength, joint contractures, and timed function tests. Results from these tests are used to track disease progression and offer insight on clinical milestones, such as the loss of ambulation and the need for ventilatory support. Both muscle weakness and joint contractures contribute to postural instability and ultimate loss of ambulation.[1] Joint angles can be objectively measured to determine the severity of contractures. Phenotypic features of postural instability and contractures in DMD and GRMD, together with underlying mechanisms, have been reviewed by the author.[2] Some comments made here were excerpted from this review.

2. SCOPE AND APPLICABILITY

Contracture and muscle strength scores for DMD usually correlate and deteriorate synchronously over time.[3] Contractures, generally speaking, are caused by inactivity and restricted motion of the affected joint,[4,5] with a subsequent increase of collagen cross-links in periarticular connective tissue.[6] Joint contractures occur more commonly in DMD than other neuromuscular diseases[7] and have long been recognized as a major factor in disease morbidity. Some studies have concluded that muscular imbalance contributes to contractures,[3,8-10] while another found no such association.[11] Those finding a relationship noted a strong negative correlation between extensor muscle weakness and flexor contracture severity in DMD. As opposing extensor muscles weakened, flexor contractures worsened.

GRMD dogs with a severe phenotype develop a characteristic plantigrade stance between 3 and 6 months, as evidenced by hyperextension of the carpus and hyperflexion of the tarsus.[12,13] Consistent with this posture, we previously reported that 6-month-old GRMD dogs positioned in dorsal recumbency for force measurements have abnormally acute (contracted) *tibiotarsal joint (TTJ) angles*.[13,14] As with DMD, these joint angle changes are associated with muscular imbalance. Dogs with weak extension and strong flexion force values tend have more severe TTJ flexor contractures. Subsequent to these early studies, we have continued to use TTJ angle as a general surrogate for broader postural changes. Concomitant with distal joint changes, the pelvic limbs shift forward, as the tuber ischium of the pelvis moves ventrally and cranially, reaching essentially a vertical plane, perpendicular to the walking surface, in severe cases.[15] To objectively characterize the pelvic vertical tilt, we also measure an angle formed by two lines extending cranially from the tuber ischium, one drawn parallel to the lumbar spine and the other extending to the midpoint of the tuber coxae. This *pelvic angle* is larger in GRMD vs. normal dogs at 6 months of age.

3. **CAUTIONS**

Dogs must be anesthetized. Otherwise, the only equipment required is a goniometer. See METHODS and EVALUATION AND INTERPRETATION OF RESULTS (below) for guidance on interpretation.

4. MATERIALS

Tibiotarsal Joint (TTJ) Angle. Dogs are anesthetized and positioned in dorsal recumbency.[13] Tibiotarsal joint angles generally are measured in dogs just prior to force measurements (see separate GRMD protocol). The pelvic limb is held so that the hip (coxofemoral) and knee (stifle) joints each form a 90° angle. The distal limb is supported with a finger placed just below the point of the TTJ (hock). The tibia should be parallel to the table. A goniometer is held so that the pivot point is centered over the lateral malleolus of the fibula, with one arm of the goniometer extending along the axis of the tibia/fibula and the other along the metatarsus (**Figure 1**).

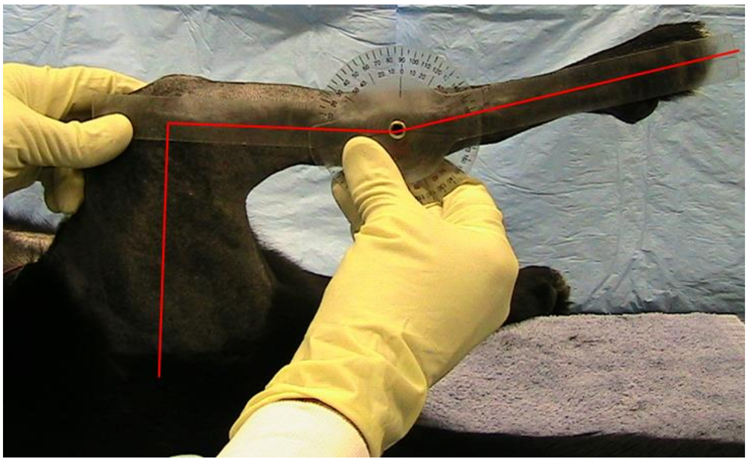

Figure 1. Placement of the goniometer to measure the tibiotarsal joint angle. The pivot point is centered over the lateral malleolus of the fibula. One arm extends along the axis of the tibia/fibula, with the other along the metatarsus. The angle here is approximately 165°.

Pelvic Angle. Dogs are anesthetized and positioned in lateral recumbence (Figure 2 A), with the line of the spine and pelvic limb at a right angle to one another. A goniometer is held so that the pivot point is centered over the tuber ischium, with one arm of the goniometer extending parallel to the spine and the other to the midpoint of the tuber coxae (**Figure 2A**). GRMD dogs with a more prominent cranioventral pelvic shift (and associated greater postural instability) have a larger (more obtuse) angle **(Figure 2B)**.

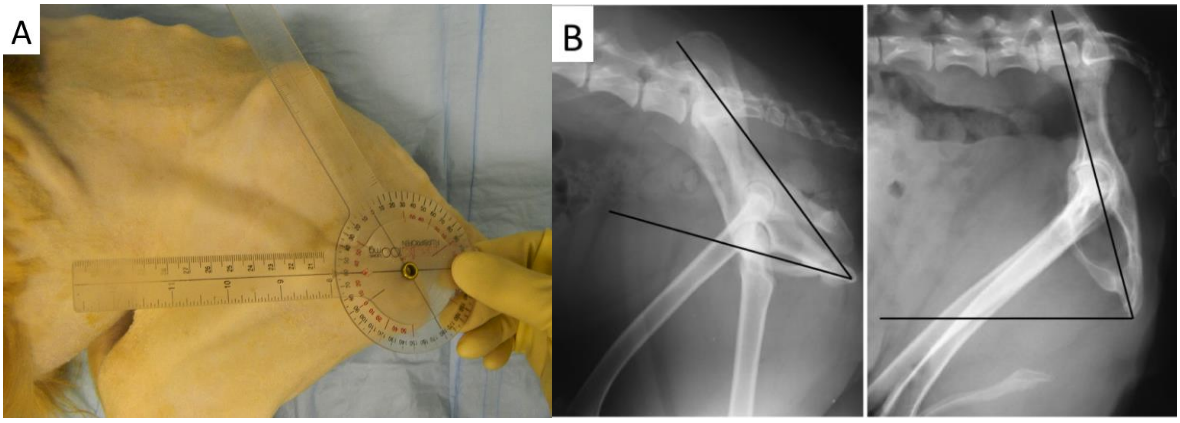

Figure 2. Placement of the goniometer to measure the pelvic angle. A. Dog positioned in dorsal recumbency and B. Radiographs of normal (left) and GRMD (right) dogs. The pivot point is centered over the tuber ischium of the ischium of the pelvis. One arm extends to the midpoint of the tuber coxae, with the other parallel to the line of the spine. The angle in A is approximately 60^{o}. Note on the normal radiograph that the line is tilted upwards so as to be parallel to the line of the spine.

5. METHODS

5.1. Anesthetic protocol (Note, in a preliminary study, mean alveolar concentration [MAC] values for isoflurane did not significantly affect force measurement values [Schueler RO, Koch J, Kornegay JN, unpublished data]).

20-30 minutes prior to anesthesia induction:

- Pre-anesthetic agents:
  - Atropine sulfate (0.04 mg/kg, IM)
  - Acepromazine maleate (0.02 mg/kg, IM) for dogs weighing greater than 5 kg
  - Butorphanol tartrate (0.4 mg/kg, IM)

Anesthetic monitoring:

During anesthesia, ECG, heart and respiratory rate, blood pressure, end tidal (Et)CO_2, and saturation of hemoglobin by peripheral oxygen (SpO_2) are monitored continuously with a pulse oximeter (Cardell Multiparameter Monitor 9405, Minrad International, Inc, Orchard Park, NY). These values, as well as capillary refill time and anesthetic setting, are recorded every 15 minutes.

Anesthetic induction:

- Anesthetic agents:
  - Propofol (up to 3 mg/kg, IV – slowly!) for dogs weighing greater than 5 kg
  - Isoflurane or sevoflurane (to effect, inhaled) (avoid masking down)

Anesthetic recovery:

Monitor affected and carrier dogs <u>closely</u> during anesthetic recovery until fully awake and in sternal recumbency.

- Naloxone (up to 0.4 mg/kg, SQ) for rapid recovery; given in ½ dose increments (1st dose given while the dog is still intubated and breathing O_2; 2nd dose, if necessary, after extubated and/or if respiration drops below 7 breaths per minute).

5.2.A. *Tibiotarsal Joint Angle*. Hold the pelvic limb so that the hip (coxofemoral) and knee (stifle) joints each form a 90° angle. The distal limb is supported with a finger placed just below the point of the TTJ (hock). The tibia should be parallel to the table. A goniometer is held so that the pivot point is centered over the lateral malleolus of the fibula, with one arm of the goniometer extending along the axis of the tibia/fibula and the other along the metatarsus **(Figure 1**). Note, the paw tends to deviate dorsally from the line of the metatarsus. The arm of the goniometer should be placed along the metatarsus and not aligned with the end of the paw.

5.2.B. *Pelvic Angle*. Position the dog in lateral recumbency **(Figure 2 A)**, with the line of the spine and pelvic limb at a right angle to one another. A goniometer is held so that the pivot point is centered over the tuber ischium, with one arm of the goniometer extending parallel to the spine and the other to the midpoint of the tuber coxae. GRMD dogs with a more prominent cranioventral pelvic shift (and associated greater postural instability) have a larger (more obtuse) angle **(Figure 2B)**.

5.3. Record the joint angle.

5.4. Repeat the measurement on the other limb.

6. EVALUATION AND INTERPRETATION OF RESULTS

Measurements of TTJ and pelvic angles are intended to serve as surrogates for broader joint contracture and postural changes in GRMD. Postural instability and leg contractures ultimately cause DMD patients to lose their ability to walk so are of special interest. The relative sequence and proportional involvement of flexor and extensor muscles is critical to the development of these changes. Early weakness of the hip (gluteus maximus) and knee (quadriceps femoris) extensors necessitates postural changes to maintain ambulation. Increased anterior pelvic tilt and lumbar lordosis are adopted to shift the center of gravity forward of the knee and behind the hip, respectively.[16] Relative preservation of hip (including the sartorius) and knee (hamstrings) flexors in DMD creates destabilizing torque forces and also contributes to contractures at both levels.[17] Toe walking is adopted to stabilize the knee and later plays a role in the development of ankle equinus.[18] Plantar flexor contractures associated with equinus are aggravated by unbalanced muscle activity at the ankle, with selective weakening of the anterior tibialis and peroneus longus muscles and relative sparing of the triceps surae (collectively, the two heads of the gastrocnemius and soleus).[3,18] These contractures may initially have beneficial effects, as tension in the gastrocnemius muscles pulls on the femoral condyles, extending the knee.[19] Iliotibial band (hip) contractures also extend the knee, providing additional stability. However, in advanced stages, heel cord and iliotibial band tightening destabilize gait, prompting the development of various corrective surgical procedures.[20,21]

Tibiotarsal joint angle was measured previously in GRMD dogs in the context of serial peroneus longus force measurements.[13,22] In that study, single pins were transversely placed proximally and distally in the tibia and were secured to metal rods mounted on a heavy plastic base. Dogs were positioned so that the tibia was parallel to the table and perpendicular to the femur. We noticed that the angle formed by the TTJ often was more acute in affected dogs. Initially, the angle formed by the flexor surface of the tarsus was traced on a transparency and then measured by use of a protractor. Tibiotarsal joint angles of GRMD and normal dogs measured using this method were not statistically different at 3 months of age.[13] However, by 6 months, GRMD angles were more acute, in keeping with flexor contractures seen when dogs are standing or walking **(Figure 3)**. An earlier paper found similar values for this angle in standing dogs.[23] Consistent with phenotypic variation or disease stabilization, older, generally mildly-affected dogs, assessed by video gait analysis, had a more upright stance, with relatively greater extension of the stifle and lesser flexion of the tarsus.[24] This posture presumably is adopted in an effort to stabilize their stance in the face of quadriceps weakness. Since our report of TTJ contractures in GRMD, others have described methods to measure tibiotarsal and other joint angles at maximal flexion and extension in normal dogs.[25,26] We now measure all pelvic limb angles based on the method of Jaegger et al[25] to gain a complete assessment.

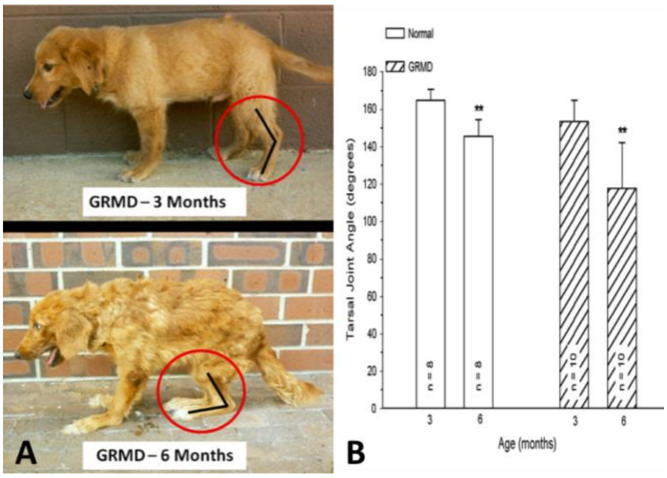

Figure 3. Tibiotarsal (Hock) Angles. Affected dog with flexor contractures at 3 (top) and 6 (bottom) months of age (A). GRMD values are similar to those of normal dogs at 3 months and more acute at 6 months (B). Note, angles in awake standing dogs, as shown in A, are more acute than those of anesthetized dogs in dorsal recumbence represented by the histograms in B. Figure A is from reference 13.

Tibiotarsal joint angles for normal dogs using our method approximate but are somewhat less than those recorded at maximal extension. The pelvic angle described here is also measured to provide an additional marker of postural instability. GRMD dogs have larger (more obtuse) hip and pelvic angles, presumably as a function of the craniopelvic shift. Hip angles and the pelvic angle appear to most effectively demonstrate treatment effect,[27,28] potentially because making these measurements is relatively straight forward and these angles better differentiate GRMD and normal dogs. These angles also correlate more strongly with additional biomarkers, such as MRI and functional measures[28,29] Others have measured joint angles and associated range of motion over time in GRMD dogs.[30]

Mechanisms contributing to postural and joint angle changes in GRMD dogs have not been defined. The cranioventral pelvic shift may be an adaptive response, as affected dogs move their pelvic limbs under the torso to maintain balance. The resultant posture is similar to that achieved by DMD boys when they shift their pelvis forward.[16] Alternatively, unbalanced hip flexor and extensor strength might play a role, with relative preservation of the hamstring muscles in GRMD dogs causing the tuber ischium to be pulled ventrally. As discussed above, considering the role that the sartorius and iliotibial band play in hip flexor contractures in DMD,[21] hypertrophy of the cranial sartorius could be playing an analogous role in GRMD. In support of a relationship, we have previously shown that body-weight-corrected cranial sartorius muscle weight and circumference correlate negatively with tarsal joint angle.[31,32] However, it's unclear whether there is truly a cause-and-effect relationship. The hypertrophied cranial sartorius could actively pull the stifle joint forward, with the tarsus passively following to assume a plantigrade position. On the other hand, cranial sartorius hypertrophy and plantigrade stance could have common root causes but no direct functional relationship. In any case, cranial sartorius hypertrophy or contracture does appear to affect the developing pelvis in young GRMD dogs, as the ilial wings from which it originates flare laterally,[15] presumably in response to unopposed torque. This is somewhat analogous to scoliosis resulting from unbalanced force applied by the dominant arm in DMD boys[33] and emphasizes the potential for disproportionate muscle size and strength to cause skeletal deformity. Local imbalance of agonist and antagonist muscles could also be playing a role in postural changes at the tarsal and carpal joints of GRMD dogs. Consistent with findings in DMD, we have shown that GRMD extensor and flexor muscles operating at the tarsal joint are differentially affected. Flexion values are especially low at 3 months, whereas extension is affected more at later ages.[14] At six months of age, the tarsal extension:flexion force ratio correlates positively with tarsal joint angle, which is to say that dogs with stronger extensors have larger joint angles and a less severe phenotype.[32]

Potential Advantages/Disadvantages of the Methodology

Advantages

Measurement of tibiotarsal and pelvic joint angles is simple and provides insight on the nature of contractures and postural instability. Tibiotarsal joint angle correlates with TTJ force, suggesting that it should accurately predict overall disease progression.

Disadvantages

There is the potential for variable placement of the goniometer by different examiners. Tibiotarsal joint angles of normal and GRMD dogs do not differ at 3 months of age. Thus, studies must be extended beyond this time point for them to be useful in demonstrating treatment benefit. While tibiotarsal and pelvic angles provide insight on broader postural and joint contractures in GRMD, other angles must be measured to gain a comprehensive assessment.

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