THE SKELETAL RESPONSE TO VARIED MODELS OF METABOLIC ACIDOSIS

Mikayla K. Moody (1), Anna K. Peterson (1), Brian Wingender (1), Katya Morozov (1), Iris Nakashima (1), Tannin A. Schmidt (1), Alix Deymier (1)

(1) Department of Biomedical Engineering
University of Connecticut Health Center
Farmington, CT, USA

INTRODUCTION

Metabolic acidosis (**MA**), a disease affecting millions annually, is clinically characterized by a decrease in blood pH and bicarbonate (**HCO_3^-**) [1-3]. MA can lead to bone loss, fueled by a dissolution of bone mineral that can restore pH homeostasis by yielding the required buffering alkali from bone mineral [1-4]. MA is most commonly observed in chronic kidney disease, diabetes, and critically ill patients; however, western diets may also cause onset acute MA [5-7]. MA is known to increase the risk of bone fractures since acidic environments denature collagen and affect the structure and composition of bone mineral, reducing bone strength, bone volume, and mineral stiffness [8-10].

Despite the prevalence and establishment of the negative effects of MA on the skeletal system, there are limited studies of murine models that recapitulate the clinical blood chemistry and bone phenotype of MA. Previously established methods induced MA in adult mice by administering 0.28M ammonium chloride (NH_4Cl) into their diet. However, these methods do not mimic the MA bone phenotype observed clinically nor maintain long-term acidemia [11-13]. We previously established a method to induce MA in adult mice by administering NH_4Cl into their diet, providing a higher graded dosage every three days. This graded diet mimicked the MA bone phenotype observed clinically and maintained MA over the course of two weeks [14]. To better understand the skeletal response to MA temporally and determine a dosage-dependent response to acid-loading in murine bones, we characterized the bone phenotype resulting from two models of MA over the course of two weeks and characterized the blood gas and bone responses.

Here, we propose a murine MA model with a graded NH_4Cl diet as an alternative to the conventional flat-dose models. We hypothesized that the use of graded acid-loading for MA induction will inhibit excessive compensatory buffering from the kidneys and lungs to exceed dietary acid loading, resulting in a bone phenotype similar to that observed in clinical MA.

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 flat-dose group, the NH_4Cl dose remained constant at 0.28M for 14 days [11,13]. 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, with the highest dose at 0.6M [14]. Mice were sacrificed following the time of blood-gas analysis indicated for each group. *Blood chemistries analysis*: For each time period assessed, 200-300 µl of blood was extracted from non-anesthetized mice through submandibular puncture procedures [15]. 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 tests. Both the flat-dose and graded models were compared to independent and concurrently run control groups. Data are mean ± standard deviation.

RESULTS

At day 1 of NH_4Cl administration, the flat-dose model exhibited a significant decrease in blood pH and subsequent increase in Ca^{2+} compared to the control mice (**Figure 1**). However, following this time point, the pH and Ca^{2+} returned to baseline, with Ca^{2+} levels again increased on day 14. HCO_3^- in the flat-dose group was not significantly altered at any time point evaluated. The flat-dose group also 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 bone volume (%) for days 1 and 3 compared to the control (**Figure 2**). Additionally, there was a significant increase in toughness from day 1 to day 14 (**Figure 3**). Resilience also increased significantly from days 1 and 3 to day 14 (data not shown). The other structural and material mechanical properties were not altered in the graded model.

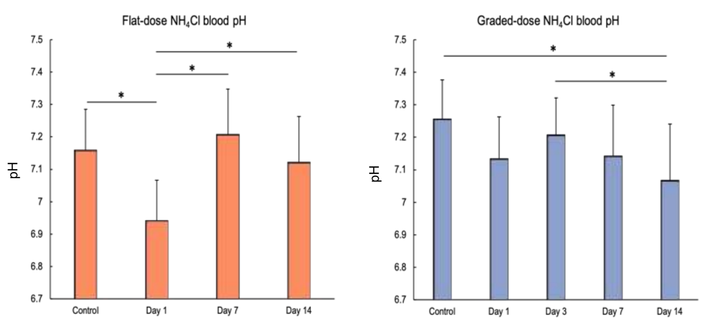

Figure 1. Blood pH data for MA across 14 days. N=16-25/day for flat and N=18-24/day for graded.

DISCUSSION

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 blood Ca^{2+} at this time point suggests that the onset of acidemia may generate bone dissolution, but phenotypic MA-induced bone loss or altered bone mechanics were not reflected in any metrics from our analysis. This suggests that compensatory buffering mechanisms, likely via the kidneys, were successfully engaged in the flat-dose model. Despite being well-established in the literature, the clinical chemistry and lack of bone phenotype renders this model of MA in adult mice unsuitable beyond an "acute" timepoint.

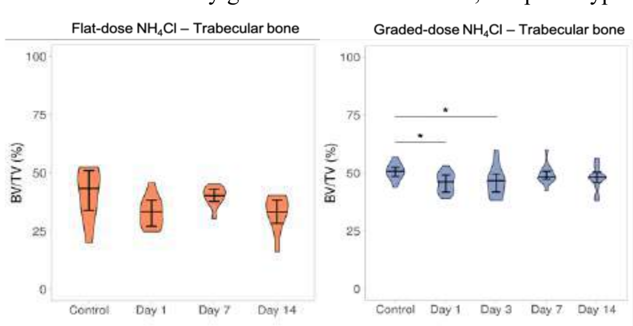

Figure 2. Bone volume (%) for MA across 14 days (N=12-14 /day).

The graded-dose model effectively induced "chronic" MA across all time points, except on day 3, based on blood pH and HCO_3^-. The return to normal blood-gas pH, Ca^{2+}, and HCO_3^- values at this timepoint implies the onset of the clinically observed phenomena of "eubicarbonatemic MA", whereby bone dissolution reestablishes blood pH and ion homeostasis [7]. Furthermore, a decrease in the percent bone volume at days 1 and 3 illustrates this dissolution. At day 14, an increased toughness and resilience suggests a greater contribution of collagen to the total bone mechanics due to bone mineral dissolution in an acidic environment. Continued bone dissolution due to acidosis could contribute to further compromised mechanics as seen clinically [16]. Future and ongoing studies aim to concretely determine whether bone composition and bone remodeling dynamics are altered in these models of MA independently. We conclude that there is both a dosage-dependent response to acid-loading in bone, and the temporal response to MA in bone drives the clinically observed phenotype. Ultimately, our model of graded MA is characteristic of the bone phenotype observed clinically and can maintain onset of MA for the course of 14 days.

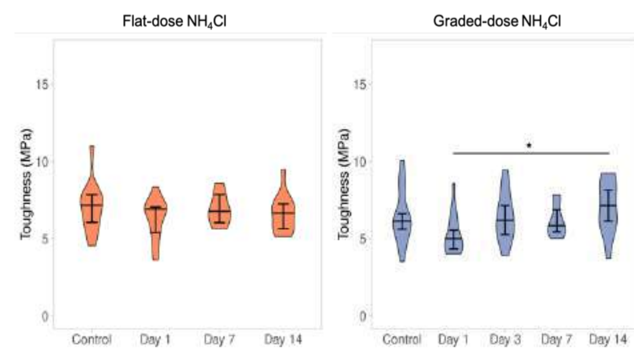

Figure 3. Toughness (MPa) for MA across 14 days (N=10-15/day).

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.

REFERENCES

[1] Kraut, J. et al., *Nat Rev Nephrol,* 6:274-285, 2010. [2] Malluche, H. H., *J Am Soc Nephrol.,* 23:525-532, 2012. [3] Kraut, J. et al., *Kidney Int,* 30:694-700, 1986. [4] Alpern, R. J. et al., *Am J Kidney Dis*., 29:291-302, 1997. [5] Kraut, J. et al., *Am J Kidney Dis*, 67:307-317, 2015. [6] Krieger, N. S. et al., *Curr Opin Nephrol Hypertens*, 13:423-36, 2004. [7] Amodu, A. et al., *Clin J Am Soc Nephrol*, 8:2034-2042, 2013. [8] Zhang, et al., *J Theor Comput Chem*, 10:245-259, 2011. [9] Usha, et al., *J Appl Polym Sci.,* 75:1577-1584, 2000. [10] Deymier, A. et al., *Biomaterials,* 127:75-88, 2017. [11] Nowik, M. et al., *Cell Physiol Biochem*, 26:1059-1072, 2010. [12] Imenez, S. et al., *Kidney Int*, 97:253-255, 2020. [13] Rector, F. C. et al., *J Clin Invest*, 34(1):20-26, 1955. [14] Peterson, A. et al., *Acta Biomater,* In Press. [15] O'Connell, K. et al., *Comp Med*, 65:96–113, 2015. [16] Sellmeyer, D.E. et al., *Am J Clin Nutr,* 73:118-122, 2001.