The Role of the Skeletal System in a Novel Murine Model of Chronic Metabolic Acidosis

Anna K. Peterson[a], Mikayla Moody[a], Brian Wingender[a], Katya Morozov[a], Iris Nakashima[a], Tannin A. Schmidt[a], Alix Deymier[a]
[a]University of Connecticut Health Center, Farmington, CT
Email of Presenting Author: anpeterson@uchc.edu

DISCLOSURES: All authors declare no potential conflict of interest.

INTRODUCTION: Metabolic acidosis (**MA**), a condition clinically characterized by a decrease in blood pH and bicarbonate (**HCO_3^-**), affects millions annually [1-3]. To reestablish pH homeostasis in MA, bone dissolution acts as a source of buffering alkali [1-4]. This aids in systemic pH balance; however, it also leads to bone loss and tissue functional limitations, and increases the risk of osteodystrophy, osteopenia, and osteoporosis [1-3]. Despite these negative effects of MA on the skeletal system, there are no well-established murine models of MA that recapitulate the clinical blood chemistry and bone phenotype. Previously established conventional methods of MA induction via administration of 0.28 M Ammonium chloride (NH_4Cl) in adult mice does not reproduce the MA bone phenotype observed clinically nor does it maintain long-term acidemia [5-7]. We propose a murine MA model with a graded NH_4Cl diet as an alternative to the conventional flat-dose models. We hypothesized that use of graded acid-loading for MA induction will be able to prevent compensatory buffering beyond the dietary acid loading resulting in a bone phenotype similar to that observed in clinical MA. To test this hypothesis, we have compared a graded murine model to the flat-dose model over the course of 2 weeks and characterized the blood gas and bone responses.

METHODS: *Induction of MA:* All animal experimental procedures were approved by the Institutional Animal Care and Use Committee at UConn Health Center and Columbia University, and comply with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Metabolic acidosis was induced in 4-6-month old CD-1 mice by replacing their drinking water with an aqueous solution of ammonium chloride and 5% sucrose. For the graded group, the NH_4Cl dosing began at 0.2M NH_4Cl and was increased by 0.1M every 3 days for 14 days [9]. For the flat-dose group, the NH_4Cl dose remained constant at 0.28M for 14 days [5,7]. *Blood chemistries analysis*: For each time period assessed, 200-300 µl of blood was extracted from non-anesthetized mice through submandibular puncture procedures [8]. Blood samples were analyzed using a Heska PoC Epoch blood-gas analyzer (Loveland) to obtain values of blood chemistries. *Whole-bone mechanics:* 3-point bend tests were used to evaluate the mechanical properties of the femurs. Load vs. displacement curves were analyzed using custom programs in MATLAB (MATLAB, R2020a, MathWorks) to determine structural and material mechanical properties. Stress vs. strain curves were calculated by normalizing the force and displacement using the beam span length, the bone centroid distance, and area moment of inertia as determined from micro-computed tomography (µCT). *Structural analysis:* After mechanical testing, the femoral samples were evaluated by µCT to obtain morphological information for the distal trabecular bone. The images were analyzed using the BoneJ toolbox of ImageJ (U.S. NIH) and the Bruker CTAn software. *Statistical analysis:* Statistical analysis was done using one-way ANOVA on Minitab software. A significance level of 0.05 was used for all tests. Comparisons between time periods were made using post-hoc Tukey tests. Both the flat-dose and graded models were compared to independent and concurrently run control groups. Data are mean±standard deviation.

RESULTS: The flat-dose model exhibited a significant decrease in blood pH and increase in Ca^{2+} at day 1 compared to the control mice (**Figure 1**). Following this time point, pH returned to normal levels. Additionally, Ca^{2+} levels returned to normal by day 7 but were increased on day 14. HCO_3^- in this flat-dose acidosis group was not significantly altered at any time point evaluated. The flat-dose group did not exhibit any changes in mechanical properties nor the structural metrics. Our graded model of MA showed continuously decreased blood pH and HCO_3^- and increased Ca^{2+} as observed in clinical MA, with the exception of day 3. For the graded group, the femurs exhibited a significant decrease in maximum strength for days 1 and 3 compared to the control. At these timepoints, bone volume (%) also decreases in the graded MA group (**Figure 2**). Additionally, there was a significant increase in toughness from day 1 to day 14 and a significant decrease in bending modulus from day 7 to day 14 (**Figure 3**). The other structural and material mechanical properties were not altered.

DISCUSSION: As predicted [5-7], the flat-dose model of diet-induced MA showed unreliable utility as a model of MA in mice as it did not meet the clinical criteria for MA of low pH and low HCO_3^- but instead only induced "acute" acidemia at day 1. The increase in Ca^{2+} at this time point suggests that the onset of acidemia may generate bone dissolution but neither MA-induced bone loss nor altered bone mechanics were reflected in any metrics from our mechanical or structural analysis. This suggests that buffering compensatory mechanisms, likely from the kidneys, are successfully engaged in the flat-dose model, rendering this an inappropriate model of MA in adult mice. The graded model successfully induced "chronic" MA across all time points with the exception of day 3. The measured increase in Ca^{2+} levels indicates bone mineral loss due to dissolution. The decrease in maximum strength within the graded group at days 1 and 3 is likely related to initial physiochemical dissolution leading to the formation of surface pits that may act as mechanical defects. Further supporting this notion, we see a decrease in the percent bone volume. Return to normal blood-gas values at day 3 indicates the onset of compensatory buffering mechanisms and the onset of "eubicarbonatemic" MA, where dissolution of bone functions to restore blood pH and ion homeostasis [4]. Beyond day 7, we observed a decrease in the femur modulus and increased toughness, which suggest that bone has adapted to the acid-loading through mineral dissolution, resulting in a greater contribution of collagen to the total bone mechanics. Future and ongoing studies aim to concretely determine whether bone composition and bone remodeling dynamics are altered in these models of MA independently. Ultimately, we conclude that our model of graded MA is representative of the bone phenotype observed clinically, and can maintain onset of MA for the course of 14 days.

SIGNIFICANCE/CLINICAL RELEVANCE: As this murine model accurately mimics endogenous acid production observed with clinical acidosis, future studies aimed to mitigate the deleterious effects of acidosis on bone or survey how comorbidities may progress these effects of metabolic acidosis can reliably utilize our model to investigate the onset, progression, and recovery from metabolic acidosis.

REFERENCES: [1] Kraut, J. et al. *Nat Rev Nephrol.* 2010. [2] Malluche, HH. *J Am Soc Nephrol.* 2012. [3] Kraut, J et al. *Kidney Int.* 1986. [4] Alpern, RJ. et al. *Am J Kidney Dis.* 1997. [5] Nowik, M. et al. *Cell Physiol Biochem.* 2010. [6] Imenez, S. et al. *Kidney Int.* 2020. [7] Rector FC JR et al. *J Clin Invest*. 1955. [8] O'Connell K., et al. *Comp Med.* 2015. [9] Peterson, A. et al. *Acta Biomater.* In review.

ACKNOWLEDGEMENTS: Funding was provided by Prof. Deymier's Startup Funds at UConn Health, fellowships from the UCHC Graduate School, the GEM Fellowship, and the Harriott Fellowship.

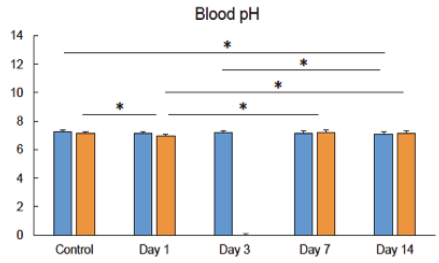

Figure 1. Blood pH data for chronic (blue) and acute (orange) acidosis across 14 days. N=16-25/day for acute and N=18-24/day for chronic.

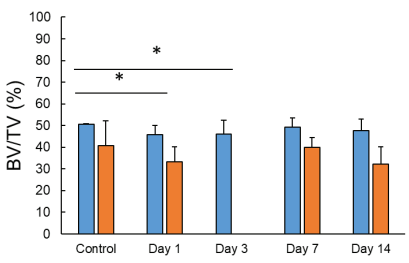

Figure 2. Bone volume (%) for chronic (blue) and acute (orange) acidosis across 14 days (N=12-14 /day).

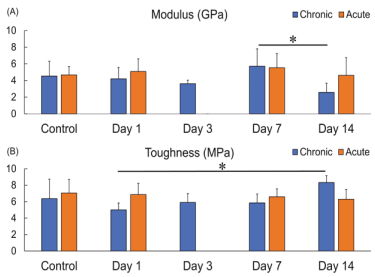

Figure 3. Mechanical properties of murine femurs. (A) Bending modulus and (B) toughness for acute (N=10-13/day) and chronic (N=5/day).