Genetics and loading history influence the tibiotarsal mechanoresponse to controlled *in vivo* loading in young female chickens

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INTRODUCTION: Characterization of the bone mechanoresponse in birds is limited. Numerous studies have reported the effects of exercise on bone in avian species including chickens, yet few studies have examined the response to controlled loading in birds, and those that have used invasive methods in the ulna of skeletally mature turkeys and roosters. More recent controlled loading studies have primarily focused on murine models and have shown that the bone's mechanoresponse is influenced by age (1), genotype (2), and loading history (3). Whether or not this extends to avian species and chicken tibiotarsi specifically, remains unclear. We therefore aimed to investigate the effect of controlled in vivo tibiotarsal loading on bone mass and microstructure in young female chickens of different genotypes and loading history (i.e. opportunity and utilization of housing). We hypothesized that controlled in vivo loading would lead to improved microstructure, and that a strain-matched stimulus would have similar effects across genotypes but would have a stronger effect in chickens with a more sedentary loading history.

METHODS: Female chickens of two genetic strains (Lohmann Brown, B; Lohmann LSL Lite, W) were raised in either cages (Cage) that only allow for standing and sitting, or two styles of aviary housing systems (Low, High), which have been shown to lead to low and high levels of physical activity, respectively (4). At 14 weeks of age, prior to onset of sexual maturity, the right tibiotarsus (n=7 chickens/genetic strain [2]/housing [3]) was subjected to controlled in vivo axial compressive loading (peak load of 170N, which engenders medial strains of -2,800 $\mu\varepsilon$, determined by strain gauging another cohort [n=10 chickens/genetic strain/housing], while the left tibiotarsus served as a non-loaded control (Fig 1). After two weeks of once daily loading (4 Hz, 216 cycles/day, 5 days/week), chickens were euthanized, and blood was collected for measurement of serum remodeling markers (TRAP5b and P1NP). Baseline serum remodeling markers were also measured from a separate cohort of chickens (n=7/chickens/genetic strain/housing), which did not undergo in vivo loading. Fluorochromes were administered during the loading period to label newly formed bone, and tibiotarsi were μCT-imaged (9um voxel size) after sacrifice to assess the mid-diaphyseal cortical and medullary bone mineral density and microstructure, bone length, and bone curvature. Results were analyzed by ANOVA (serum markers) or repeated measures ANOVA (bone density, microstructure, length, curvature, histomorphometry), followed by Tukey's post-hoc pairwise comparisons, with significance at $p \leq 0.05$.

RESULTS: Loading did not influence bone curvature or bone length and led to lower bone mass and microstructure. Compared to the non-loaded limb, we observed lower mass and structural parameters in the loaded limbs of B Low (Ct Ar, T.Ar, Ct.vTMD, Ct.Imax, Ct.J, Md.vTMD), B High (Md.BV/TV), W Cage (Ct.Th, Ct.Ar, T.Ar, Ct.Ar/T.Ar, Ct.Imin, Md.vTMD, Md.BV/TV), W Low (Md.BV/TV), and W High (T.Ar). The effect of loading was influenced by genetic strain and housing: loading led to lower Ct.Th in W chickens, but not B when reared in Cage, but not Low or High. The effect of loading on Md.vTMD and Md.BV/TV was influenced by genetic strain. B chickens had higher Ct.Imax, Ct.Imin, Ct.J, Ct.Ar, and T.Ar, and lower Ct.ar/T.Ar, Ct.vTMD, Ct.Th, and Md.BV/TV, compared to W, and housing influenced Ct.Ar/T.Ar and Ct.Th (Cage $\leq$ Low $\leq$ High). In W chickens, serum TRAP5b was decreased in loaded chickens compared to the baseline group, while P1NP was unchanged (Fig. 2). Analyses of serum remodeling markers for B chickens and histomorphometric analysis at the tibiotarsal midshaft are ongoing (Fig. 3).

DISCUSSION: Our results surprisingly show a negative effect of in vivo loading on mid-diaphyseal tibiotarsal microstructure and density despite there being decreased circulating TRAP5b in the loaded compared to baseline W chickens. These results warrant further investigation of bone microstructure at other bone regions, such as the proximal and distal metaphyses, which may have a different strain environment during loading compared to the mid-diaphysis; microCT analyses at these other sites and finite element modeling is ongoing. Future parametric studies are also necessary to determine how load magnitude, frequency and duration may influence the mechanoresponse in lower limb bones of chickens.

SIGNIFICANCE/CLINICAL RELEVANCE: This is the first study to report the effects of non-invasive controlled loading in an avian specie, and the first study to assess the effect of controlled loading on medullary bone volume and mineral density. Genetics and loading history have been shown to influence the bone mechanoresponse in murine studies, and our findings are novel in showing that this is extended to chickens. By considering differences in physiological design and priorities between chickens and other vertebrates, it is possible to create novel comparative scenarios to understand the relationship between bone function and form and the forces driving mechanoadaptation throughout the lifetime of an organism.

REFERENCES: (1) A. I. Birkhold et al., Biomaterials, 2014; (2) N. Holguin et al., Calcified Tissue International, 2013; (3) L. B. Meakin et al., Bone, 2013; (4) Pufall et al., Animals, 2021

IMAGES AND FIGURES:

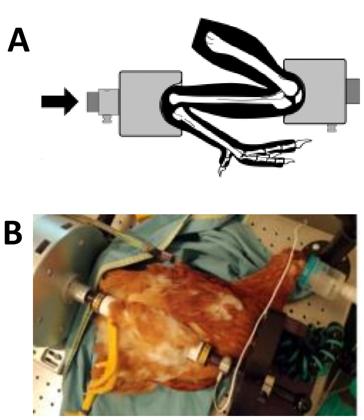

Fig. 1 A) Illustration and B) image of loading of a chicken tibiotarsus

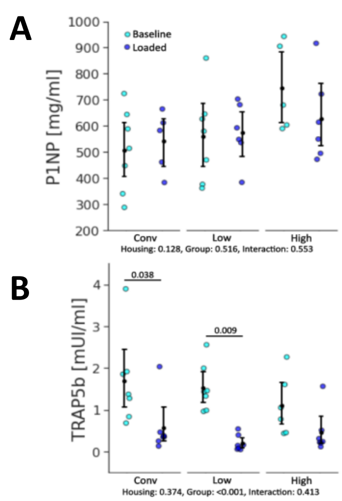

Fig. 2 Serum concentrations of A) P1NP and B) TRAP5b in baseline and loaded W chickens.

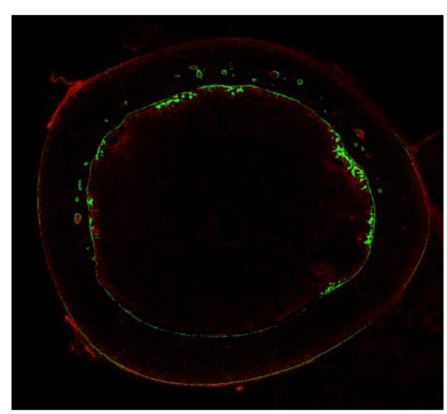

Fig. 3 Confocal image of calcein and alizarin-labelled loaded tibiotarsus