Ionizing radiation from *ex vivo* sterilization diminishes collagen integrity and vertebral body mechanics

Shannon R. Emerzian[1], Megan M. Pendleton[1], Alfred Li[2], Jennifer W. Liu[3], Simon Y. Tang[3], Joshua S. Alwood[4], Grace D. O'Connell[1], Tony M. Keaveny[1]

[1]University of California, Berkeley, CA, [2]University of California, San Francisco, CA, [3]Washington University, St. Louis, MO, [4]NASA Ames Research Center, Moffett Field, CA

INTRODUCTION: Bones can be exposed to a wide range of ionizing radiation levels, from accumulative 50 Gy for cancer treatment to acute 35,000 Gy for allograft sterilization. Ionizing radiation has been shown to decrease bone quality[1,2] and degrade collagen integrity[2,3] in cortical samples, but its effects on mechanical or biochemical properties of whole bones are not fully understood. Therefore, we conducted an *ex vivo* radiation study to investigate the non-cellular effects of ionizing radiation on collagen network structure (crosslinks and fragmentation) and monotonic compressive strength of whole murine vertebral bodies.

METHODS: Mice were euthanized and lumbar vertebrae were extracted. Endplates, posterior processes, surrounding musculature and soft tissues were removed to isolate the vertebral bodies. Specimens were randomly assigned to one of five groups, each with a different dose *ex vivo* x-ray irradiation: 0 (control), 50, 1,000 17,000 or 35,000 Gy. After irradiation, vertebral bodies were subjected to monotonic compressive loading to failure at 0.01 mm/s. Adjacent vertebral levels were used for collagen network assessment: a fluorometric assay quantified relative amounts of collagen crosslinks[2], and a bioanalyzer was used to evaluate collagen chain molecular weight distribution using automated electrophoresis.

RESULTS: Collagen crosslinks increased significantly for all irradiated groups ($p<0.0001$, Fig.1A). By contrast, the radiation effects on collagen chain molecular weight distribution and monotonic strength were only evident for the doses of 17,000 and 35,000 Gy. At those doses, the amount of smaller collagen chains increased, indicating a rise in collagen fragmentation (Fig.1B), and the monotonic strength decreased by 50–67% ($p<0.0001$, Fig.1C).

DISCUSSION: Our new findings of irreversible mechanical degradation and collagen network damage are observed beginning at an *ex vivo* dose of 17,000 Gy, which is two-fold lower than standard allograft sterilization (35,000 Gy). It is not clear at what dose the damage starts, since we did not include doses between 1,000 and 17,000 Gy. Previous work showed either increased collagen crosslinking[2] or fragmentation[3] to be the underlying cause of mechanical degradation. Here, we demonstrate increases in collagen crosslinking and fragmentation occur in parallel with decreased strength. Taken together, these findings raise questions regarding the mechanical integrity of sterilized bone allografts.

REFERENCES: [1] Wernle, J +, *J.Biomech*, 2010; [2] Barth, H.+, *Biomaterial*s, 2011; [3] Burton, B.+, *Bone*, 2014

ACKNOWLEDGEMENTS: NSF GRF 1752814, NASA PECASE, NSTRF NNX14AM56H, NIH AR069804.

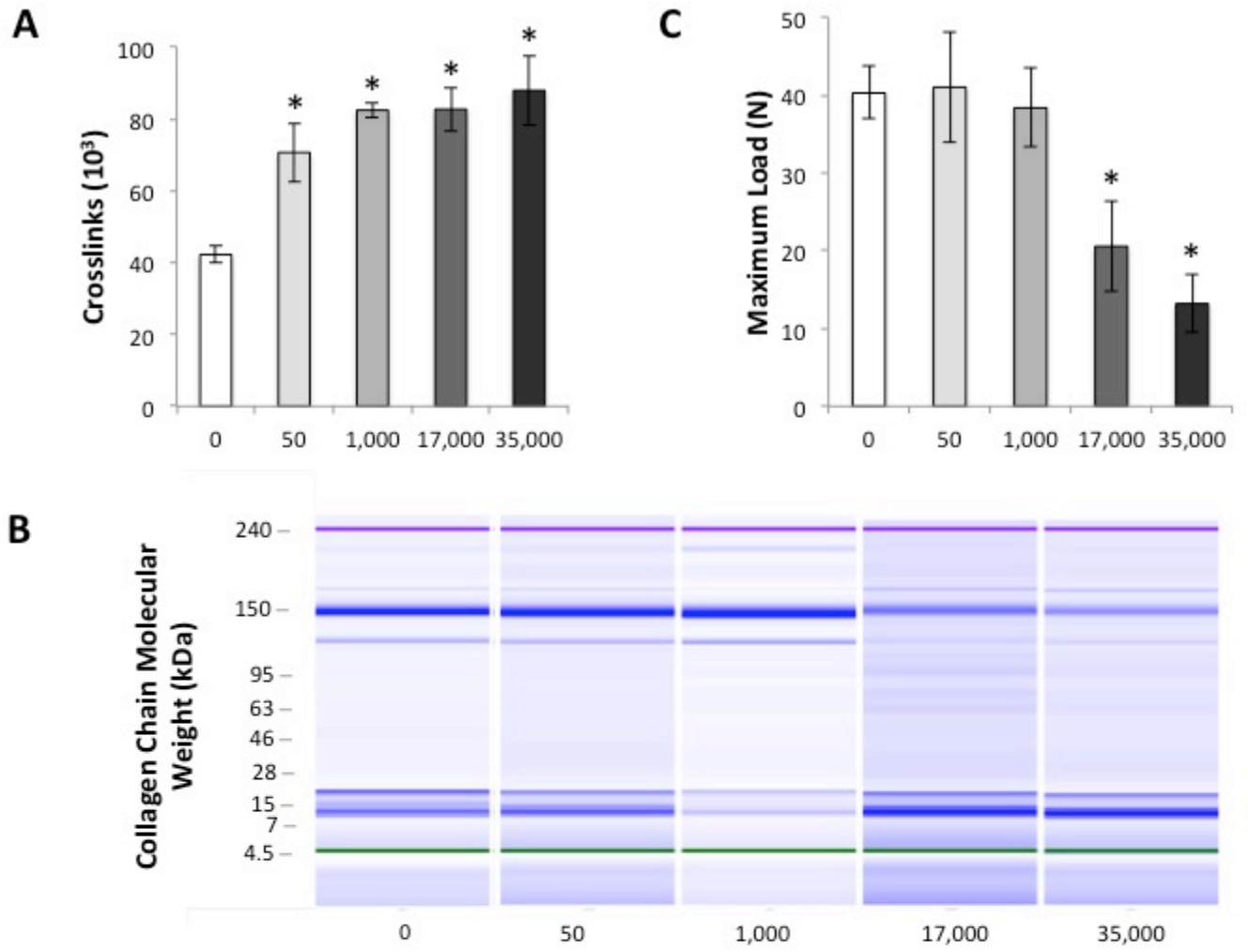

Figure 1: (A) Total number of fluorescent (non-enzymatic advanced glycation end products) collagen crosslinks [ng quinine/mg collagen]. The number of crosslinks was significantly increased for all irradiated groups, but these groups were not significantly different from one another ($p>.05$); **(B)** An example image of the bioanalyzer gel from demineralized, pepsin-digested murine bone collagen; y-axis represents collagen molecule molecular weight distribution in kDa. Irradiated bone collagen ≥17,000 Gy had a less dense band at 140-150 kDa and smearing at lower molecular weight, which is indicative of collagen fragmentation increasing due to irradiation; **(C)** Maximum load at failure for monotonic testing; [x-axis is radiation dose in Gy; error bars represent standard deviation; * = $p<.0001$ vs. 0 Gy control for all]

Figure 1: (A) Total number of fluorescent (non-enzymatic advanced glycation end products) collagen crosslinks [ng quinine/mg collagen]. The number of crosslinks was significantly increased for all irradiated groups, but these groups were not significantly different from one another ($p>.05$); **(B)** An example image of the bioanalyzer gel from demineralized, pepsin-digested murine bone collagen; y-axis represents collagen molecule molecular weight in kDa. Irradiated bone collagen ≥17,000 Gy had (a less dense band at 140-150 kDa and smearing at lower molecular weight, which is indicative of collagen fragmentation increasing due to irradiation; **(C)** Maximum load at failure for monotonic testing; [x-axis is radiation dose in Gy; error bars represent standard deviation; * = $p<.0001$ vs. 0 Gy control for all]