Metabolic dysfunction Associated Fatty-Liver Disease (MAFLD) is associated with sex-dependent skeletal changes in DIAMOND mice

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Introduction: Metabolic dysfunction Associated Fatty-Liver Disease **(**MAFLD) has enormous prevalence, affecting roughly 25% all persons globally.[1] It is associated with increased rates of osteoporosis and fracture via unknown mechanisms.[2] The recently developed DIAMOND (diet-induced animal model of non-alcoholic fatty liver disease) mouse is unique in developing MAFLD from diet alone, mimicking the human fatty liver disease phenotype in its cause, presentation, and onset. However, MAFLD associated effects on skeletal mass and strength have not been studied using this DIAMOND mouse model. Here, we describe an early, novel, investigation into MAFLD-associated skeletal fragility.

Methods: Male and female DIAMOND mice were obtained from VCU IACUC approved, ACUP-supervised studies. The mice were fed a high-fat, high-carbohydrate "Western" diet (WD) (Harlan TD.88137 and sugar water (18.9 g/L d-glucose, 23.1 g/L d-fructose)) or a control diet (CD) (Harlan TD.7012 and normal water) from 10 weeks of age to 46-48 weeks before sacrifice. The mice, at this stage, develop severe hepatic steatosis, hepatitis, and fibrosis. Tibias and femurs were isolated, imaged using micro-CT (μCT, Bruker SkyScan 1276, 7 μm resolution), and analyzed as described elsewhere.[3,4] Mechanical properties were assessed in 3-point bending (Bose ElectroForce 3200) using a 1 mm/min loading rate and an outer span of 8 mm for femurs and 10 mm for tibias. Mechanical properties were modeled using previously described methods.[5] Inter-group differences for diet and sex were assessed via a two-way ANOVA with Tukey's post-hoc testing. Multiple comparisons were controlled using the Šidák correction.

Results: Exposure to Western diet resulted in significant increases in body mass compared to CD mice (**Table 1**). In the tibia, females on WD experienced increases in cortical area fraction and ultimate stress compared to CD controls, while males experienced a decrease in cortical thickness (**Fig 1A**). Tibias from male mice from both diet groups bore greater ultimate load than female counterparts, but lower ultimate stresses (**Fig 1A**). In femurs from both male and female mice, the WD resulted in decreases in cortical area fraction and ultimate stress, with no change to ultimate load (**Fig 1A-B**).

Discussion: All bones from male mice on WD exhibited changes consistent with decreases in skeletal integrity, while femurs and tibias from female mice on WD exhibited opposing effects. No mice on WD exhibited significant increases in ultimate load, despite a ~50% increase in body mass, and much greater forces exerted on their limbs, suggesting the presence of a defect of anabolic bone formation. Indeed, osteogenesis is profoundly accelerated when adipocytes are ablated.[6] Broadly speaking, MAFLD in humans is associated with greater losses in skeletal integrity among males than females.[2] Here, we observe similar sexual dimorphism among DIAMOND mice, and evidence of a protective mechanism among females. The DIAMOND mouse is a promising preclinical candidate for the study of skeletal fragility in MAFLD. To address the retrospective nature of the study described here, we are conducting a prospective mechanical, morphologic, and molecular investigation in DIAMOND mice fed the Western diet.

Significance/Clinical Relevance: MAFLD affects 1 in 4 people globally, among whom, rates of osteopenia and risk of fracture are significantly elevated. This work will result in the development of novel therapeutics and surgical strategies for prevention and management of fracture in this important disease population.

References: [1]Younossi et al., BMJ Open. 2017. [2]Su et al., J Bone Miner Res. 2023 [3]Bouxsein et al., J Bone Miner Res. 2010. [4]Buettmann et al., Bone. 2023. [5]Turner, Burr. Bone. 1993. [6]Zou et al., Cell Metabolism. 2020.

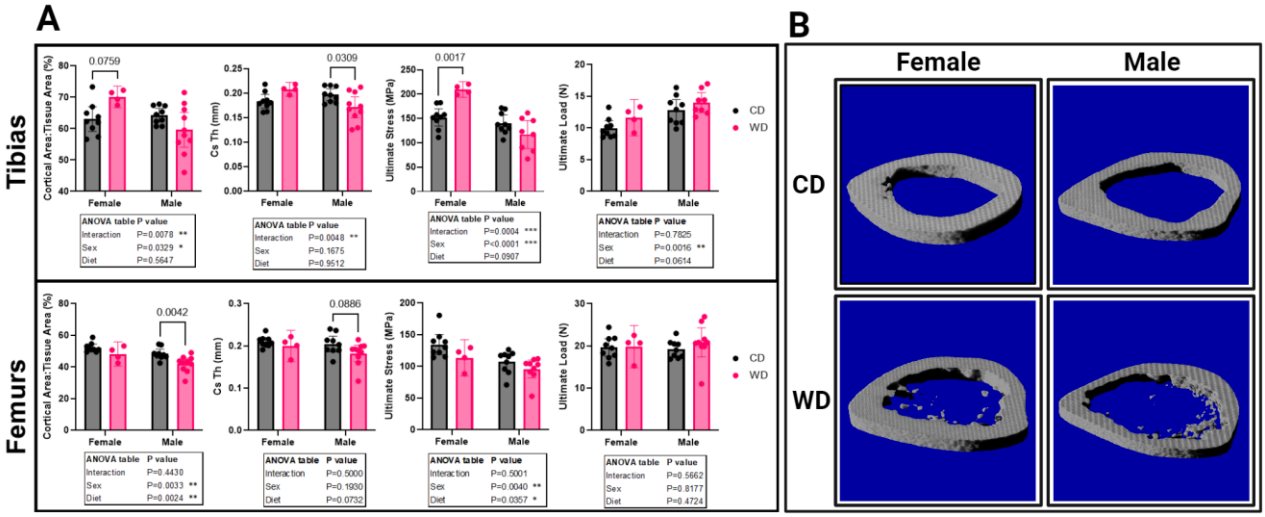

Figure 1: (A) Cortical area to total area ratio and cortical cross-sectional thickness of femurs and tibias from DIAMOND mice analyzed at a 180 μm section of the mid diaphysis. Also shown are ultimate stress and ultimate load borne in 3-point bending. (B) Representative sections of left femurs of male and female DIAMOND mice at the mid diaphysis. Reported p-values are adjusted for multiplicity.

Mouse Group	*Sample Size*	*Body Weight (g)*
Chow Diet (CD) Female	9	24 ± 2
Western Diet (WD) Female	4	36 ± 7 *
Chow Diet (CD) Male	9	27 ± 3
Western Diet (WD) Male	10	42 ± 4 ***

Table 1: Sample size and body weight data for the mice described in this study. * $p < 0.05$, *** $p < 0.001$ when compared to age- and sex-matched controls.