Total Body Irradiation Drives Deficits in Vertebral Trabecular Bone Structure but Not Mechanical Properties in Male Rhesus Macaques

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INTRODUCTION: Exposure to ionizing radiation for oncological therapy is known to increase risk for late-onset fractures in survivors. Specifically, survivors of localized radiation treatment during adulthood and survivors of total body irradiation (TBI) during childhood are at increased risk of fracture and reduced bone mineral density for many years following exposure [1,2,3]. In parallel with these observations, preclinical murine models of localized and total body irradiation have revealed compromised bone structure, altered bone turnover, and diminished mechanical behavior following radiation exposure in the short-term [4,5,6]. Limited work has been done to assess TBI's impact on bone in large-animal models, which better approximate human physiology and permit the study of the long-term effects of TBI. The primary aim of this study was to quantify the late effects of TBI on bone microstructure and trabecular bone mechanical behavior in rhesus macaque (*Macaca mulatta*) non-human primates (NHPs). A secondary aim was to quantify the effects of age on bone microstructure and mechanical behavior.

METHODS: Lumbar vertebrae were obtained post-mortem from 3 groups of skeletally-mature male NHPs: TBI (n=7, median age 19.7 years, [IQR: 18.0, 21.8]), age-matched non-irradiated (NR) controls (n=4, 19.2 [18.3, 20.2] years), and NR young NHPs (n=6, 9.1 [9.0, 9.2] years). The TBI group received an acute dose of 6.0-6.75 Gy at a median age of 8.6 years. Tissues were harvested ~12yrs after TBI. The lumbar vertebrae (L2) were collected for analysis of whole bone trabecular bone microarchitecture via microCT (μCT40, Scanco Medical, 18 μm^3 isotropic voxel size) and mechanical behavior via compression testing of a vertebral trabecular bone core (5 mm diameter, 10 mm length). Cores were embedded into shallow brass endcaps using PMMA, and apparent material properties were assessed via monotonic compression testing on a servohydraulic system (Instron 8511). The testing regimen consisted of a 10 N preload and subsequent compression to 10% strain at 0.5% strain/s. Force, displacement, and microCT geometry data were used to calculate apparent material properties of the trabecular bone, including apparent compressive modulus (GPa), ultimate stress (MPa), and toughness to ultimate stress (mJ/mm^3). Kruskal-Wallis and Dunn's post-hoc tests were used to assess group effects. If significant differences were detected, Dunn's post-hoc p-values are reported, with percent differences calculated between group medians.

RESULTS: Body mass did not vary significantly between groups (p=0.091) but tended to be higher in young NHPs and lower in older NHPs (control, TBI). TBI resulted in vertebral trabecular architectural deficits, with lower bone mineral density (**Fig. 1,** -22%, p=0.04) and bone volume fraction (**Table 1,** -31%, p=0.09) compared to NR Controls. Trabecular number trended lower in TBI, but this difference did not reach statistical significance; other microarchitecture parameters were similar among the groups (**Table 1**). There were no differences in trabecular bone apparent compressive modulus, ultimate stress (**Fig. 2**), or toughness to ultimate stress (**Table 1**).

DISCUSSION: This study is the first to examine the late-effects of TBI in a large-animal model via microCT and mechanical testing. In this NHP model of TBI, radiation exposure was associated with diminished vertebral trabecular bone density but no differences in apparent material properties a decade after exposure. Aging alone did not influence vertebral microarchitecture or mechanical behavior. The timing of TBI could explain the observed lack of change in apparent material properties. Radiation administered to children and adolescents prior to skeletal maturity has been shown to interfere with normal bone mass acquisition, elevating their long-term risks of fracture and osteoporosis [7]. In this study, however, all NHPs in the TBI group received treatment after achieving peak bone mass, which occurs by age 7 in male rhesus macaques [8,9]. Despite the limited sample size, our results suggest that TBI *after* acquisition of peak bone mass may negatively impact trabecular bone microarchitecture but does not appear to influence the apparent tissue-level properties.

SIGNIFICANCE/CLINICAL RELEVANCE: While the burden of skeletal fragility among patients undergoing radiation therapy is growing, there is an incomplete understanding of how radiation increases fracture risk. These results indicate that the lumbar vertebrae of long-term survivors of adulthood TBI are resilient, retaining or recovering their mechanical integrity over a decade post-treatment despite losses in trabecular architecture. Further investigations in skeletally mature and immature bone with larger sample sizes are needed to elucidate the mechanisms by which TBI increases fracture risk.

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Table 1. Whole-bone trabecular microarchitecture and apparent material properties in Control, TBI, and Young NHP lumbar vertebrae.

Characteristic	Young N = 6[1]	Control N = 4[1]	TBI N = 7[1]	p-value[2]
Whole Bone Trabecular Microarchitecture				
Tb.BMD (mg/cm^3)	255 (247, 266)	264 (262, 268)	206 (191, 249)	**0.034**
BV/TV (%)	0.25 (0.23, 0.27)	0.26 (0.25, 0.27)	0.18 (0.16, 0.24)	**0.047**
Tb.N (1/mm)	1.97 (1.76, 2.18)	2.02 (1.97, 2.17)	1.55 (1.48, 1.87)	0.086
Tb.Th (mm)	0.146 (0.134, 0.162)	0.141 (0.128, 0.149)	0.125 (0.113, 0.143)	0.301
Tb.Sp (mm)	0.56 (0.50, 0.64)	0.55 (0.52, 0.55)	0.66 (0.56, 0.71)	0.219
Conn.D (1/mm^3)	14 (8, 18)	16 (14, 22)	16 (7, 18)	0.837
Vertebral Trabecular Core Mechanical Properties				
σ_{ult} (MPa)	33.6 (31.0, 36.7)	26.9 (23.3, 31.9)	24.0 (21.9, 32.8)	0.316
E_{app} (GPa)	1.07 (1.00, 1.26)	0.78 (0.67, 0.94)	1.03 (0.82, 1.11)	0.230
U (mJ/mm^3)	1.13 (1.02, 1.27)	1.07 (0.72, 1.46)	0.68 (0.56, 1.17)	0.436

Abbreviations: Bone mineral density (Tb.BMD), bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (Conn.D) ultimate stress (σ_{ult}), apparent compressive modulus (E_{app}), toughness to ultimate stress (U).

[1] Median (IQR); [2] Kruskal-Wallis rank sum test; p<0.05 in bold

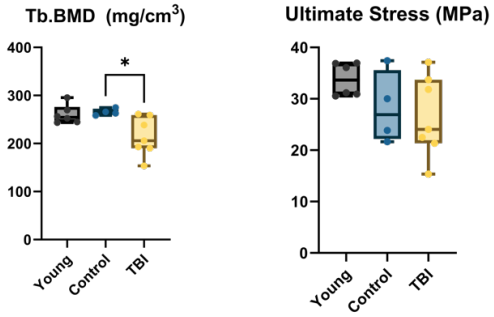

Figure 1: Whole lumbar vertebral trabecular volumetric bone mineral density (Tb.BMD, left) and trabecular bone ultimate stress (right) associated with aging and total body irradiation (TBI) in non-human primates. Data shown as median±IQR; whiskers extended from minimum to maximum. Statistically significant differences between groups denoted as * for p<0.05.