Loss of the Involucrin Skin Barrier Protein Impairs Bone Microstructure and Strength

Ram N. Yadav[1], Daniel Oravec[1], Autumn R. Hicks[1], Angelica Guardia De Diego[1], Harika Ileni[1], Cristina de Guzman Strong[1,2], Yener N. Yeni[1,2]
[1]Henry Ford Health, Detroit, MI, [2]Henry Ford Health + Michigan State University Health Sciences, Detroit, MI
ryadav1@hfhs.org

Disclosures: Ram N. Yadav (N), Daniel Oravec (N), Autumn R. Hicks (N), Angelica Guardia De Diego (N), Harika Ileni (N), Cristina de Guzman Strong (N), Yener N. Yeni (N)

INTRODUCTION: Involucrin is a scaffold protein specific to the epidermis and encases differentiated epidermal keratinocytes for the skin barrier. We previously identified reduced Vitamin D Receptor (*Vdr*) activity and decreased *Vdr* regulated gene expression in *involucrin* knockout (*Ivl* -/-) mice, thus revealing a regulatory relationship between involucrin and vitamin D signaling [1]. Vitamin D and *Vdr* play crucial roles in calcium absorption, which is essential for maintaining strong and healthy bones. Low involucrin expression is associated with several skin inflammatory diseases including atopic dermatitis [2], which is accompanied by diminished bone mineral density (BMD) [3] and a more than 50% increased risk of osteoporotic fracture [4]. These studies suggest a role for involucrin to not only modify skin but bone health as well. Here we test the hypothesis that involucrin affects bone quality. Therefore, the aim of this study was to determine the extent to which involucrin affects bone microstructure and strength in *Ivl* -/- mice.

METHODS: Tibias, femurs and vertebrae (L2-L6) were isolated from female adult *Ivl* -/- and wild-type (WT) C57BL/6 mice (n=8 per group, age 11 weeks) with IACUC approval and stored at -20^{o}C in saline soaked gauge prior to imaging studies. The present study included only female mice due to its preliminary nature. All bones were µCT-scanned at 14 µm voxel size in a 1% agarose medium along with a density phantom. Cortical bone parameters were calculated for tibias and femurs in a 1 mm long mid-diaphyseal section using the Slice Geometry plugin of BoneJ (Figure 1A). Cortical thickness (Ct.Th), cortical cross-sectional area (Ct.Ar), and total area (Tt.Ar: sum of cortical and bone marrow area) were measured for cortical bone (Figure 1B). Trabecular microstructure was analyzed in a 1-mm long section, offset 0.5 mm from growth plate in distal femurs (Figure 1C) and proximal tibias. For vertebrae, trabecular microstructure was analyzed within the central region, offset 0.5 mm from both growth plates. Trabecular bone volume fraction (BV/TV), thickness (Tb.Th), number (Tb.N) and separation (Tb.Sp) were measured using CT Analyser (Skyscan, v1.23.0.2). The femurs and tibias were then loaded in 3-point bending to failure to measure bone strength (F) (Figure 1D). Femoral bones were loaded in the anterior-posterior direction [5] while tibial bones were loaded in the medial lateral direction [6]. Linear mixed effect models were utilized to compare microstructural and mechanical properties of bones between WT and *Ivl* -/- mice.

RESULTS: Bone length and trabecular and cortical tissue densities were not different between *Ivl* -/- and WT mice for femurs or tibias ($p>0.2$ to $p>0.8$). Trabecular bone of *Ivl* -/- mice had lower BV/TV (20-39%, $0.001<p<0.03$), Tb.N (16-38%, $0.002<p<0.05$), and Tb.Th (2-10%, $0.004<p<0.2$), and higher Tb.Sp (11-57%, $0.002<p<0.05$) compared to WT mice at the femur, tibia (Figure 1C, Table 1), and spine (Table 2). Similarly, *Ivl* -/- mice had lower Ct.Th (9-10%, $0.02<p<0.05$, Figure 1B) and Ct.Ar (8-13%, $0.04<p<0.08$) than WT mice, while no difference in Tt.Ar (0-9%, $p>0.2$) was observed between the two groups (Table1). Strength was 9.5% ($p>0.1$) and 14.6% ($p<0.006$) lower in *Ivl* -/- mice than in WT for the femur (Figure 1D) and tibia, respectively (Table 1).

DISCUSSION: In summary, we observed impaired bone microstructure and reduced bone strength in *involucrin* knockout mice. The phenotypes that we observed in these mice differed between cortical and trabecular compartments. The difference in bone quantity between *Ivl* -/- and WT was more pronounced in the trabecular compartment (20–39% in BV/TV) than in the cortical compartment (8–13% in Ct.Ar). In trabecular bone, decreased bone quantity was primarily due to a lower trabecular number (16–38%) and to a lesser extent, trabecular thinning (2–10%). In cortical bone, the main contributor to decreased bone quantity was cortical thinning. As bone growth does not seem to be affected, the observed bone loss is likely due to increased bone resorption that warrants further investigation. Nevertheless, our results are similar to the observed bone defects in vitamin D receptor knockout models, which show substantially lesser bone in both trabecular (50–95%) and cortical (11–58%) compartments [7-9]. Osteoblast-specific *Vdr* knockout mice exhibited greater bone loss than our *Ivl* -/- mice as well as impaired bone growth [7]. Involucrin deficiency reduces *Vdr* signaling [1] but does not eliminate it, which could explain the milder bone phenotype compared to full *Vdr* knockout models. Interestingly, involucrin knockout mice did not exhibit changes in overall bone dimensions, a finding similar to that in the intestine-specific *Vdr* knockout model [7].

These similarities suggest a regulatory interaction between involucrin and vitamin D signaling pathways that involve the skin-gut axis with an impact on bone microarchitecture and strength. In addition, inflammatory skin conditions involving reduced involucrin expression [2], for example atopic dermatitis, have been associated to increased risk of bone fracture and lower BMD [3-4]. Further studies are needed to understand the mechanisms by which involucrin influences bone loss in males and females, and to evaluate bone quality in patients with skin conditions characterized by low involucrin expression.

SIGNIFICANCE/CLINICAL RELEVANCE: The present study found that lack of involucrin expression impairs bone microstructure and strength. Decrease in involucrin expression occurs in certain inflammatory skin diseases, and may contribute to deteriorated bone health in these conditions.

REFERENCES: [1] Schmidt 2023, J Invest Dermatol 143(6):1052-61, [2] Kim 2008, Clinical Immunology 126:332-37, [3] Wu 2021, Ann Transl Med 9(1):40, [4] Lowe 2020, J Allergy Clin Immunol 145(2):563-71, [5] Jepsen 2015, JBMR 30(6):951-66, [6] Kuruvilla 2008, J Musculoskelet Neuronal Interact 8(1):71-8, [7] Jiang 2023, Endocrinology 164: 1-11, [8] Yamamoto 2013, Endocrinology 154:1008-20, [9] Cui 2012, JBMR 27(10): 2097-2107.

ACKNOWLEDGEMENTS: NIH R01AR079888 (CGS). This is under a provisional patent.

IMAGES AND TABLES

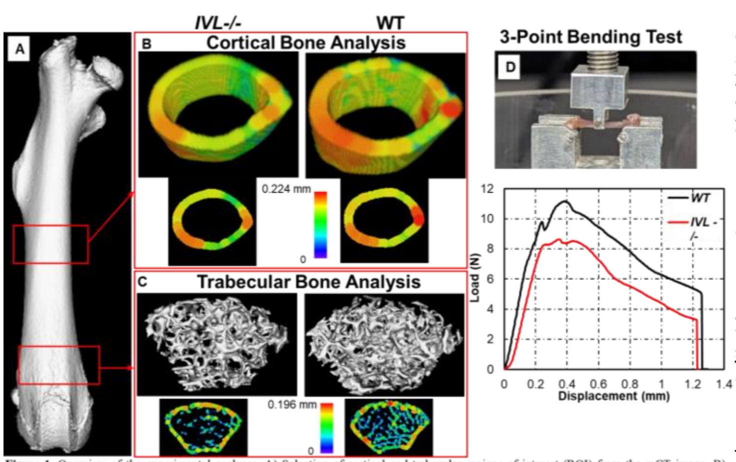

Figure 1. Overview of the experimental analyses. A) Selection of cortical and trabecular regions of interest (ROI) from the mCT image. B) Representative cross-sectional images comparing cortical bone morphology in *IVL-/-* and WT mice, with corresponding cortical thickness (Ct.Th) map. C) Representative images of trabecular microarchitecture with corresponding trabecular thickness (Tb.Th) map, highlighting lower cortical thickness, and porous and thinner trabecular structure in *IVL-/-*. D) Representative three-point bending load–displacement curves from femurs, illustrating differences in mechanical performance between *IVL-/-* and WT mice.

Table 1: Comparison of trabecular and cortical bone microstructural parameters and mechanical strength of the femur and tibia between *IVL-/-* and WT mice. Data are presented as mean ± SD, percentage difference, and corresponding p-values.

	Femur				Tibia			
Variable	***IVL-/-***	**WT**	**% Diff**	**P value**	***IVL-/-***	**WT**	**% Diff**	**P value**
Trabecular Bone								
BV/TV(%)	14.2±3.9	21.6±3.4	-34.3	**0.029**	11.7 ± 3.017	19.3 ± 2.7	-39.5	**0.013**
Tb.Th (mm)	0.056±0.002	0.058±0.002	-3.4	**0.011**	0.058 ± 0.003	0.059 ± 0.004	-1.7	0.154
Tb.N (1/mm)	2.562±0.723	3.740±0.646	-31.5	**0.046**	2.022 ± 0.502	3.275 ± 0.510	-38.3	**0.003**
Tb.Sp (mm)	0.230±0.042	0.178±0.020	29.2	**0.045**	0.357 ± 0.091	0.227 ± 0.041	57.3	**0.013**
Cortical Bone								
Ct.Ar (mm^2)	0.659±0.033	0.715±0.034	-7.8	**0.032**	0.493 ± 0.039	0.565 ± 0.034	-12.7	0.079
Ct.Th (mm)	0.169±0.005	0.186±0.009	-9.1	**0.006**	0.190 ± 0.014	0.210 ± 0.007	-9.5	**0.041**
Tt.Ar (mm^2)	1.646 ± 0.079	1.647 ± 0.046	-0.1	0.744	0.863 ± 0.058	0.948 ± 0.064	-9.0	0.280
F (N)	9.379±0.652	10.363±0.883	-9.5	0.199	7.739 ± 0.861	9.063 ± 0.673	-14.6	**0.005**

Table 2: Comparison of average bone microstructural parameters of spine (L2-L6) between *IVL-/-* and WT mice. Data are presented as mean ± SD, percentage difference, and corresponding p-values.

Variable	***IVL-/-***	**WT**	**% Diff**	**P value**
BV/TV(%)	16.4±2.4	21.8±2.9	-24.8	0.001
Tb.Th (mm)	0.056±0.004	0.060±0.005	-7.2	0.004
Tb.N (1/mm)	2.940±0.293	3.634±0.275	-19.1	0.002
Tb.Sp (mm)	0.232±0.020	0.203±0.013	14.5	0.002