

Factor VIII Deficient Mice Have Reduced Bone Mass: Chronic Effects of Factor Deficiency Without Injury and Acute Bone Loss in Injured Joints

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INTRODUCTION

While joint damage is the primary co-morbidity of hemophilia A, osteopenia and osteoporosis are highly prevalent. **This study investigates bone loss of the knee joint in skeletally mature factor VIII knockout (FVIII^{-/-}) mice. The goal was to understand the relative contribution of both chronic factor deficiency and acute joint injury on bone mass and bone turnover.** Changes in bone mass were examined by DXA and microCT. Bone turnover was assessed by serum levels of osteocalcin (as a marker of bone formation) and TRAP5b (indicating bone resorption).

METHODS

This study consisted of 4 groups: 1) Uninjured (UINJ) WT, 2) Injured (INJ) WT, 3) UINJ FVIII^{-/-}, and 4) INJ FVIII^{-/-}. Injured mice were subjected to a unilateral joint hemorrhage of the left knee at 22-weeks of age. All mice were euthanized at 24-weeks of age. DXA scans were performed at 16-, 22-, and 24-weeks with bone mineral content (BMC) analyzed. Serum was collected at 22- and 24-weeks for ELISA analysis of osteocalcin and TRAP5b. Hind limbs were processed for microCT; surface smoothness ratio, volumetric bone mineral density (vBMD) and trabecular thickness were assessed at the proximal tibia adjacent to the injured joint at two weeks after hemarthrosis.

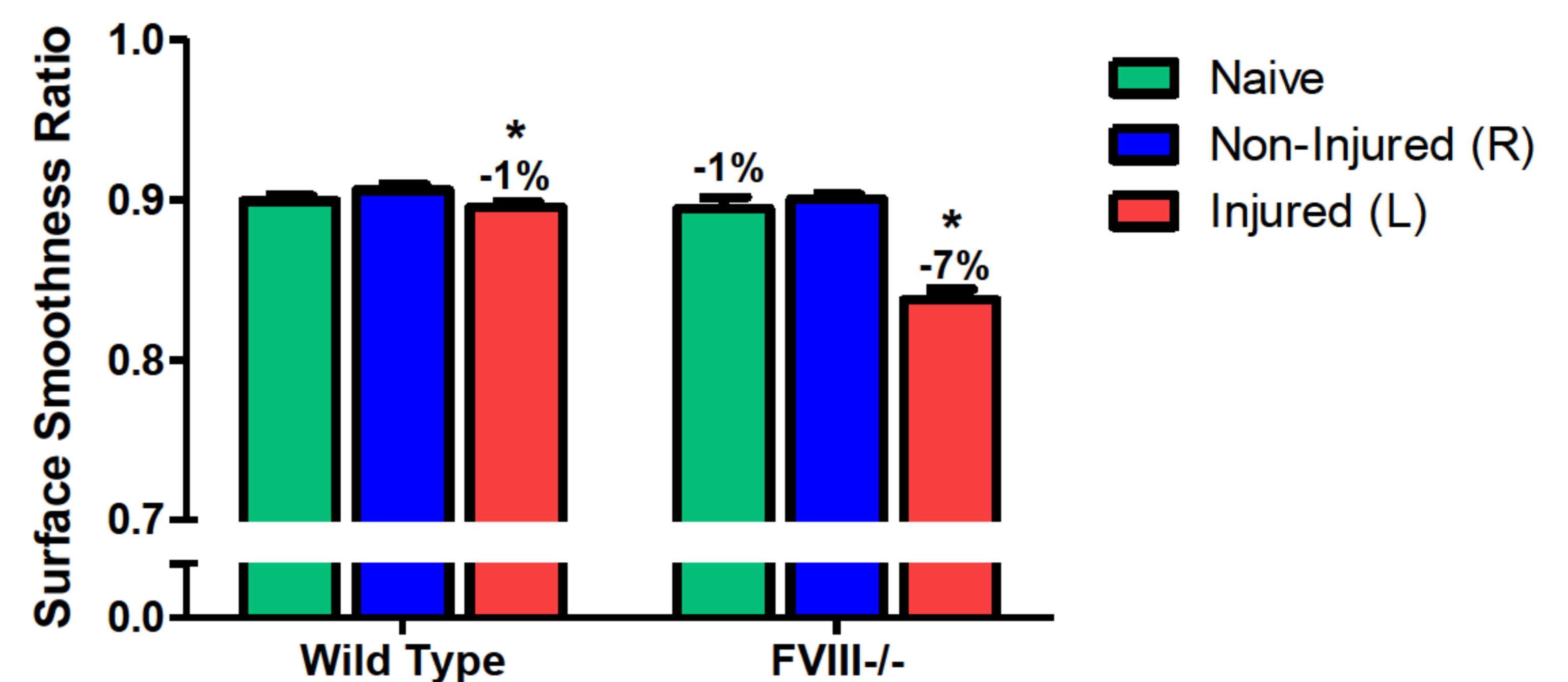
CONCLUSION

This study suggests that hemophilia A results in a skeletal phenotype that is compromised by both genotype and joint injury. By 16-weeks of age, FVIII^{-/-} mice have reduced whole body BMC that is exacerbated by joint injury. When combined with lower pre-injury bone mass, injured FVIII^{-/-} mice cumulatively have half the trabecular BMD of uninjured WT mice in the bone adjacent to the involved joint. There were not changes in circulating osteocalcin and TRAP5b which suggests that developmental and injury-induced bone loss occurs earlier than the time points examined.

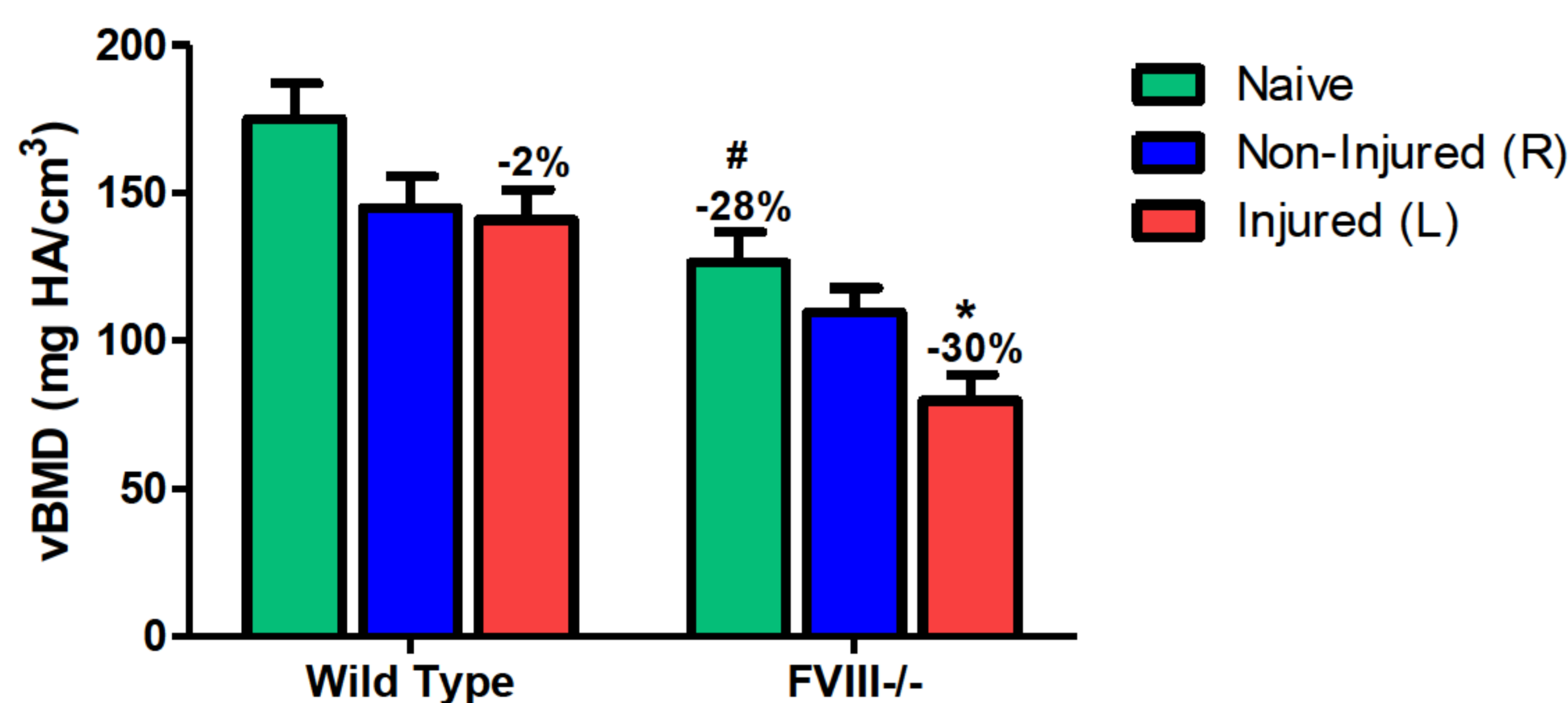
RESULTS

DXA Scanning: Small differences in bone density/content associated with hemophilia A (compared to WT) are magnified following hemarthrosis

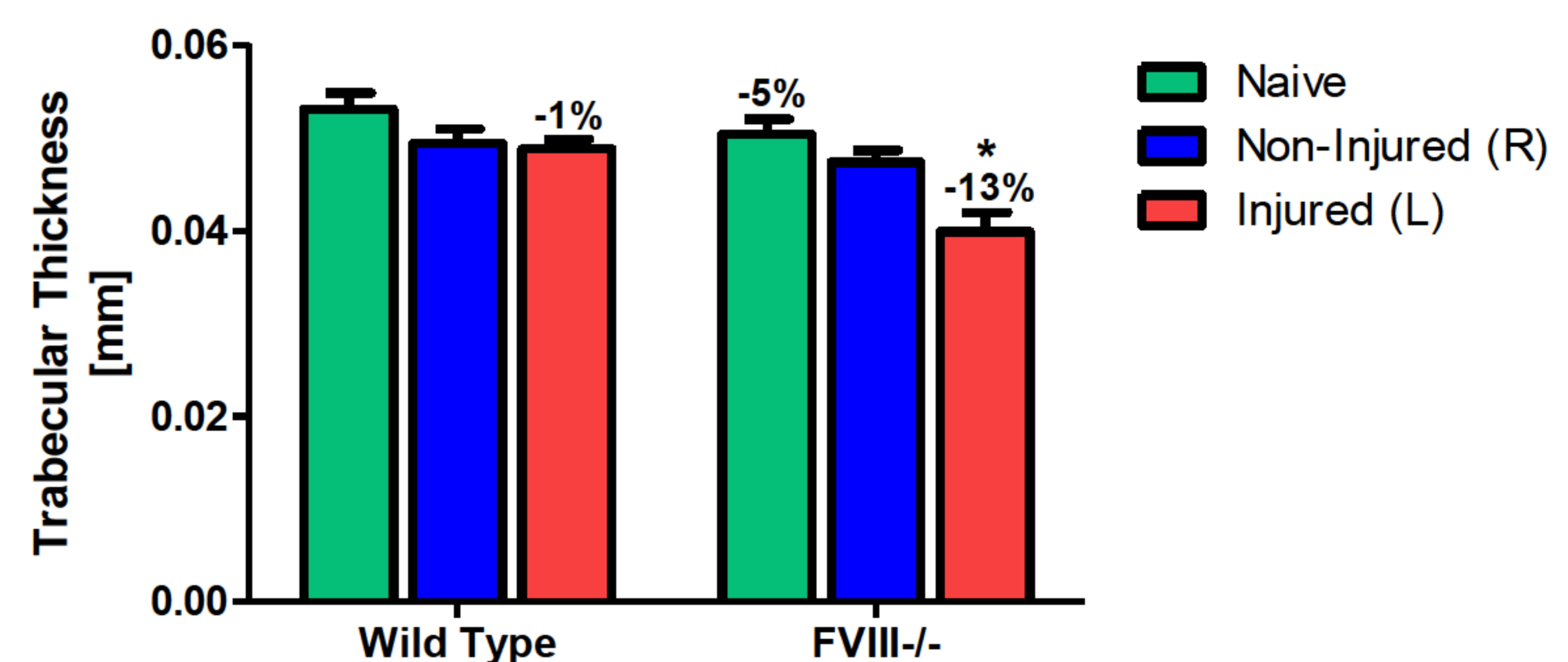
BMC(g)	WT mice	FVIII ^{-/-} mice	%	P
16 weeks of age	460.8±48.0 (n=44)	436.3±42.7 (n=47)	-5.32%	0.011
22 Weeks of age PRIOR TO joint hemorrhage	488.4±45.7 (n=44)	467.6±43.8 (n=46)	-4.25%	0.031
24 weeks of age, NO hemarthrosis	488.2±57.7 (n=16)	461.5±51.1 (n=14)	-5.47%	0.192
24 weeks of age, Two weeks AFTER unilateral knee hemarthrosis	498.5±51.7 (n=28)	456.8±42.2 (n=33)	-8.36%	0.001



- A theoretically perfectly smooth surface without either elevations (depositions) or depressions (erosions) assigned a value of 1.0. The "Surface Smoothness Ratio" is a quantitative expression of the relative loss of integrity of the articulating surface of the bones of the knee joint that has experienced hemarthrosis. Deviations from perfectly smooth surface result in values of less than 1.0.
- "Wild Type" refers to hemostatically normal littermates of FVIII^{-/-} mice.



Loss of volumetric Bone Mineral Density as measured by microCT at 10 micron resolution. Green bar ("Naive") = MicroCT findings from the proximal tibia of 24 week old mice that were NOT subjected to induced joint hemorrhage in the adjacent knee joint. Red bar = MicroCT of the proximal tibia adjacent to the injured LEFT knee from mice at two weeks after an induced left knee joint hemarthrosis. Blue bar = MicroCT of the proximal tibia adjacent to the uninjured RIGHT knee from mice that experienced an induced joint hemarthrosis in the contralateral left hindlimb knee joint two weeks earlier.



In addition to Bone Mineral Density, trabecular thickness is decreased in skeletally mature hemophilia A mice when compared to hemostatically normal littermates. Hemarthrosis acutely (within 2 weeks) results in marked further loss of trabecular bone density and trabecular thickness. Similar trends were seen in the ratio of trabecular bone to total bone volume (BV/TV) and trabecular number (data not shown).

