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MEETING REPORT

Session on Bone Aging at the 2012 Annual Meeting of the Orthopedic Research Society

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Meeting Report from the Orthopaedic Research Society Annual Meeting 2012, San Francisco, CA, USA, 4–7 February 2012

The 2012 annual meeting of the Orthopedic Research Society convened a session on Bone Aging with 7-min oral presentations that concerned the effects of age on a range of topics from intracellular signaling to extracellular matrix composition and quality to skeletal responsiveness to exercise.

Mutyaba *et al.*[1] compared proliferation, differentiation, and signaling in pools of mesenchymal progenitor cells (MSCs) isolated from 5-month and 25-month-old C57BL/6 mice from the NIA aged-rodent colony. After 4 days *in vitro*, there was a greater number of MSCs from the young than from the old mice. There was less differentiation of both osteoblast and adipocyte lineages in MSCs from the old mice than from the young mice. Based upon the authors' previous interest in the effects of Jagged-1 to activate Notch signaling, they seeded MSCs from young and old mice onto wells with and without pre-bound Jagged-1. There was greater basal expression of Notch target genes in MSCs from young mice. Because there was equivalent responsiveness to Jagged-1 in both populations of MSCs, contrary to expectations, they concluded that Jagged-1 would not be an approach to rescue the proliferation and differentiation defects in MSCs from old mice.

Kitay *et al.*[2] tested whether Wnt signaling in fracture callus differed in 7-week-old and 8- to 9-month-old mice. They showed that maturation of fracture callus was impaired in the old mice, with significantly greater immunohistochemical staining for β-catenin at day 10, compared with callus in the young mice. There was differential expression of some Wnt pathway genes at early timepoints that may account for the endurance of the cartilage phase of healing in the old mice.

Glowacki *et al.*[3] showed an age-associated increase in spontaneous *in vitro* osteoclast differentiation of marrow cells from subjects between 36 and 87 years of age. Accordingly, there were age-related increases in constitutive expression of several osteoclast regulatory factors, including the pro-osteoclastogenic receptor activator of nuclear factor-κB ligand (RANKL), its receptor RANK, and c-fms (the receptor for the pro-osteoclastogenic factor M-SCF), and a decrease in osteoprotegerin (OPG), an inhibitor of osteoclastogenesis. The ratio of RANKL/OPG in human marrow stromal cells was increased with age and may be a target for rejuvenation. Consistent with this, it was previously reported that marrow from subjects taking a bisphosphonate had a lower RANKL/OPG ratio and generated 21% of the osteoclasts in control cultures from age-matched subjects.[4]

Turunen *et al.*[5] used Fourier transform infrared (FTIR) microspectroscopy to unravel the effects of age (26–82 years) and anatomical location on the molecular composition of human trabecular bone. They chose the femoral neck, trochanter major and calcaneus as sites subjected to different mechanical loading, respectively, compressive forces, tensile forces, and impact loads. Analysis of mineral/matrix and carbonate/matrix ratios indicated that they increase with age in the trochanter and calcaneus. The effects of age on the composition and structure were correlated for the trochanter major and calcaneus. The composition of the femoral neck did not change with age. It was concluded that mechanical forces in different anatomical sites influence bone turnover and remodeling rates that lead to age-related changes in bone composition.

Karim *et al.*[6] used femurs from seven age groups of C57BL/6J mice between 3 weeks and 2.6 years of age to follow changes in fracture toughness and bone content of osteopontin (OPN), osteocalcin (OC), and advanced glycation end-products (AGEs). Fracture toughness was calculated at fracture initiation and propagation. There was novelty in the method to create a notch on the femur as a model for a pre-existing crack followed with load-deformation curves. There were wide variations in all parameters and gender effects were not included. Fracture toughness and OPN and OC content showed a trend for a peak at 64 weeks and a trend to decline thereafter. There was greater AGE content in bone from the oldest group, leading to an inverse correlation with fracture toughness. The authors reported statistically significant correlations for both OPN and OC with fracture toughness; the authors highlighted the importance of those non-collagenous proteins in bone's fracture properties.

Shirazi-Fard *et al.*[7] used 6-month-old male Sprague–Dawley rats to determine the effects of exercise on bone loss follow-

ing double bouts of disuse with the unloaded hindlimb model. Following 4 weeks of unloading, test rats were trained to an exercise regimen of jumping with gradually (7 weeks) increasing weights in a vest strapped to their backs. As expected, bone mineral content (BMC) and volumetric bone mineral density (vBMD) were decreased following disuse. Exercise significantly enhanced recovery of BMC and vBMD to a greater extent than in rats that did not exercise following disuse. Both BMC and vBMD were increased with exercise and were maintained after a second bout of unloading, in contrast to the bone loss in rats without exercise subjected to a second bout of unloading and to another control group that received the first bout at that later timepoint. Thus, adding exercise not only improved recovery following disuse, it also inhibited bone loss after the second bout of disuse. The benefits of exercise were site-specific; both BMC and vBMD were enhanced for the proximal tibia metaphysis but only the BMC was elevated at the femoral neck. The endurance of the beneficial effects was striking.

Two papers from one research team in the session on microRNA pertained to mouse models of skeletal aging.[8,9] In both, Zhang *et al.* summarized as-yet unpublished data of an age-dependent increase in micro RNA (miRNA)-214 in human bone and an inverse correlation between miRNA-214 and bone formation. One of the major targets of miRNA-214 is ATF4, a key transcription factor in osteogenesis. Those clinical studies inspired the mouse studies presented at the meeting. Both studies tested the hypothesis that therapeutically targeting miRNA-214 would stimulate osteogenesis in models of diminished bone formation. With a recently published delivery system,[10] Antagomir-214 or a negative control Antagomir or vehicle was administered intravenously (daily for 3 days and then fortnightly three times) to 18-month-old mice that had been ovariectomized at age 6 months. Evaluation by μCT and dynamic bone histomorphometry showed bone loss and reduced bone formation in control mice. Trabecular bone mass, trabecular bone architecture, and bone formation parameters were significantly increased in mice treated with Antagomir-214. Treatment with Antagomir-214 significantly increased ATF4 protein and decreased miRNA-214 levels in bone. The Antagomir-214 was also tested in mice subjected to hindlimb suspension.[9] Mice received three injections of Antagomir-214 or a negative control Antagomir or vehicle prior to hindlimb suspension for 4 weeks. MicroCT analysis showed loss of trabecular bone mass and architecture in the control mice. Treatment with Antagomir-214 reduced the loss of bone and increased bone formation parameters. Treatment with Antagomir-214 significantly increased serum OC, mRNA for OC and alkaline phosphatase, and the amount of ATF4 protein in bone and decreased miRNA-214 levels in bone. In summary, administration with Antagomir-214 was effective for *in vivo* gene silencing and increased bone formation in mice with established post-OVX osteopenia and in mice subjected to hindlimb suspension.

In conclusion, these presentations indicate progress in applying state-of-the-art technologies, such as signaling pathway analyses, microRNA arrays, antagomir delivery, and FTIR microspectroscopy, to discover mechanisms of skeletal aging. Site-specific effects of age on trabecular bone composition and of beneficial effects of exercise show the importance of descriptive baseline data to optimize study design. Animal studies included comparisons of young and old mice and their cells, and the validated models of post-OVX osteopenia and hindlimb unloading. Although investigations with mouse and rat models continue to predominate, studies with human bone cells, marrow cells and matrix showed their value for testing hypotheses for relevance to human aging.

Conflict of Interest

The author declares no conflict of interest.

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