Modeling Chick Embryo Tendon Strains Experienced During Development

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INTRODUCTION: Abnormal fetal movements are implicated in common musculoskeletal birth defects, including arthrogryposis and clubfoot. Fetal paralysis effects on bone and cartilage development have been studied, whereas less is known about tendon development. Using the chick embryo (equivalent of fetus in mammal), we recently discovered that fetal paralysis inhibits tendon mechanical development, whereas hypermotility improves mechanical properties (Pan et al., 2018). The study revealed the importance of mechanical loading for tendon development but could not provide information such as tendon strains experienced during movements. We have developed a computer simulation model (forward dynamics) to examine relationships between mechanical loading and tendon development in the chick embryo. Limb movement begins after embryo day (D)6-D8 and peaks during D12-D17 (Wu et al., 2001). These movements involve not only kicks but also impacts with the egg wall (Bradley et al., 2005). The role of these wall impacts in tendon development is not well understood. Chick embryos grow rapidly and are likely only able to impact the wall for a limited period. Since tissue strength is in theory regulated by the most prevalent strains experienced (Seedhom, 2006), we hypothesize that (i) the period when the limb is able to impact the wall coincides with the period when the most embryo movement occurs, and (ii) calcaneal tendon strains during limb motions are much greater when impacting the egg wall versus when not impacting the wall. This is a first report on using computer simulation to analyze strains that tendons experience throughout fetal development. This model can be expanded to evaluate strains experienced by cartilage, bone, and other tissues during fetal movements under normal as well as aberrant conditions. This model provides a powerful tool to enable new insights into the mechanobiology of musculoskeletal tissue development.

METHODS: Fertilized White Leghorn chicken eggs were incubated until D10 to D19. Embryos were sacrificed and staged according to anatomical features. The leg was dissected from the body at the hip joint, and then segmented into the thigh, shank, and foot. Each leg segment was placed on a Kimwipe for 10 s, flipped 180 degrees and placed on a Kimwipe for another 10 s, placed in a capped 50 mL conical tube to prevent evaporation, and weighed. Four distinct length measurements were taken using calipers: thigh, shank, foot minus toes, and longest toe. Each measurement was aligned with the bone for consistency. A sagittal plane musculoskeletal model of the chick embryo limb was developed using MotionGenesis software and forward dynamics simulations of *in ovo* kicking at D10-19 were performed in MATLAB software. The model consisted of four rigid segments (thigh, shank, foot, and toes) connected at pin joints (**Fig. 1**). Segment lengths and masses were assigned from the chick embryo measurements, resulting in 10 models of different size, one for each embryonic day. Each joint was actuated by a three-component Hill-based muscle model (Miller et al., 2018) representing the extensor muscles of the hip, knee, and foot/ankle complex. Muscle geometry and masses were referenced from Hutchinson et al. (2004) and scaled to the size of the present model. Calcaneal tendon stiffness was based on Navarro et al. (2022) and damping was set so that the tendon energy return was approximately 93% (Alexander et al., 2002). Contact between the egg and wall was possible at the distal and proximal ends of the toes segment, where Hunt-Crossley contact springs were defined (Sherman et al., 2011). Viscous forces applied to the segment mass centers modeled embryonic fluid drag. Egg dimensions were defined from measurements at the widest section in the sagittal plane. The hip was fixed to the back wall of the egg and the joints, when possible, were initially posed in their neutral resting angles with the muscles deactivated and all forces in static equilibrium. This initial pose was chosen because it was the same joint angles for all models, removing the effect of different initial poses. Kicks were simulated by inputting a 50-ms twitch excitation to each extensor muscle. The outcome variable of each simulation was peak strain of the ankle extensor's series elastic component, representing the calcaneal tendon. Ten simulations were performed, with the model's dimensions and masses set to the average measurements on each day from D10-19.

RESULTS: Leg segment masses increased nonlinearly, whereas leg segment lengths increased linearly during development (**Fig. 1**). Before D14, the chick model's leg was too short to impact the wall. After D17, the leg segments were too long to set the neutral initial pose, and a more flexed pose with the toes already resting against the egg wall had to be used. From D14-17, the leg was able to impact the wall during the kicks, and developed peak calcaneal tendon strains roughly twice as high as those from kicks where the wall was not impacted. Peak strains ranged from 0.60-0.72% when the embryo leg was too small to impact the wall (D10-D13), 1.10-1.38% when able to impact the wall (D14-D17), and 0.72-0.76% when too large to impact the wall (D18-D19). Peak tendon strain correlated well with the absolute difference between leg length (thigh+shank+foot) and egg diameter at its widest point (r = 0.78). The large strains from egg wall impacts occurred during the incubation time when chick leg motility is typically greatest (Wu et al., 2001) (**Fig. 2**).

DISCUSSION: Mechanobiology studies of tendon development have been limited by the inability to directly measure tendon strains during fetal movement. The eggshell is too opaque to see through, and views of the embryo though a window in the shell are obstructed by the yolk and vascularized chorioallantoic membrane. We have developed a computer simulation model to predict movements involved in kicking, impacts with the egg wall, and calcaneal tendon strains with each kick from D10-D19. The model was informed by empirically measured leg masses and dimensions averaged across biological replicates (future studies will perform simulations for each biological replicate). Estimated strains during development approximately doubled when the limb was large enough to forcefully impact the wall during muscle twitch contractions. Potential to develop high strains during late development when the muscles are largest and strongest was limited by the constrained space within the egg. The simulations can also test other factors, such as the pose of the body and limb, muscle contraction intensity, paralysis, friction against the egg wall, and the role of the air pocket at the top of the egg. While this first study focused on the calcaneal tendon, it can be extended to analyze other tendon types and body parts, and other tissues including ligaments, cartilage, bone, meniscus, and more.

SIGNIFICANCE: This model provides a powerful tool to gain new insights into the mechanobiology of musculoskeletal tissue development, which has the capacity to inform the development of fetal interventions and therapeutics for musculoskeletal birth disorders.

REFERENCES: Alexander et al., *Comp. Biochem. Physiol. Part A,* 2002; Bradley et al., *J. Neurophysiol.,* 2005; Hutchinson et al., *J. Morphol.,* 2004; Miller et al., *Handbook of Human Motion*, 2018; Navarro et al., *J. Biomech.*, 2022; Seedhom et al., *Rheumatology*, 2006; Sherman et al., *Symposium on Human Body Dynamics*, 2011, Pan et al., *Phil. Trans. R. Soc.*, 2018; Wu et al., *J. Exp. Zool.*, 2001

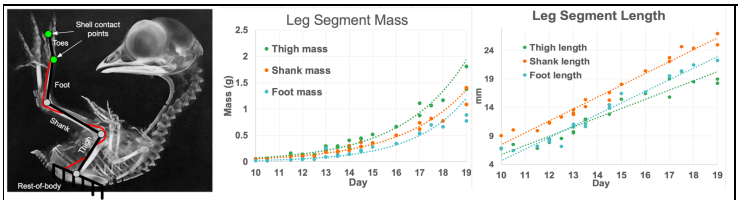

Fig. 1*. Limb with 4°of freedom (hip, knee, ankle, toe joints), actuated by 3 extensor muscles (red lines) (**left**). Contact with egg possible at 2 points defined on distal and proximal ends of toes segment. Leg masses increased non-linearly (r^2>0.91) (**middle**) and leg lengths (**right**) increased linearly (r^2>0.92).*

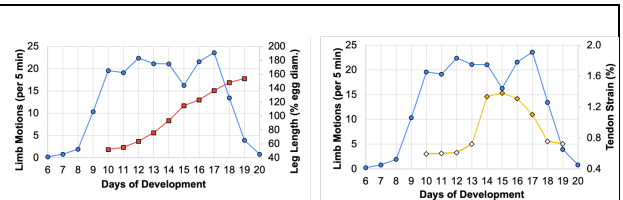

Fig. 2*. Fully extended limb length (thigh+shank+foot) (red) at D10-19 (**left**). Peak calcaneal tendon strains (yellow) during limb kicking simulations at D10-19 (**right**). Leg motility data (blue) from Wu et al. 2001.*