INTRODUCTION:
Older humans and animals have inferior fracture healing capabilities than younger individuals, but the biologic factors responsible are not well understood. We first hypothesized that the primary deficit with age-related impaired fracture healing occurs from decreased osteogenesis. Osteogenesis is critical in bone bridge formation, so pathways affecting osteoblast differentiation may be implicated. The canonical Wnt pathway is important for the induction of osteoblastogenesis and the stimulation of pre-osteoblast replication. This pathway plays an important role in fracture healing and it may be involved in aging processes in bone. Despite its involvement in bone repair and aging, the role of the Wnt pathway in age-related impaired fracture healing has not been explored. Our second hypothesis was that differential regulation of the Wnt signaling pathway plays a role in impaired fracture healing capacity of older mice.

METHODS:
We used a previously established open femoral shaft fracture model to explore age-related changes in endochondral fracture healing. After IACUC approval, fractures were surgically created in the right hindlimbs of C57BL/6 young (7 wk old, n=45) and old (8-9 mo old, n=45) mice followed by internal fixation with intramedullary 25-gauge needles. Mice were euthanized on post-operative days 1, 3, 5, 10, and 14 for analysis. To explore age-related changes in endochondral fracture healing, after IACUC approval, fractures were surgically created in the right hindlimbs of C57BL/6 young (7 wk old, n=45) and old (8-9 mo old, n=45) mice followed by internal fixation with intramedullary 25-gauge needles. Mice were euthanized on post-operative days 1, 3, 5, 10, and 14 for analysis by microcomputed tomography (μCT), histology, immunohistochemistry (IHC), or gene microarray.

RESULTS:
The total mineralized callus was selected and analyzed for total mineralized callus volume (TV, mm³), bone volume (BV, mm³), bone volume fraction (BV/TV), bone mineral density (BMD, mg/cc), and tissue mineral density (TMD, mg/cc). Total callus size was estimated from callus dimensions in each age group at days 10, 14, and 21 (n=8/gp). Diameters were measured in triplicate and averaged. The cross sectional area was calculated at the level of the maximum diameter assuming an oval area. For histology, the specimens were fixed (10% NBF), decalcified, paraffin embedded, sectioned, and stained with Safranin-O and Masson’s trichrome. IHC localization (n=8/gp) of the active form of β-catenin was used as a marker for canonical Wnt activity. IHC was performed using a mouse monoclonal antibody against the active, unphosphorylated form of β-catenin (Millipore, Billerica, MA). A negative control was included in each run. The area of B-catenin-positive staining was quantified (Osteo II, Bioquant, Nashville, TN).

DISCUSSION:
Old mice had impaired hard callus and bone bridge formation based on our μCT and histology data. Despite similar total mineralized callus volumes at day 14, old mice had lower bone volumes and bone volume fractions, suggesting deficiencies in bone formation (d10 and d21 analysis pending). TMD was also reduced, indicating that the bony calluses of old mice are less mineralized that those of their younger counterparts. Old calluses were still largely cartilaginous at later time points with bone formation on histology than young mice. Thus, old mice were less capable of transforming soft callus into hard callus and relied on large, cartilaginous calluses for fracture stabilization, accounting for the larger total callus cross sectional areas at later time points. The age-related differences in active β-catenin IHC staining implicates differential Wnt activity in the impaired healing process in old mice. Canonical Wnt activity is known to vary during the different stages of endochondral fracture healing. β-catenin activity is high at early stages of chondrogenesis, low as chondrocytes become hypertrophic, and high again when osteoblasts populate the callus tissue. Perhaps the increased day 5 β-catenin IHC staining in the old mice represented delayed endochondral healing, as the chondrocytes were not yet hypertrophic and still had high levels of active β-catenin. As hard callus formation progresses and β-catenin activity is high in osteoblasts, we expect young mice to have increased staining at days 14 and 21 (d14 and d21 IHC pending). Our gene microarray data further supports our hypothesis that differential Wnt regulation plays a role, and validation is currently underway with qPCR. Further mechanistic work will clearly elucidate the relationship between differential Wnt regulation and deficits in hard callus formation with aging.

SIGNIFICANCE:
Differential Wnt activity plays a role in age-related impaired fracture healing, which suggests that we may be able to target Wnt effectors as means of enhancing bone repair in the elderly.

ACKNOWLEDGMENTS: Funding provided by NIH R01-AR056802

REFERENCES: