Characterization of Gait Kinematics and Muscle Function in Becker Muscular Dystrophy Pigs: a pilot study

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Abstract:

Vertebrate animal models of Becker muscular dystrophy (BMD) have been developed. Here, we characterized the gait kinematics and muscle function of a naturally occurring BMD pig model of dystrophin insufficiency. BMD pigs tended to have alterations in hip range of motion (ROM): hip (67%, 95% CI -0.64 to 14.12 degrees). While parameters were unaltered in extensor muscles, the dystrophin levels in flexor tibiotarsal joint muscles correlated with fatigue index as well as reduced isometric force (48%, 95% CI -1.86 to -0.61 N-m), and a 33% increase in fatigue index (95% CI -36.25 to 96.71 percent); the extensor muscles had no observable reductions in muscle force, with a 48% increase in fatigue index (95% CI -232.6 to 472.6 percent). Histological analysis of muscle biopsies supported a BMD phenotype in the flexor muscles of BMD pigs, with a 75% (95% CI -55.14 to -15.66 percent) decrease in large and a 43% (95% CI 17.74 to 57.38 percent) increase in small muscle fiber cross-sectional area. Dystrophin protein abundance was 28% less in flexor muscles from BMD pigs (95% CI -49.63 to 11.41 arbitrary units). Together, our model may serve as a clinically relevant model of BMD to assess safety and efficacy of therapeutics.

Key Words

Porcine, Skeletal Muscle Regeneration, Gait Analysis, Preclinical Model, Duchenne muscular dystrophy, Large animal model.

Introduction

Becker muscular dystrophy (BMD) describes a spectrum of rare X-linked genetic disease caused by mutations in the *DMD* gene, resulting in the production of a truncated and/or insufficient accumulation of functional dystrophin protein for 1 in 18,000 male births (Gorgoglione et al., 2025; Magot et al., 2023; Malhotra et al., 1988). As the milder allelic variant of Duchenne muscular dystrophy (DMD), BMD typically presents later in childhood/adolescence, progressing slower with reduced severity, though symptoms are widely heterogenous among affected populations (Le Rumeur, Winder, & Hubert, 2010; Mercuri, Bönnemann, & Muntoni, 2019). Common phenotypes of BMD patients include progressive muscle weakness and degeneration of the pelvic and shoulder girdle, gait abnormalities, and an increased risk of cardiomyopathy (Flanigan, 2014; Salari et al., 2022). Despite the clinical significance, BMD remains poorly understood compared to DMD, with limited therapeutic development and fewer dedicated clinical studies (Straub & Guglieri, 2023). Given the prominence of gene and exon skipping therapies aimed to lessen the severity of DMD to a Becker-like phenotype (Duan, 2018; Straub & Guglieri, 2023), there is a need to develop translatable preclinical models of BMD.

Several DMD animal models have been developed for therapeutic testing, including multiple murine models, rodent, dogs, and pigs, but only recently was a murine model characterized for BMD (Blasi et al., 2025; Wilson et al., 2017). A porcine model of BMD would possess several advantages over rodent and dog models as their anatomy, physiology, and genome more similarly mimic those of humans (Hollinger et al., 2014; Stirm, Klymiuk, Nagashima, Kupatt, & Wolf, 2024).

Our group has characterized the *DMD* gene in-frame mutation of a naturally occurring BMD porcine model that appears to be caused by a nucleotide change in Exon 41, which disrupts the amino acid sequence resulting in an insufficient accumulation of the dystrophin protein (Hollinger et al., 2014; Nonneman, Brown-Brandl, Jones, Wiedmann, & Rohrer, 2012; Selsby,

Ross, Nonneman, & Hollinger, 2015). At 12 months of age, these BMD pigs had reduced dystrophin expression in the diaphragm (70% reduction), the longissimus (90% reduction), and the psoas (45% reduction) compared to healthy littermates, as well as varied muscle histopathology (Hollinger et al., 2014; Selsby et al., 2015). Furthermore, preliminary investigations in step length and step time demonstrated gait abnormalities indicative of disease-related compensation and elevated creatine kinase (CK) levels (Selsby et al., 2015). We also observed cardiomyopathy and electrocardiogram abnormalities (Selsby et al., 2015). A heterogenous phenotype is observed across investigated skeletal muscles, likely arising from differences in the quadruped gait cycle. The initial, limited characterization of this model suggests some similar disease manifestations as human BMD patients, yet further investigations are required to characterize this model as an effective large animal, preclinical model for therapeutic safety and efficacy testing prior to human clinical trials.

The purpose of this study was to further investigate characteristics of a naturally occurring BMD porcine model of dystrophin insufficiency. We assessed hindlimb gait kinematics, hindlimb muscle function, histopathology, and blood chemistry in Healthy and BMD (Affected) NIH minipigs, 8-10 months of age. Herein, we report further abnormalities in the BMD pig hindlimb muscles, providing additional insight into the pathology of a large animal, preclinical model of BMD.

Materials and Methods

Ethical Statement

All protocols in this study were approved by Animal Care and Use Committees at Texas A&M University (AUP 2023-0025). Animal care was in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011)*. Animals were coded prior to experimentation and data analysis was conducted by blind investigators.

Animals

Male and Female NIH minipigs (Healthy, n=2 and Affected, n=5) were bred at Iowa State University and transported to Texas A&M University and maintained in approved kennels. Boars were single housed to prevent fighting but were able to visually see other animals in the bay. Animal numbers were limited by age and availability.

Surgical Muscle Biopsy

Animals were sedated with butorphanol, masked, and maintained with inhalant isoflurane maintained at 2% unless an increase was required up to 4% and 100% oxygen. Following blood collection, the surgical site was aseptically prepped. A 3-4 cm incision was made in the skin and a 1x1x1cm open muscle biopsy was taken from the vastus lateralis, biceps femoris, cranial tibial (flexor), and lateral head of the gastrocnemius (extensor). The incision was closed with 2-0 Vicryl, the animal recovered from anesthesia and returned to its home cage with post operative monitoring and analgesic as needed.

Isometric muscle testing

Methods were adapted from our internationally established canine protocols and modified based on published torque measures in pigs (Blasi et al., 2025; Corona, Rivera, Dalske, Wenke, & Greising, 2020). Animals were placed under general anesthesia as indicated above. An Aurora Scientific, custom large animal testing machine was used for analysis (model # 701C for high phase stimulator; model # 892A-HD for the custom muscle testing rig, Aurora, Ontario, CA,). A pelvic limb was secured to a leg brace and the hooves/tarsus secured to a foot pedal with Vet Wrap (Marietta, GA). The foot pedal was connected to a transducer (Model # 492A-AMP). Percutaneous needle electrodes were inserted around the fibular nerve to stimulate (100 Hz, 0.1ms pulse, over 800 millisecond) the cranial tibial compartment to flex (pull the foot pedal) the tibiotarsal joint (90 degrees). The same stimulation was applied to the tibial nerve to stimulate the

gastrocnemius and digital flexors to extend (push the foot pedal) the tibiotarsal joint (90 degrees). Twitch and tetanic contractions were performed in each muscle group to evaluate torque. All assessments were performed in flexion before moving to extension or vice versa. Due to limitations in the hardware and software, eccentric contraction decrements could not be made. A fatigue protocol was developed that delivered one tetanic contraction (100 Hz) per second for 60 replicates at 800 ms train durations with 1 second in between each replicate to induce fatigue in each muscle group. Data were recorded using DMA Software for 615A model number. Area under the curve (AUC) of fatigue replicates was analyzed between groups. Fatigue index (FI) was calculated as : FI = [(initial force – final force)/initial force) x 100].

Gait Analysis

Beginning at 4-5 months of age, pigs underwent 2-3 months of acclimation training on a treadmill at a speed of 1 m/sec prior to motion capture and video recording. A custom treadmill with enclosed sides was used for both training and testing. The right side of the treadmill featured a transparent plexiglass panel, enabling the capture of gait kinematics. Due to the equipment set-up, the treadmill had a constant incline of 3 degrees during all walking trials. A digital video camera (GoPro 11 Black, GoPro, Inc., San Mateo, CA, U.S.) was positioned perpendicular to the treadmill to record the two-dimensional kinematics of the pigs' gait (**Figure 1**). The camera was 1.4 meters from the treadmill at a height that centered the treadmill within the frame. To minimize interlacing and lens distortion, video recording was captured at 60 frames per second with 4K resolution, using the "Linear + Horizon Lock" setting on GoPro. Two additional lights were positioned near the treadmill at a 45-degree angle to improve visibility of the pig's hind legs (**Figure 1**). A yardstick was placed on the treadmill parallel to the belt for image calibration.

Pigs' right pelvic limb and pelvis were modeled into four distinct segments: pelvis, femur, shank, and foot using five anatomical markers placed on tuber coxae, greater trochanter, knee joint, tibiotarsal joint, and metatarsophalangeal joint. Specifically, pelvis segment was defined by

markers on the tuber coxae and greater trochanter; femur was defined by markers on the greater trochanter and knee joint; shank segment was defined by markers on the knee joint and tibiotarsal joint; and the foot segment by markers on the tibiotarsal joint, and metatarsophalangeal joint. One day before data collection, a veterinarian palpated these anatomical landmarks and outlined each landmark by drawing a one-inch diameter circle using a black Sharpie. Hair around these areas was shaved to enhance marker visibility.

Procedure

Gait data collection was conducted between 9:00 to 11:00 am. Pigs were transported individually via enclosed cart from their housing units to the treadmill located on the same hallway. The animal handler ensured that the pig was positioned at the center of the treadmill before gradually increasing the speed of the belt to 1 m/sec. Once a stable walking pattern was observed, a research technician initiated video recording. Each pig was recorded for one minute, yielding more than 60 steps for gait analysis. After recording, the pig was returned to its housing unit in an adjacent room.

Data Analysis

Raw video recordings were exported from the GoPro camera and imported into Kinovea (v. 2023.1, Kinovea.org) for two-dimensional coordinate data extraction. Video image calibration was performed using a yardstick placed on the treadmill. A coordinate frame was established by placing the origin at the bottom left corner of the treadmill in the video. The coordinates of all five markers were extracted relative to this origin.

Marker positions were determined using a semi-automatic tracking approach. In the first video frame, a square area containing the marker was selected as a reference feature for Kinovea's point tracking tool. This tool identified marker positions in subsequent frames by comparing pixel similarity across frames. After automatic tracking, a research technician visually

inspected and manually corrected any tracking deviations. A second research technician independently reviewed the tracking results to ensure accuracy and consistency. For each recording, two-dimensional marker position time series data of the five markers were exported to Matlab (v. 2024a, MathWorks, Natick, MA) for further kinematic analysis.

Steps were classified as non-walking steps when irregular walking movements, such as kicking or jumping, were identified through comparison with the video recordings, and removed. For all remaining steps, comprehensive gait characteristics were quantified using spatiotemporal parameters and joint angle measurements. Spatiotemporal parameters included step time, stance time, swing time, swing and stance phase percentages within a step cycle, and step length.

Stride was defined as described (Boakye et al., 2020): a stride was the interval between two consecutive hoof contacts with the same hoof; the stance phase was between initial hoof contact to hoof lift-off; and the swing phase extended from hoof lift-off to the next hoof contact. Joint angles were computed based on vectors formed by two adjacent limb segments. To enable step-to-step comparisons, joint angle data were normalized for time to 100 data points per step. All joint angle plots were visually inspected to exclude non-walking steps from the final analysis. Joint angle measurements exported included maximum, minimum, and range of motion (ROM) for the hip, knee, and ankle joints.

Standard deviations from each gait analysis parameter were converted to a percentage of the healthy group and then collectively pooled into healthy and affected groups to draw conclusions around gait consistency.

Cryosectioning, Hematoxylin and Eosin Staining, and Light Microscopic Imaging

Cranial tibial and gastrocnemius muscle samples were taken by biopsy from BMD and wild-type pigs, immediately frozen in isopentane cooled in liquid nitrogen, and stored at -80ºC. Frozen skeletal muscle samples were mounted in Tissue-Plus™ O.C.T. Compound (Scigen, Fischer Scientific, Hampton, NJ, USA) and sectioned (thickness, 10 μm) in a Cryostar NX50

Cryostat (Epredia, Kalamazoo, MI, USA) at -17ºC onto a microscope slide, followed by H&E staining (SelecTech H&E Staining System, Leica Biosystems, Deer Park, IL, USA). Tissues were stained in Hematoxylin for 30 seconds, washed in tap water, differentiated in 0.3% acetic acid for 30 seconds, blueing agent for 1 minute and 30 seconds, then washed again in tap water. Sections were then washed with ethanol at 70%, 95%, 95% for 1 minute each, stained with Eosin for 2 minutes, and washed again with 95% and 100% ethanol. The sections were cleared in xylene and mounted using Permount™ Mounting Medium (Electron Microscopy Sciences, Hatfield, PA, USA). Images were captured with a 10x (N.A., 0.50) objective using a Zeiss Axioplan upright microscope equipped with a Zeiss Axiocam HRc brightfield microscope camera (Zeiss Microscopy, White Plains NY, USA). Myofiber cross-sectional area (CSA) was analyzed from approximately 350 fibers per muscle using NIH ImageJ (RRID: SCR_003070, NIH, Bethesda, MD, USA) as described (A. B. Morton et al., 2018).

Creatine Kinase and Biochemical Analysis

Creatine kinase and analytes (Total Protein, Albumin, Calcium, Phosphorus, Glucose, BUN, Creatinine, Bilirubin, ALKP, AST, ALT, Globulins, Albumin: Globulin ratio, Sodium, Potassium, Chloride, Sodium: Potassium ratio) were determined on a blood chemistry panel via colorimetric assays, ion selective electrodes (ISE) (Na, K, and Cl), and calculations (globulins, A:G ratio, Na:K ratio) on a Beckman Coulter DxC 700 AU analyzer through an AAVLD-accredited laboratory.

Western Blot Analysis

Cranial tibial and gastrocnemius biopsy samples from BMD and wild-type porcine were homogenized in lysis buffer (pH 6.8) containing 75 mM Tris-HCl, 10% SDS, 10 mM EDTA, and 5% 2-mercaptoethanol as described (Fiorillo et al., 2015). Homogenates were centrifuged at 10,000 *g* for 10 minutes at 4 °C. The supernatant (soluble fraction) was aspirated, and its protein

concentration assessed using the Bradford method (Sigma-Aldrich, St. Louis, MO, USA). Protein samples (50 µg) were blotted and analyzed using Image Studio Lite as described (A. Morton et al., 2025). Primary antibody used is a 1:1 mix of DYS-1 (1:1000, RRID: AB_442080) + DYS-2 (1:1000, RRID: AB_442081) monoclonal antibodies against dystrophin (Leica Biosystems, Deer Park, IL, USA). Membrane was exposed to IRDye 800CW Goat anti-Mouse IgG (1:5000, RRID: AB_621842, Cat.# 926-32210) (Li-Cor Biotechnology, Lincoln, NE, USA) and scanned with a Li-Cor Odyssey DLx Imager (Li-Cor Biotechnology, Lincoln, NE, US). Blots were normalized to total protein (A.U.) using Revert total protein stain (Cat.# 926-11011, Li-Cor Biotechnology, Lincoln, NE, USA). Results reported as percent of Healthy.

Analysis

Summary data are reported as percent change and 95% CI from non-parametric T-Tests. Given limited numbers of healthy pig littermates, correlations were generated combining all 7 pigs using JASP (University of Amsterdam, Amsterdam, The Netherlands) and plotting individual outcome measures from each pig against Dystrophin 1 & 2 expression from both flexor and extensor muscles before analyzing respective slopes for deviation from 0. $P \leq 0.05$ was considered statistically significant.

Results

General Measures

Gait was 30% less consistent in Affected pigs over Healthy (95% CI 3.68 to 54.4) **(Figure 2A)** while body weights at 9 months of age were similar between Healthy and Affected pigs **(Figure 2B),** means 50.6 kg (95% CI 49.9 to 51.3) and 50 kg (95% CI 42.2 to 57.2) respectively, (n=2-5/group).

Gait Analysis

Gait results demonstrated alterations to spatiotemporal and joint angle kinematics in Affected pigs compared to Healthy **(Figure 3)**. Spatiotemporal analysis of the pig gait cycles revealed swing time was decreased 12% (95% CI -0.08 to 0.01), stance time was decreased 17% (95% CI -0.16 to 0.01), and step time was decreased 15% (95% CI -0.24 to 0.01) in Affected pigs compared to Healthy **(Table 1)**. Notably, joint angle analysis demonstrated that Hip ROM and SD were increased by 40% (CI -0.64 to 14.12) and 50% (CI -0.03 to 1.67), respectively, in Affected compared to Healthy pigs **(Figure 3A)**, whereas Knee (8%, CI -17.14 to 12.13) **(Figure 3B)** and Ankle (11%, CI -15.88 to 8.46) ROM declined slightly in Affected compared to Healthy, respectively **(Figure 3C)**.

In Vivo Muscle Function

At 9 months of age (n=2-5), flexors in Affected pigs had reduced absolute maximum torque compared to Healthy pigs (**Figure 4A**, 48%, 95% CI -1.86 to -0.61). Fatigue curves for Affected appeared more linear and reduced compared to Healthy (**Figure 4B**). Area under the curve for flexor torque was 58% lower (95% CI -55.56 to -20.51) (**Figure 4C**). In addition, the fatigue index was 33% greater (95% CI -36.25 to 96.71) in Affected vs. Healthy (**Figure 4D**). For extensor muscles, maximum torque and fatigue metrics were similar between groups (**Figure 4 E-H**).

Muscle Fiber Histopathology

Muscle fiber CSA was determined in cranial tibial (flexor) and gastrocnemius (extensor) muscles (**Figure 5**). CSA was partitioned into 2000 μm^2 increments, creating relative frequency histogram plots for Healthy and Affected pigs (**Figure 5A**). Flexor muscles of BMD pigs shifted the CSA frequency curve to the left (**Figure 5A, B**). There were 43% (95% CI 17.74 to 57.38) more small fibers (≤6000 μm^2) and a 75% (95% CI -55.14 to -15.66) reduction in large fibers

(≥8000 μm^2) (**Figure 5C, D**). However, extensor muscle fiber CSAs appeared similar across fiber sizes (**Figure 5E-H**).

Dystrophin Expression

Relative dystrophin protein decreased by 34% (95% CI -87.9 to 20.2) in cranial tibial muscles (flexors) and 37%, with significant variability, (95% CI -138.1 to 62.73) in gastrocnemius (extensors) of Affected pigs versus Healthy (**Figure 6A, B,** n=2-5/group).

Correlation of Dystrophin Expression to Functional Measures

Linear regression was used to discern whether there was a correlation between relative dystrophin protein abundance from flexor and extensor muscles of Healthy and Affected pigs to markers of muscle function (**Figure 7, Supplementary Figure 1-3**) (n=2-5). Flexor torque was significantly correlated with dystrophin expression (**Figure 7A**. P=.049) while fatigue index was inversely correlated with relative dystrophin abundance in flexor muscles (**Figure 7B.** P =.013) but not extensor muscles (**Figure 7D-F**).

Blood Panel Analysis

A comprehensive blood chemistry panel revealed that CK concentration was increased by 250%, (95% CI -215 to 1973) in Affected pigs compared to Healthy pigs. All other measured circulating factors were similar between groups (**Table 2,** n=3-5).

Discussion

In this pilot study, we present a phenotypic characterization of a naturally occurring porcine model of BMD, focusing specifically on hindlimb skeletal muscles and gait analysis. This porcine

model may fill a critical gap in translational research as large animal models of BMD are limited. These findings build upon our group's previous work, investigating the functional, histological, and biochemical markers to determine the validity in recapitulating the phenotype of human BMD patients (Hollinger et al., 2014; Selsby et al., 2015). Moreover, given the increasing focus on gene and exon skipping therapies aimed at converting DMD into a milder BMD phenotype (Nakamura, 2019), the population of BMD and BMD-like presentations in humans may rise.

Among the most significant findings of our pilot study were the potential gait abnormalities seen in Affected pigs, specifically hip ROM. Standard deviation from all gait measures demonstrate an almost 30% greater inconsistency in Affected pigs, while overall spatiotemporal parameters and joint angles of the gait cycle showed modest deviations, the 40% (95% CI -0.64 to 14.12) increase in hip ROM mirrors key biomechanical phenotypes observed in human BMD patients where compensatory hip movements are required to maintain forward propulsion due to weakness in the proximal leg muscles (Gao et al., 2025). These findings suggest that the hindlimb musculature in Affected pigs may exhibit functional maladaptation to underlying muscle weakness like human patients (Hou, Du, & Wu, 2022; Stirm et al., 2021). The lack of observed differences in knee and ankle ROM may reflect early or milder disease progression, typically seen in BMD where distal muscles are initially spared (Thada, Bhandari, Forshaw, & Umapathi, 2025). These gait alterations further suggest this model may recapitulate the human BMD phenotype (Nakamura et al., 2023) reinforcing the translational potential in evaluating functional endpoints for therapeutic intervention. Importantly, these kinematic disruptions provide a non-invasive, longitudinal marker that can be leveraged to monitor disease progression and response to treatment.

Muscle function testing and histopathological analyses further suggested the presence of a Becker-like phenotype in Affected pigs. *In vivo* muscle torque assessments revealed reductions in the flexor muscles in Affected pigs compared to Healthy, with no evident changes in extensor function. This asymmetry may suggest differential muscle involvement based on anatomical

usage of a quadruped and disease burden, aligning with prior studies indicating a heterogenous pathology across muscle groups in BMD (Bello et al., 2016; Comi et al., 1994). Histopathological analysis of muscle CSA aligned with cycles of degeneration and regeneration common to dystrophinopathies (Darras, Urion, & Ghosh, 2022), a spectrum of X-linked muscle diseases. While the extensor muscles of Healthy and Affected showed no difference in muscle fiber morphology, the flexor muscles of Affected pigs displayed a leftward shift in fiber size distribution, with increases in the percentage of small muscle fibers and reductions in the percentage of large muscle fibers. This change in muscle fiber size is consistent with muscle injury and repair frequently observed in dystrophinopathies indicating ongoing cycles of degenerative and regenerative activity (Ripolone et al., 2022). Furthermore, average CK levels in blood were 2.5-fold greater in Affected pigs, a common biomarker of muscle fiber membrane instability and damage. Taken together, these findings suggest the BMD pig model exhibits functional deficits and histopathological hallmarks of disease in select muscles, similar to observations in humans with BMD.

Analysis of dystrophin revealed moderate reductions in both extensor and flexor hindlimb muscles of Affected pigs. Although this reduction in dystrophin is less severe than previous investigations into the diaphragm, longissimus, and psoas (Selsby et al., 2015), these values are consistent with the in-frame nature of the mutation in Exon 41, likely permitting some dystrophin expression, but, given previous reports and data presented herein, not at levels sufficient to prevent disease pathology. The presence of dystrophin at reduced levels aligns with the intermediate clinical progression of BMD, where dystrophin is partially functional but unable to maintain normal muscle structure and function (Anthony et al., 2011; Bushby et al., 1993; Hoffman et al., 1989). Given the spectrum of severity noted in humans with BMD, modest reductions in dystrophin, when viewed alongside preliminary functional and histological deficits support the conclusion that this model may provide a translational perspective for therapeutic development. In addition, dystrophin expression was highly correlated with peak torque and fatigue index from

flexor muscles. This may be due, at least in part, to a greater reliance on intermediate/slower, more fatigue resistant muscle fiber types and a reduction in the contribution of fast fatigable fibers to functional measures in peak torque concomitant with declines in dystrophin as demonstrated by others (Webster et al., 1988).

Limitations to the current study were that we were only able to study eight pigs (5 with BMD and 3 healthy from the same background). Although the power of such a study is low, observations in Affected pigs were comparable to recent published results in human BMD patients.

Conclusion

This pilot study provides a gait characterization of a naturally occurring porcine model of BMD with an in-frame Exon 41 mutation resulting in dystrophin insufficiency. Our findings indicate a Becker-like phenotype including disease mediated alterations in hip ROM, reduced flexor muscle function and muscle fiber CSA, and elevated CK concentrations. Dystrophin expression was inversely correlated with fatigue index in flexor muscles. In contrast, extensor muscles did not display a Becker-like phenotype across all parameters, suggesting heterogeneity in muscle pathology. These abnormalities closely mirror the human BMD phenotype, particularly in functional and histopathological features of the hindlimb musculature. Together, these results further suggest the BMD pig as a translationally relevant large animal model for studying disease progression and evaluating the safety and efficacy of emerging therapeutic advancements.

Data Availability Statement

The data that supports the findings of this study are available at the following link. https://doi.org/10.7910/DVN/ZOX424.

Competing Interests

The authors have no competing interests.

Author Contributions

Conceptualization: A.B.M., P.P.N., Data Curation: A.B.M., J.A.K., S.G., J.M.Y., P.P.N., Formal Analysis: A.B.M., J.A.K., S.G., J.M.Y., P.P.N., , Funding Acquisition: A.B.M., P.P.N., Investigation: A.B.M., J.A.K., M.M., S.G., J.T.S., P.P.N., , Methodology: A.B.M., M.M., K.C., J.M.Y., P.P.N., J.T.S., J.W.R., Z.E.K., Project Administration: A.B.M., P.P.N., Resources: A.B.M., Z.E.K., J.W.R., J.M.Y., J.T.S., N.E.P.D., P.P.N., Validation: A.B.M., P.P.N., Visualization: A.B.M., P.P.N., Writing – original draft: A.B.M., J.A.K., Writing – review and editing: A.B.M., J.A.K., J.M.Y., P.P.N., J.T.S., All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work ensuring that questions related to accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

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Table 1. Gait Analysis for Healthy and Affected pigs.

	Healthy	Affected		Healthy	Affected
Hip ROM (degrees)	10.11	16.85	***SD Swing Time (seconds)***	0.019	0.021
Hip ROM SD (degrees)	0.821	1.642	***Mean Stance Time (seconds)***	0.449	0.371
Knee ROM (degrees)	29.54	27.03	***SD Stance Time (seconds)***	0.039	0.041
Knee ROM SD (degrees)	2.170	2.806	***Mean Step Time (seconds)***	0.745	0.631
Ankle ROM (degrees)	33.90	30.19	***SD Step Time (seconds)***	0.040	0.044
Ankle ROM SD (degrees)	3.178	4.960	***Mean Stance Percent (percent)***	0.601	0.586
Mean Swing Time (seconds)	0.296	0.260	***SD Step Time (seconds)***	0.028	0.036

Gait analysis data for Healthy and Affected groups at 8-9 months of age. Values are means, n=2-5/group.

Table 2. Blood panel for Healthy and Affected pigs.

Blood Marker	Healthy	Affected	Blood Marker	Healthy	Affected
Creatine Kinase (pg/dL)	558.3	1438.0	***ALKP (U/L)***	70.0	76.3
Total Protein (g/dL)	7.4	7.0	***AST (U/L)***	70.0	109.4
Albumin (g/dL)	4.8	4.3	***ALT (U/L)***	46.5	46.4
Calcium (mg/dL)	12.5	11.6	***Globulins (g/dL)***	2.7	2.7
Phosphorous (mg/dL)	9.0	8.8	***Albumin: Globulin Ratio***	1.8	1.6
Glucose (mg/dL)	138.5	102.6	***Sodium (mEq/L)***	180.5	160.6
BUN (mg/dL)	21.0	18.6	***Potassium (mEg/L)***	6.1	10.4
Creatinine (mg/dL)	1.5	1.4	***Chloride (mEq/L)***	133.0	117.2
Bilirubin - total (mg/dL)	0.1	0.1	***Sodium: Potassium Ratio***	29.7	20.7

Blood panel data for Healthy and Affected groups at 8-9 months of age. Values are given in picograms (pg), milligrams (mg), or grams (g) per deciliter (dL), or units (U) or milliequivalents (mEq) per liter (L). Values are means, n=2-5/group.

Figure Legends

Figure 1.

Experimental set up and marker placement for motion capture analysis. A) *Marker Placement*, Markers were placed at joints (tuber coxae, hip joint, knee joint, ankle joint, mtp joint) on the right hindlimb of walking pigs. B and C) *Room Layout*, Two lights were placed on either side of a digital camera mounted upon a tripod placed at a 90º angle to the joint markers placed upon pigs, B) Top View, C) Subject View.

Figure 2.

General descriptive measures. A) Pooled standard deviation (SD) reported as percent Healthy from all kinematic measures reported n=16 measures from Healthy and n=40 measures for Affected suggesting step pattern inconsistency. AB Average body weight of Healthy and Affected pigs at 9 months of age were similar. C) Body weight changes at 8-9 months of age were similar.

Figure 3.

Joint angle range of motion (ROM) comparison for Healthy and Affected pigs. A) ROM for hip, B) knee, and C) ankle joints during walking test. (n= 2-5/group).

Figure 4.

Muscle torque from Healthy and Affected flexor and extensor muscles. A) peak flexor torque, B) flexor fatigue curves, C) flexor fatigue Area Under the Curve (AUC), D) flexor fatigue index. E) peak extensor torque, F) extensor fatigue curves, G) extensor fatigue Area Under the Curve (AUC), H) extensor fatigue index. (n= 2-5/group).

Figure 5.

Histology of flexor and extensor muscles of Healthy and Affected pigs. A) Representative images of Healthy and Affected extensor muscle cross sections. Scale bars = 200 μm. B) Frequency histogram from binned cross-sectional areas (CSA) of extensor muscles. C) Summary data of small and D) large diameter fibers from extensor muscles. E) Representative images of Healthy and Affected flexor muscle cross sections. Scale bars = 200 μm. F) Frequency histogram from binned (0 - >12,000 in 2,000 μm^2 increments) cross-sectional areas (CSA) of flexor muscles. G) Summary data of small and H) large diameter fibers from flexor muscles. (n= 2-5/group)

Figure 6.

Dystrophin 1 and 2 expression in Affected compared to Healthy pigs reported as percent of Healthy. A) Representative blot image at 427 kDa (top), mean densiometric data (middle), and total protein (bottom) for A) Extensor muscles and B) Flexor muscle. (n= 2-5/group).

Figure 7.

Correlation of Dystrophin protein expression to direct muscle function from Healthy and Affected pigs. *Flexor Muscles* A) Torque and B) fatigue index are significantly correlated with Dystrophin expression while the C) areas under fatigue curves (AUC) were not. *Extensor Muscles* D-F) None of the functional measures in extensor muscles correlated with dystrophin expression.

Supplementary Figure 1.

Correlations of Dystrophin protein expression from Healthy and Affected extensor and flexor muscles to joint range of motion (ROM) variables. *Flexor Dystrophin* A, B, E, F, I, J) joint ROM variables were not well correlated with dystrophin expression. In addition, dystrophin expression in *Extensor Muscles* C, D, G, H, K, L) was also not well correlated with ROMs.

Supplementary Figure 2.

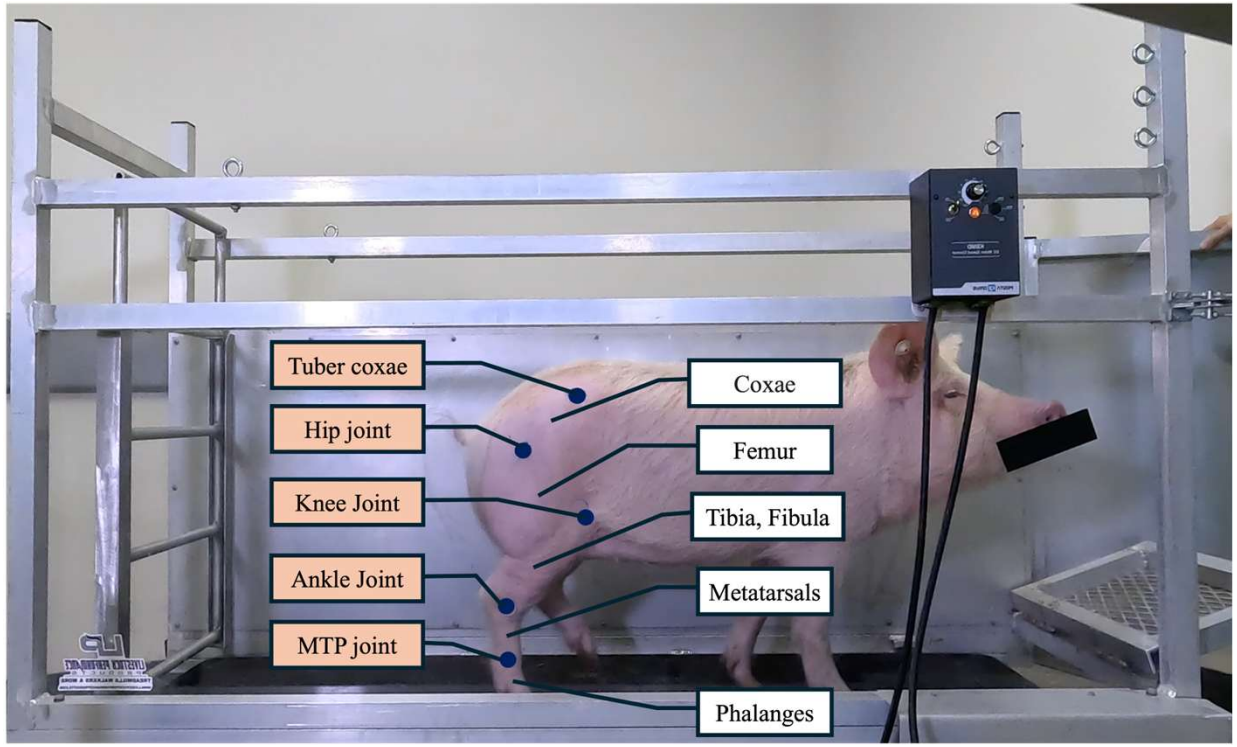
Correlations of Dystrophin protein expression from Healthy and Affected extensor and flexor muscles to temporal variables in the gait cycle. *Flexor Dystrophin* A, B, E, F, I, J) temporal variables were not well correlated with dystrophin expression. In addition, *Extensor Muscles* C, D, G, H, K, L) were also not well correlated.

Supplementary Figure 3.

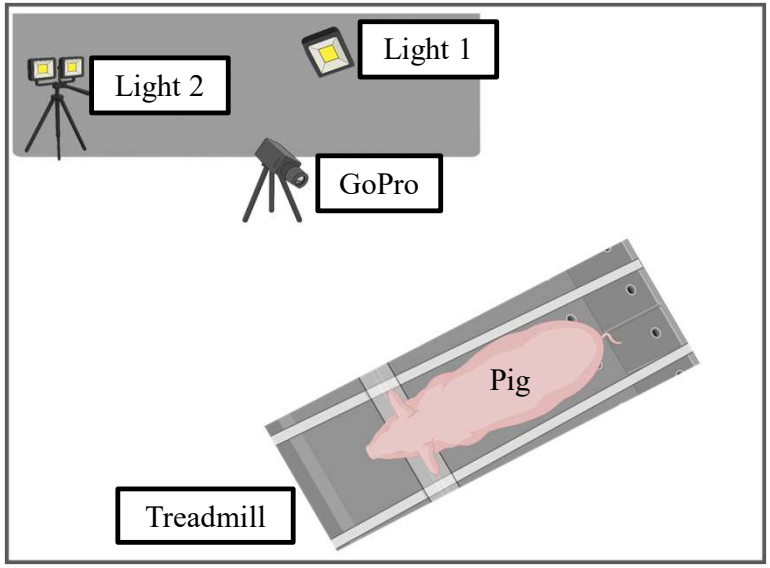
Evaluation of stance percent parameters compared to dystrophin expression in either flexor (A and B) or extensor (C and D) muscles.

Figure 1

A


Tuber coxae
Hip joint
Knee Joint
Ankle Joint
MTP joint
Coxae
Femur
Tibia, Fibula
Metatarsals
Phalanges

B


Light 1
Light 2
GoPro
Pig
Treadmill

C

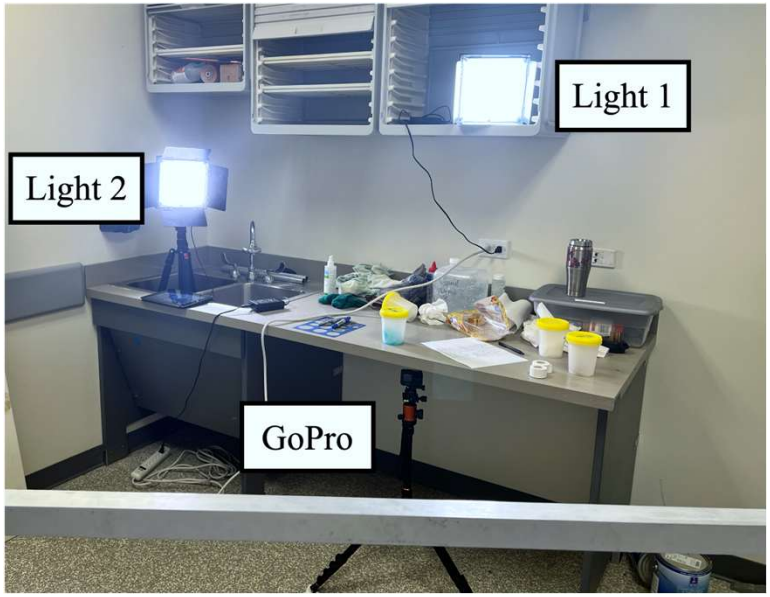

Light 1
Light 2
GoPro

Figure 2

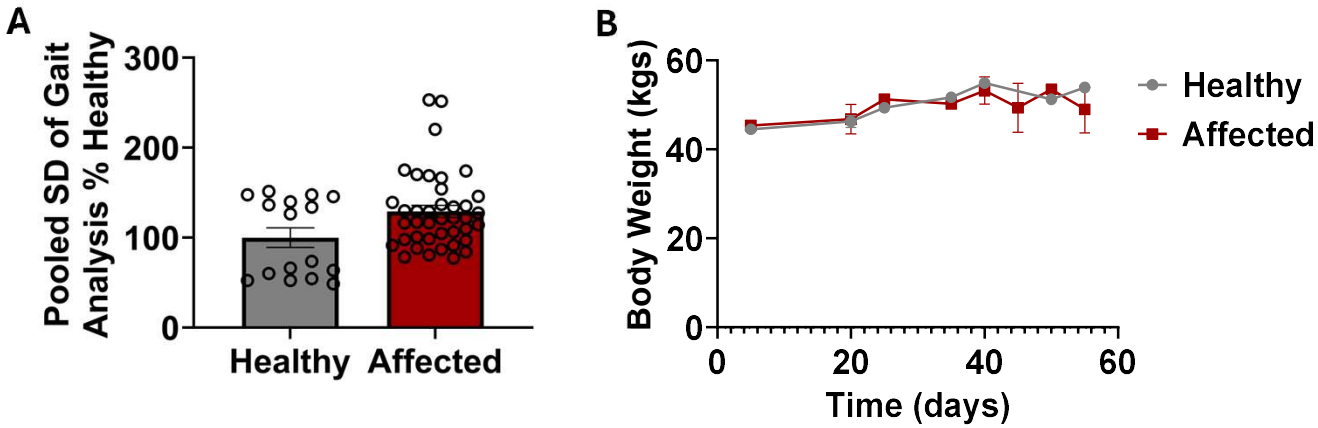

A
Pooled SD of Gait Analysis % Healthy
300
200
100
0
Healthy
Affected
B
Body Weight (kgs)
60
40
20
0
0
20
40
60
Time (days)
Healthy
Affected

Figure 3

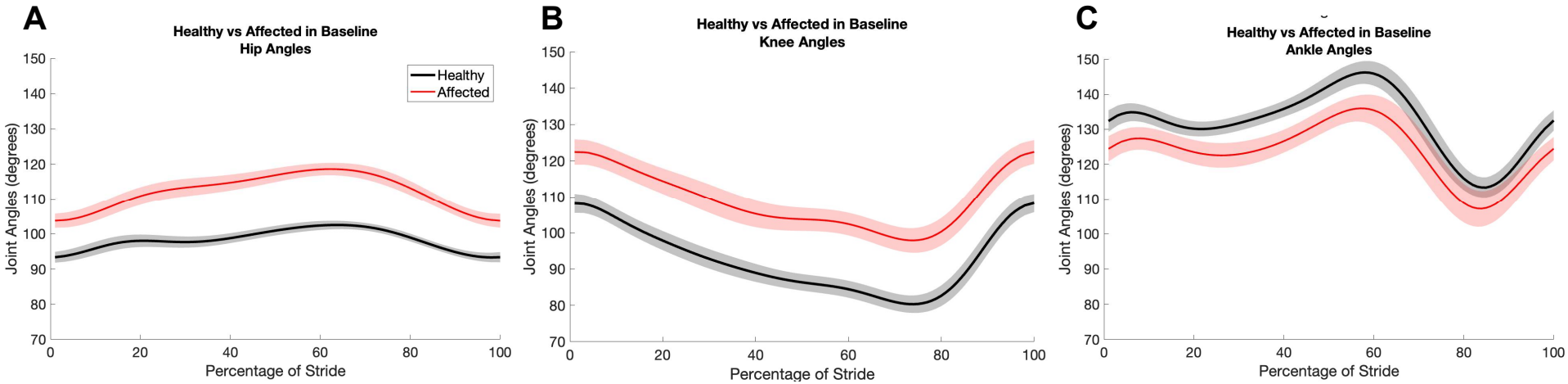

A
Healthy vs Affected in Baseline
Hip Angles
Healthy
Affected
Joint Angles (degrees)
Percentage of Stride
B
Healthy vs Affected in Baseline
Knee Angles
Joint Angles (degrees)
Percentage of Stride
C
Healthy vs Affected in Baseline
Ankle Angles
Joint Angles (degrees)
Percentage of Stride

Figure 4

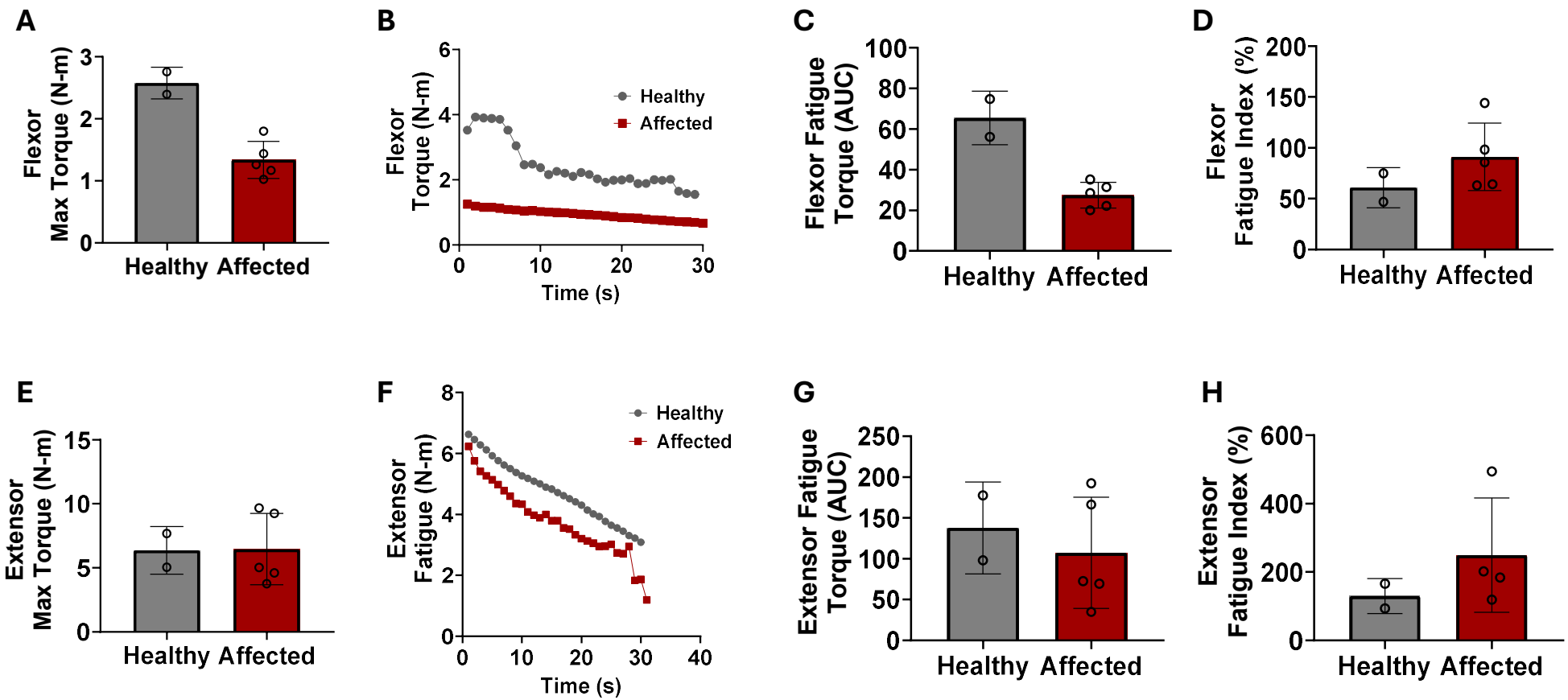

A
Flexor Max Torque (N-m)
0
1
2
3
Healthy
Affected
B
Flexor Torque (N-m)
0
2
4
6
0
10
20
30
Time (s)
Healthy
Affected
C
Flexor Fatigue Torque (AUC)
0
20
40
60
80
100
Healthy
Affected
D
Flexor Fatigue Index (%)
0
50
100
150
200
Healthy
Affected
E
Extensor Max Torque (N-m)
0
5
10
15
Healthy
Affected
F
Extensor Fatigue (N-m)
0
2
4
6
8
0
10
20
30
40
Time (s)
Healthy
Affected
G
Extensor Fatigue Torque (AUC)
0
50
100
150
200
250
Healthy
Affected
H
Extensor Fatigue Index (%)
0
200
400
600
Healthy
Affected

Figure 5

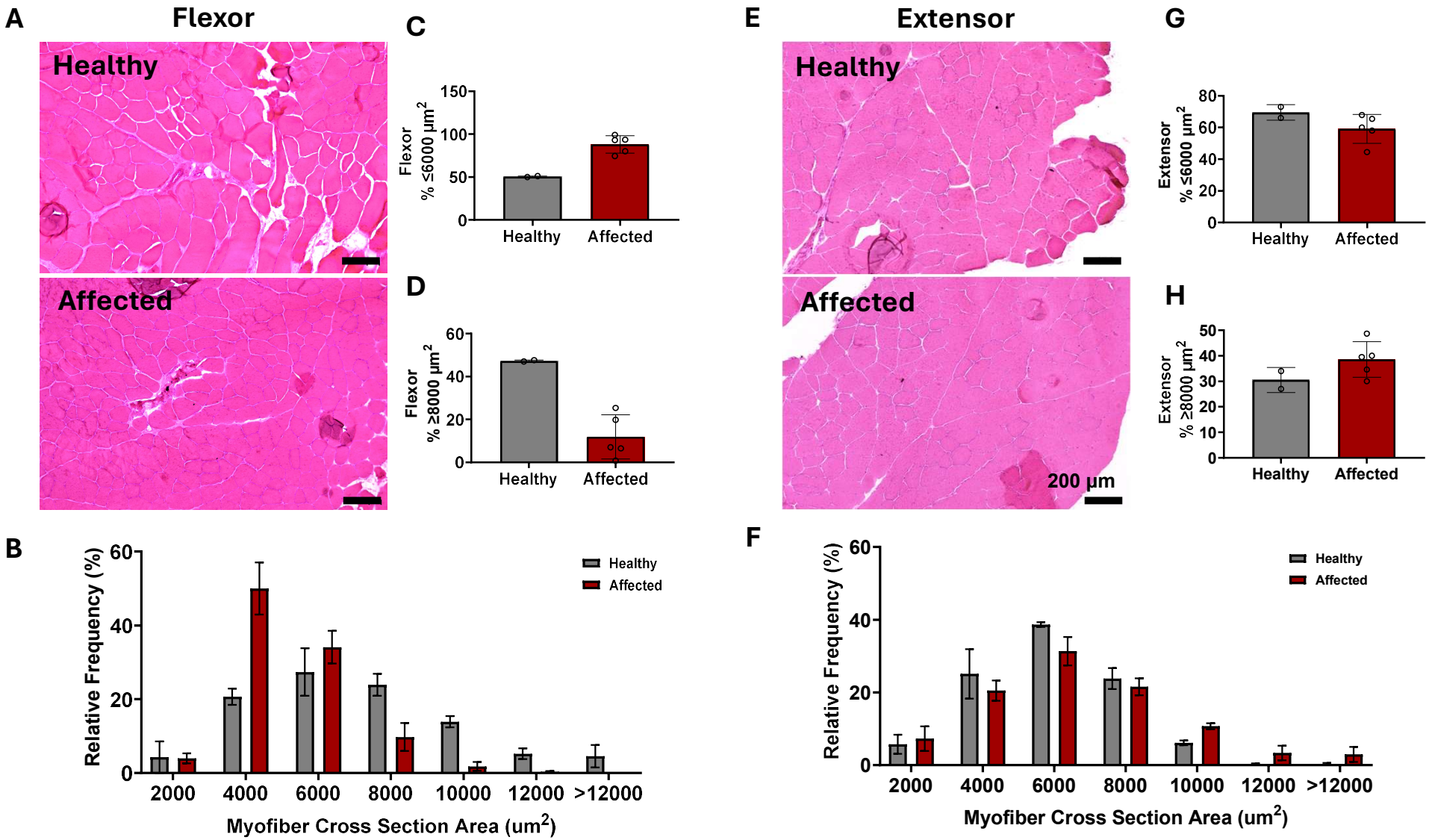

A
Flexor
Healthy
Affected
C
Flexor % ≤6000 µm²
150
100
50
0
Healthy
Affected
D
Flexor % ≥8000 µm²
60
40
20
0
Healthy
Affected
E
Extensor
Healthy
Affected
200 µm
G
Extensor % ≤6000 µm²
80
60
40
20
0
Healthy
Affected
H
Extensor % ≥8000 µm²
50
40
30
20
10
0
Healthy
Affected
B
Relative Frequency (%)
60
40
20
0
Healthy
Affected
2000
4000
6000
8000
10000
12000
>12000
Myofiber Cross Section Area (um²)
F
Relative Frequency (%)
60
40
20
0
Healthy
Affected
2000
4000
6000
8000
10000
12000
>12000
Myofiber Cross Section Area (um²)

Figure 6

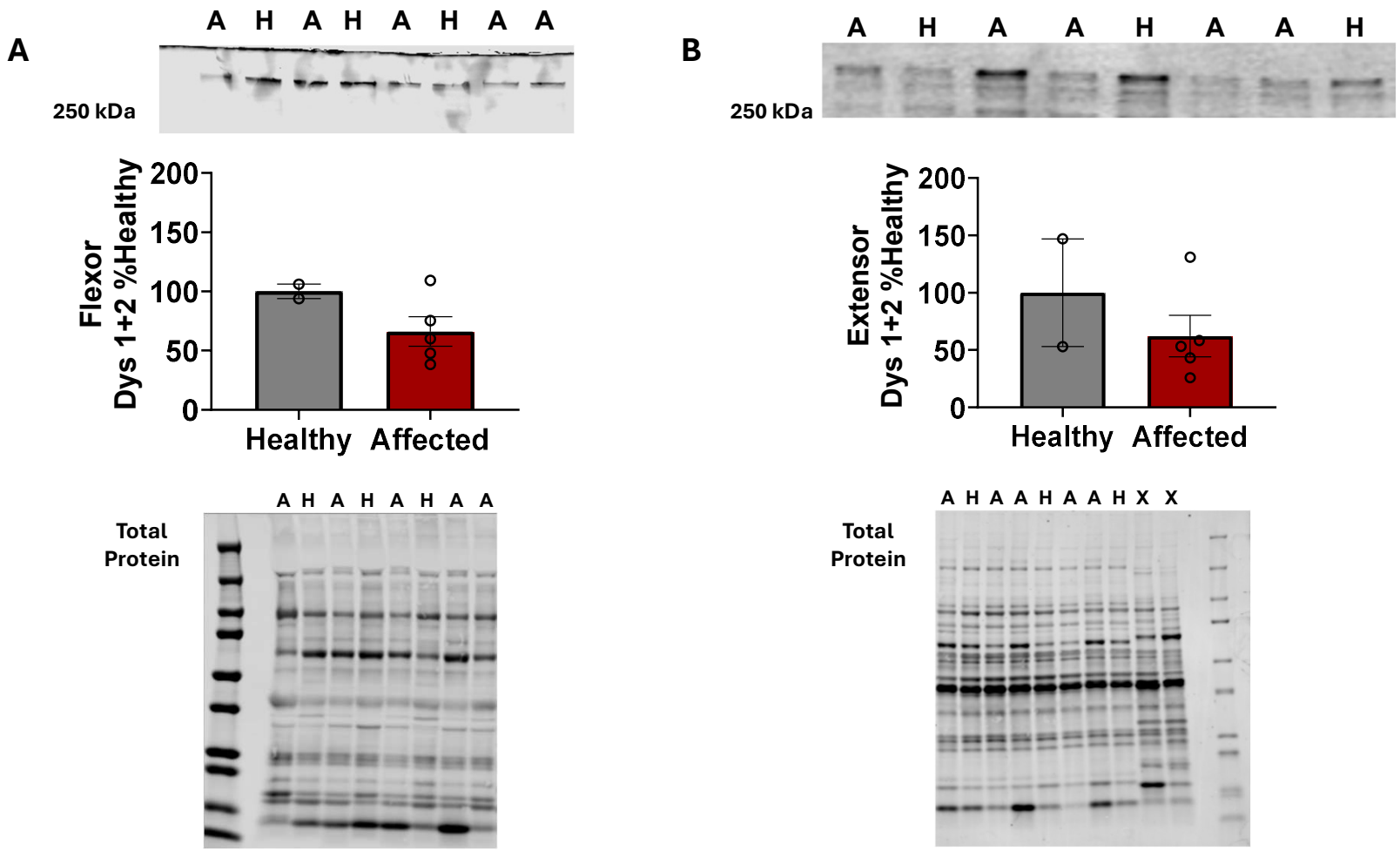
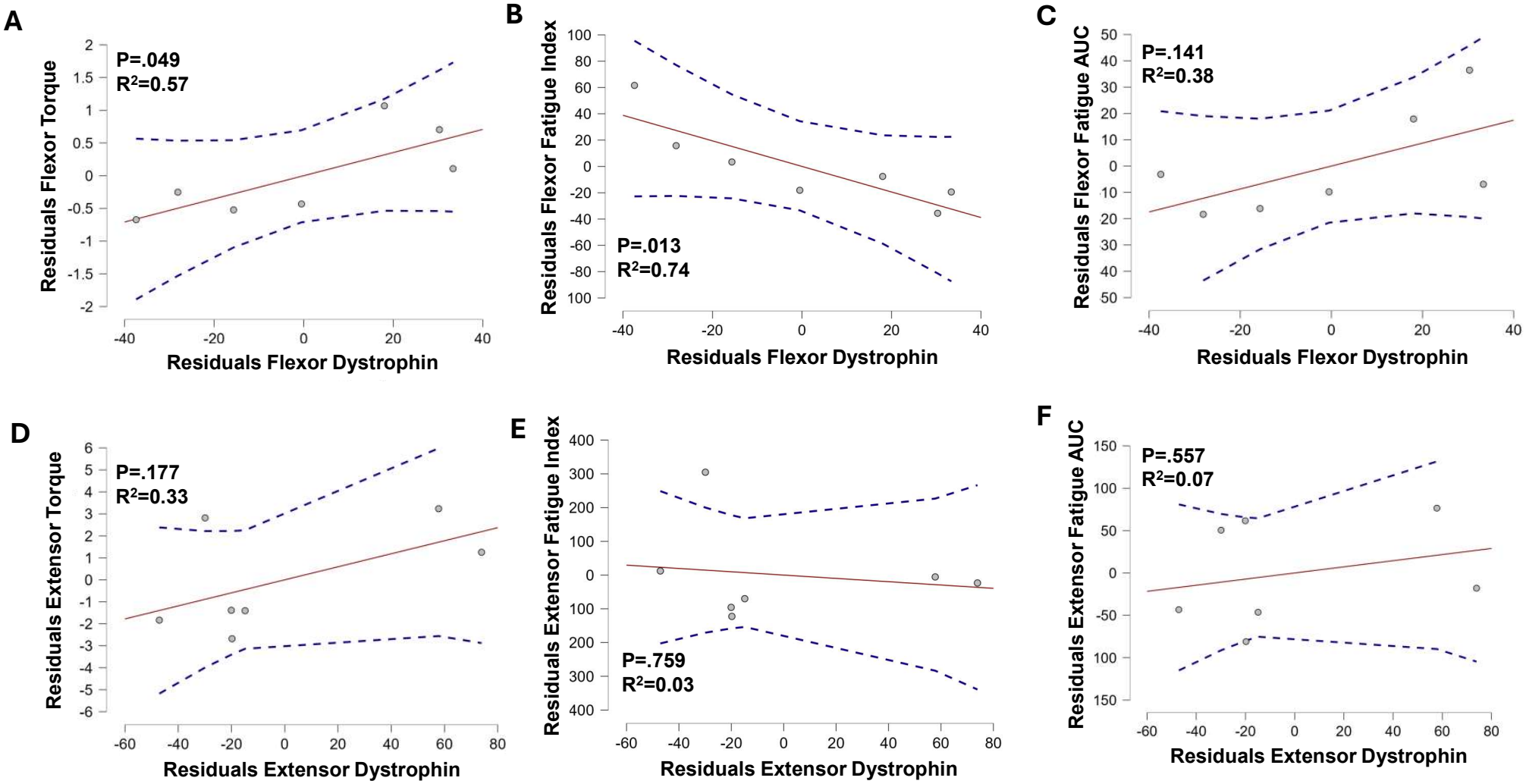
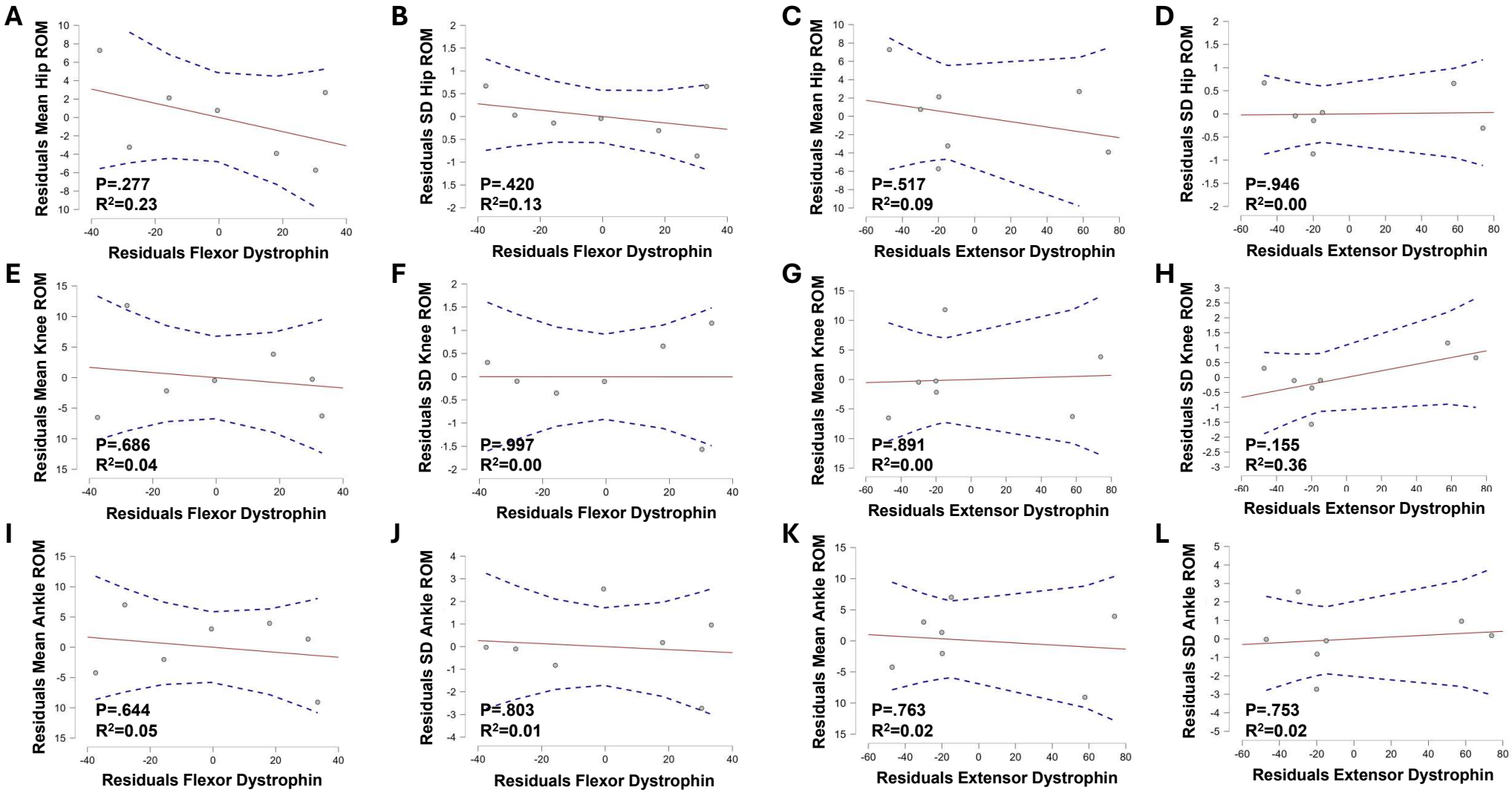

A
A H A H A H A A
250 kDa
Flexor
Dys 1+2 %Healthy
200
150
100
50
0
Healthy
Affected
Total
Protein
A H A H A H A A
B
A H A A H A A H
250 kDa
Extensor
Dys 1+2 %Healthy
200
150
100
50
0
Healthy
Affected
Total
Protein
A H A A H A A H X X

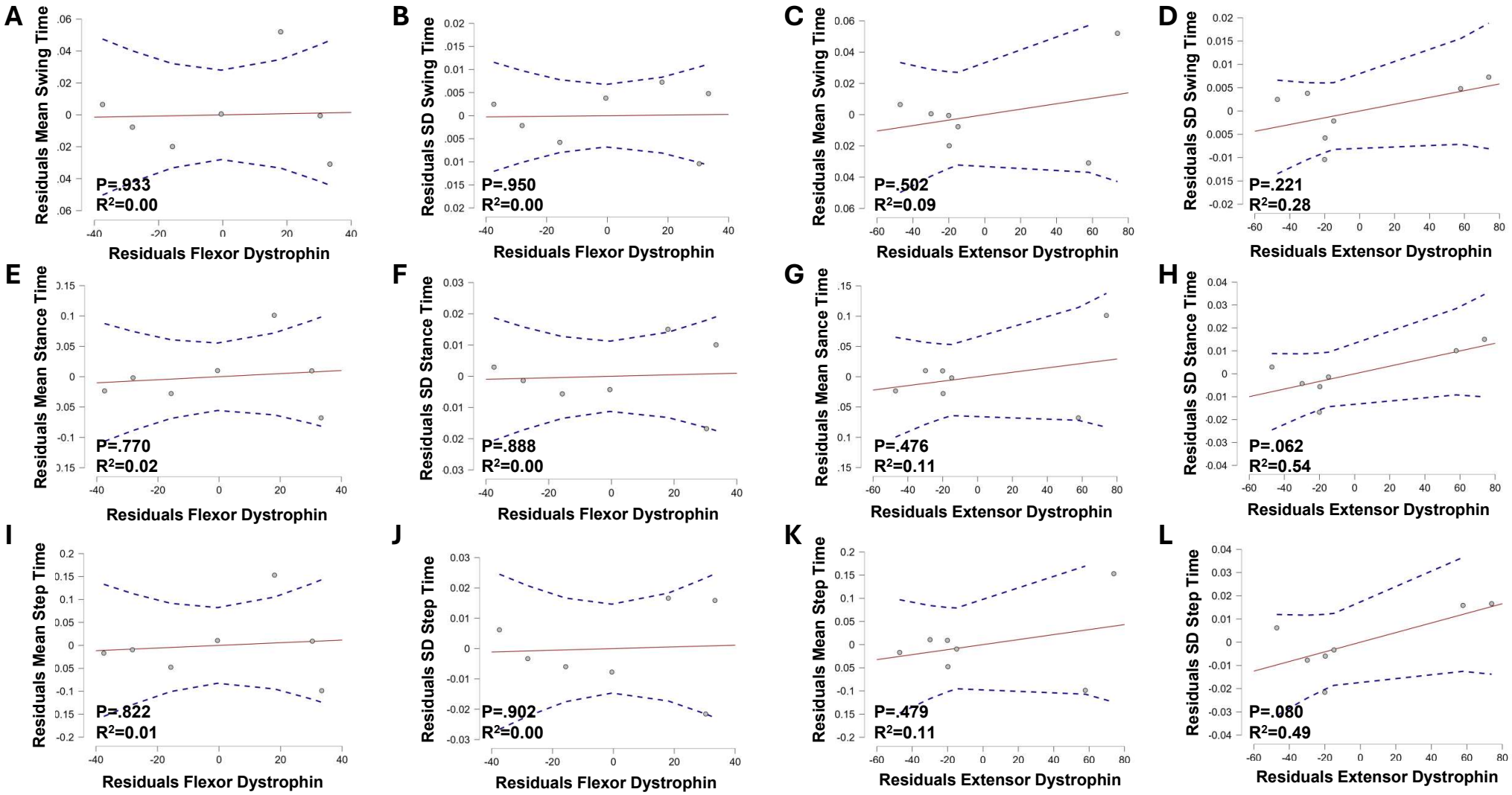
Figure 7


A
P=.049
R²=0.57
Residuals Flexor Torque
Residuals Flexor Dystrophin
B
P=.013
R²=0.74
Residuals Flexor Fatigue Index
Residuals Flexor Dystrophin
C
P=.141
R²=0.38
Residuals Flexor Fatigue AUC
Residuals Flexor Dystrophin
D
P=.177
R²=0.33
Residuals Extensor Torque
Residuals Extensor Dystrophin
E
P=.759
R²=0.03
Residuals Extensor Fatigue Index
Residuals Extensor Dystrophin
F
P=.557
R²=0.07
Residuals Extensor Fatigue AUC
Residuals Extensor Dystrophin

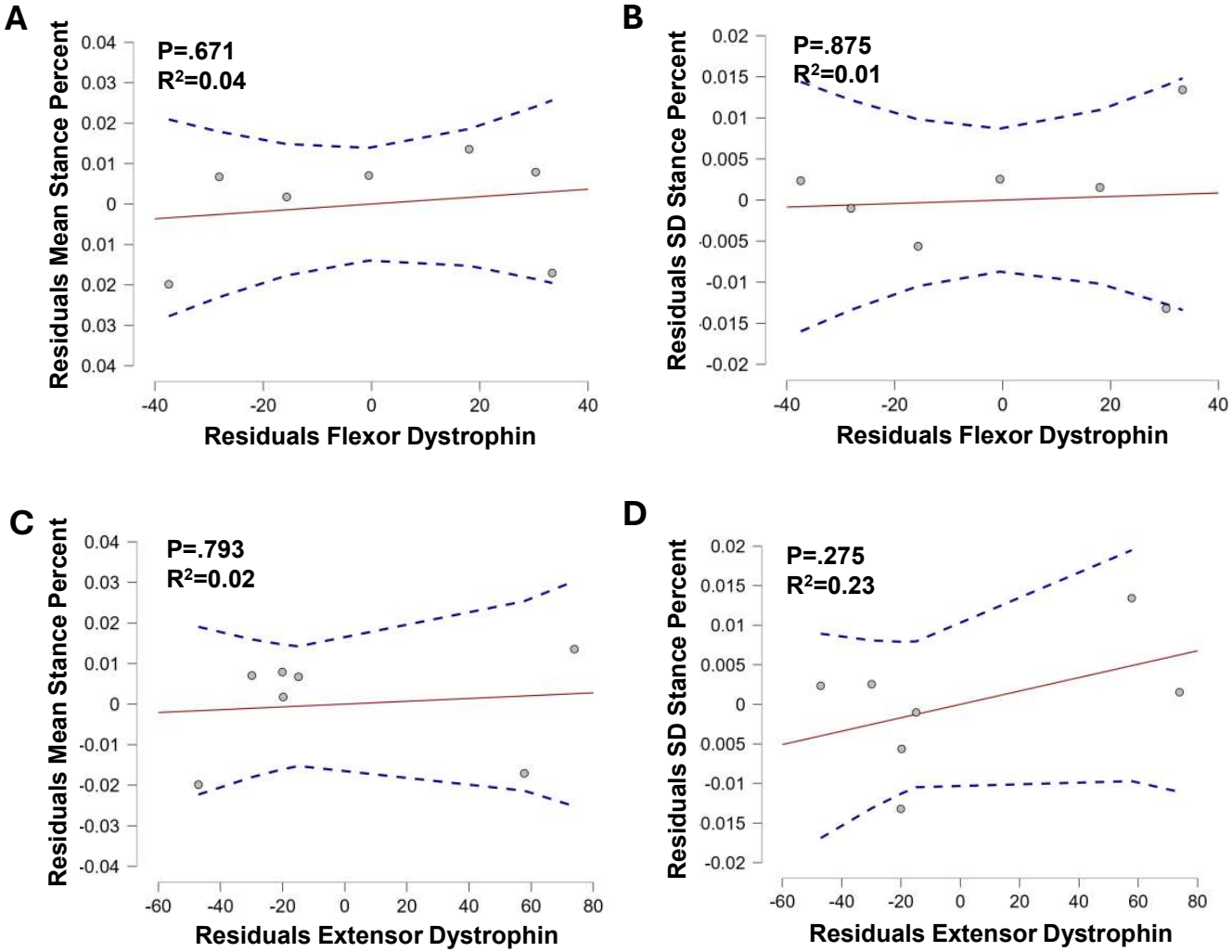
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Stated in the "Animals" section and statistics. Single Animal
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Individual Stated in "Animals" section
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Diseased large animal subjects were selected as available data points were not excluded 'n' for each experiment is shown
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Randomization was used between healthy and Affected pigs Randomization of muscle function, biopsies, and gait analysis was used to perform each on different days.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Two researchers were in charge of coding. Data analysis was conducted by blinded research staff.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Dystrophin expression and muscle function correlations Methods
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Analysis section No methods were used to test if the assumptions of the statistical approach were met.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Animals Section, and Results Blood Panel, Table 2
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Individual Methods section
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results Results/Figure Legends