Clinically Relevant Severe Acute Compartment Syndrome in Turkey Model.

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INTRODUCTION: Acute Compartment Syndrome (ACS) is a common and severe condition in which sustained elevation of intracompartmental pressure (ICP) compromises tissue perfusion, leading to irreversible muscle damage following extremity trauma. To study muscle injury mechanisms, we previously developed a turkey ACS model applying 50 mmHg of ICP, which successfully induced mild-to-moderate muscle damage. While that model demonstrated mild-to-moderate muscle damage, it did not reproduce the severe muscle necrosis or functional deficits typically seen in clinical ACS, such as those leading to gait dysfunction. One potential reason is the significant disparity between the applied pressure (50 mmHg) and the turkey's native systolic blood pressure (140 mmHg), which may have limited ischemic insult. In the present study, we increased the applied ICP from 50 to 100 mmHg to develop a more clinically relevant model of ACS that better simulates the extent of muscle damage observed in clinical cases.
METHODS: We used a total of 12 Bourbon Red Heritage turkeys (equal ratio of males and females; Little Bend Heritage Farm, LLC, Chatfield, MN), about 1 year old (10-15 kg) and skeletally mature. Turkeys were divided into 2 groups (n=6, Fig.1A): Group 1 had the ICP maintained at 50 mmHg for 6 hours, and Group 2 at 100mmHg. Once each animal was under general anesthesia, the left or right leg, selected randomly, was prepared for ACS creation. The tibialis cranialis (TC) muscle, which corresponds to tibialis anterior muscle in humans, was identified with ultrasound by extending the ankle and foot (Fig. 1B). A 19-gauge slit catheter with intracompartmental pressure monitor (Stryker, Kalamazoo, MI) was inserted into the center of the muscle to measure and monitor the intracompartmental pressure (ICP) continuously throughout the procedure. An 18-gauge needle with a catheter was inserted into the deep anterior compartment and 5% solution of chicken albumin was infused continuously with a rotating IV pump (Harvard pump, Harvard Apparatus, South Natick, MA) (Fig. 1C). Infusion speed was adjusted based on the desired ICP and each pressure level was maintained for ten minutes (Fig 1A). Pedobarographic and kinetic data of turkeys were collected weekly including the pre-ACS stage using a portable walkway system (emed-xl, Novel, Munich, Germany). Stance was calculated using designated software (Novel Projects, Novel, Munich, Germany) (Fig 1D). Two weeks post-ACS, isometric tetanic muscle force (ITF) testing was performed on the TC muscle under general anesthesia, following ITF testing, all turkeys were euthanized. The fibular nerve was fully exposed bilaterally, and the nerve branch of the common peroneal nerve was identified. A miniature bipolar electrode (Harvard Apparatus, Holliston, MA) was attached to the peroneal nerve to stimulate the TC muscle. The distal TC tendon was released and attached to a force transducer (MDB-0.5, Transducer Techniques, Temecula, CA). The contraction force of the TC muscle was measured by stimulating the peroneal nerve at 10 V, duration 0.4 s and delay 2 ms with preload at 100 N (Fig 1E). After euthanizing, the TC muscle was dissected and fixed in 10% formalin solution, then sliced sequentially and H&E-stained. Stained sections were observed using an optical microscope (BZX-800, Keyence, Osaka, Japan).

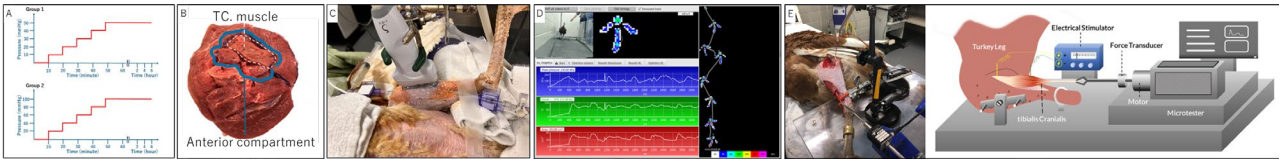

Fig 1. Experimental setups. A) Elevation of ICP: ICP was elevated in the same stepwise manner to 50 mmHg in Group 1 and 100 mmHg in Group 2 then maintained for 6 hours. B) TC muscle of the turkey lower limb: the TC muscle is in the anterior compartment beneath superficial muscle. C) Setup for ICP elevation: a 19G slit catheter was inserted into the center of the TC muscle to measure and monitor ICP, and an 18G needle was inserted into the anterior compartment, through which a 5% solution of chicken albumin was infused. D) Gait analysis using a pressure-sensitive walkway: pedobarographic and kinetic data of the turkeys were collected weekly during pre-ACS stage. E) ITF test: the peroneal nerve was stimulated electrically, and the isometric tetanic muscle force of TC muscle was measured.

RESULTS: Quantitative gait analysis revealed that the 100 mmHg ACS group had significantly longer stance time in the ACS limb at 1 week post-ACS (3542.56±908.78 ms) compared to the 50 mmHg group (833.6±121.60 ms, $p < 0.01$); also 2 weeks post-ACS, the stance time in the 100 mmHg group (2964.66 ±501.59 ms) approached that of the 50 mmHg group (2044.44±176.43 ms) but was still significantly longer ($p = 0.031$) (Fig 2A). ITF testing revealed a significant decrease in tetanic force with increasing ICP. The control leg (non-surgical) exhibited the highest force (2173.72± 925.27 mN), followed by the 50 mmHg group (1521.55±130.23 mN), with the 100 mmHg group exhibiting the lowest force (664.91±77.31 mN). The 100 mmHg group showed a statistically significant reduction in muscle force compared to both the control ($p = 0.012$) and the 50 mmHg group ($p = 0.021$) (Fig 2B). Upon dissection, it was observed that the TC muscle had turned green indicating necrosis of the muscle (Fig. 2C). Histological evaluation of the tibialis cranialis muscle in the 2-week ACS model revealed pressure-dependent pathological changes. In the 100 mmHg group, extensive muscle fiber necrosis, interstitial edema, and inflammatory cell infiltration were observed. The 50mmHg group exhibited relatively preserved muscle architecture with minimal fiber degeneration. On the control side, muscle histology appeared normal with no notable abnormalities in either pressure group (Fig. 2D).

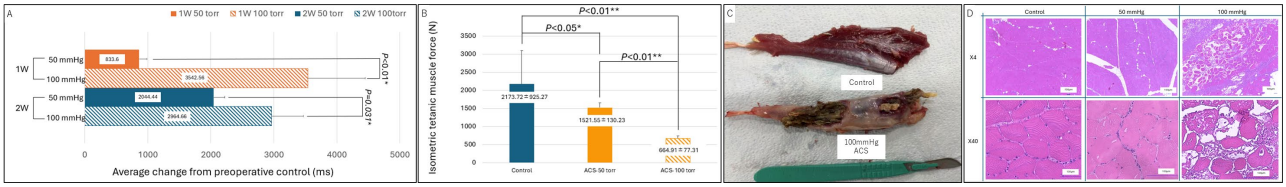

Fig 2. Results. A) Stance time of the ACS-side leg: the 100 mmHg group stance time was significantly longer than that of the 50 mmHg group. B) Isometric muscle force: the 100 mmHg group exhibited significantly lower force than that of the 50 mmHg group. C) Picture of the dissected TC muscle of the 100 mmHg group: extensive muscle atrophy and necrosis were observed. D) H&E-stained cross-sections of the TC muscle: muscle fibers in the 100 mmHg group showed severe degeneration and necrosis, including inflammatory infiltration and loss of normal architecture.

DISCUSSION: The gross green discoloration of the TC muscle observed in the 100 mmHg group is consistent with the pathophysiology of avian "green muscle disease" (deep pectoral myopathy), a naturally occurring ischemic myopathy in turkeys caused by exertion-induced compartment-like pressure elevation. In both conditions, ischemia within a noncompliant fascial compartment leads to muscle fiber necrosis, edema, and inflammatory infiltration, with the characteristic green hue resulting from biliverdin accumulation during heme breakdown. Although our model targets the hindlimb and uses controlled intracompartmental pressure elevation rather than exertion, the shared mechanism supports the face validity of this model for replicating severe ACS. The presence of green muscle changes, combined with functional and histologic findings, underscores the model's clinical relevance for studying pathophysiology and evaluating interventions for severe ischemic muscle injury.
SIGNIFICANCE/CLINICAL RELEVANCE: In the ACS turkey model, setting the ICP to 100 mmHg yields results closer to those seen in actual clinical practice.