Ionizing Radiation from *Ex Vivo* Sterilization Diminishes Fatigue but Not Static Murine Vertebral Body Mechanics

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INTRODUCTION: For a variety of medical and scientific reasons, human bones can be exposed to ionizing radiation. At relatively high doses (30,000±5,000 Gy), *ex vivo* ionizing radiation is commonly used to sterilize bone allografts. However, ionizing radiation in these applications has been shown to increase risk of fracture clinically [1, 2] and decrease bone quality [3, 4]. Previously, we observed a significant decrease in compressive static strength and fatigue life of *ex vivo* whole bones exposed to x-ray radiation at 17,000 Gy and above; no changes in compressive mechanical properties were observed for radiation doses of 1,000 Gy and below [5]. The gap in doses between no mechanical change (1,000 Gy) and significant mechanical degradation (17,000 Gy) is large, and it is unclear at what dose mechanical integrity begins to diminish in whole bones, and if its effects differ in response to static versus cyclic mechanical loading. This is a major clinical concern, as trabecular and cortical bone allografts are commonly used in structural, load-bearing applications [6].

To gain insight into the effect of ionizing radiation from 1,000-17,000 Gy, we conducted an *ex vivo* radiation study on the static and fatigue mechanical properties of the vertebral whole bone. Our objectives were to: (1) quantify the effect of exposure to *ex vivo* ionizing radiation on the mechanical integrity (compressive static and fatigue) of whole bones; and (2) evaluate, if there are observed differences in mechanics, if they differ in magnitude for static versus cyclic properties. The results of this study will give insight into the need for changes in protocols for bone allograft radiation sterilization procedures.

METHODS: 20-week old female mice were euthanized and the lumbar spine was dissected using IACUC approved protocols. The lumbar vertebrae (L4-L5) were extracted from the spine and specimens were randomly assigned to one of three groups for *ex vivo* radiation exposure: x-ray irradiation at 5,000 (n = 5) or 10,000 Gy (n = 5), or a 0 Gy control (n = 4). Following irradiation, the vertebrae were imaged using microcomputed tomography (micro-CT) and then subjected to either static compressive loading to failure (L4) or uniform cyclic compressive loading (L5). During cyclic testing, samples were loaded in force control to a force level that corresponded to a strain of 0.46%, as determined in advance by a linearly elastic micro-CT-based finite element analysis for each specimen [7]. Tests were stopped at imminent fracture, defined as a rapid increase in strain. The main outcomes were the static strength (maximum force) for the static test and the fatigue life (log of the number of cycles of loading at imminent failure) for the cyclic test. Following a one-way ANOVA, group means were compared with controls using Dunnett's post-hoc test when significance was found ($p < 0.05$).

RESULTS: Compressive fatigue life was lower after irradiation, being 18% ($p < 0.01$) and 37% ($p < 0.0001$) lower for 5,000 and 10,000 Gy doses, respectively, compared to the control (5.0 ± 0.4 log(cycles); **Figure 1A**). We detected no significant effect of radiation dose for any of the compressive static mechanical properties, either for strength ($p = 0.12$; **Figure 1B**), stiffness ($p = 0.62$), or maximum displacement ($p = 0.51$).

DISCUSSION: Consistent with previous reports [8, 9], we saw a greater effect of irradiation exposure on the fatigue life than the static strength. Though lumbar vertebrae tested statically (L4) exhibited no observable difference in static compressive properties, adjacent vertebrae (L5) in the same animals exhibited a significant difference in compressive fatigue life when exposed to radiation. Despite the relatively small sample size (n = 4-5 per group), our ability to observe the detrimental effect of irradiation on fatigue life confirms that the random assignment of mice to each group had sufficient power to show an effect. Our new findings suggest that irradiation has unique effects on cyclic behavior that are not manifested in static behavior.

Understanding the mechanism driving reduced fatigue life is critical to ultimately preventing mechanical degradation with ionizing radiation exposure. Damage to collagen integrity can result in degraded mechanical performance [9, 10]. Thus, ongoing work is investigating if ionizing radiation at these doses damaged collagen through the presence of non-enzymatic collagen crosslinks [9] or cleavage of the collagen backbone [10]. Identifying the mechanism will give insight into the development of targeted radioprotectants, which could be used to maintain allograft mechanical integrity during sterilization with ionizing radiation.

Clinically, our results have implications for how allografts are most safely sterilized. Doses of 11,000 Gy have been proposed as a safer sterilization dose for allografts, as this dose achieves the same sterility level as the current standard dose of 30,000±5,000 Gy [11]. However, our new results suggest that fatigue life can be reduced at 11,000 Gy, and even at half that dose. It remains to be seen if even lower doses (2000–5000 Gy) can achieve biological sterilization without compromising the bone quality, and in doing so, we recommend that both static and cyclic behavior be assessed.

SIGNIFICANCE: These findings suggest that doses well below sterilization standards (30,000±5,000 Gy) and a proposed alternative (11,000 Gy) may compromise the mechanical integrity of bone allografts, making them more susceptible to failure under cyclic loading.

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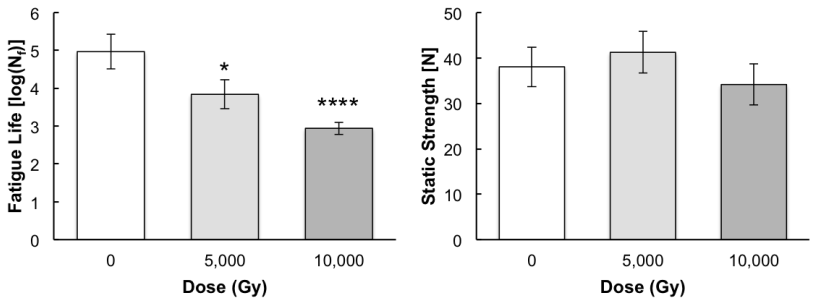

Figure 1: Effect of *ex vivo* x-ray radiation exposure on cyclic (A) and static (B) mechanical properties of mouse lumbar vertebrae. Data are shown as least-square means. Error bars denote 95% confidence intervals. * $p < 0.05$, **** $p < 0.0001$, using Dunnett's post-hoc test.