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## MEETING REPORT

# Biomechanics & mechanobiology (ASBMR 2012)

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### Diffuse Damage Repair

Linear microcracks in bone that form during repetitive loading are repaired via targeted remodeling, triggered by osteocyte apoptosis. But what about bone damage that happens at a smaller length scale, that is, diffuse damage? Seref-Ferlengez *et al.*[1] created diffuse damage (without microcracks) by loading rat ulnae *in vivo*, and found that acutely loaded bones were less stiff ($-15\%$) than control bones. After 14 days the amount of diffuse damage was significantly reduced, and, most importantly, the stiffness was back to normal levels, that is, the damage was 'repaired'. As it was recently reported by the same research group that diffuse damage does not trigger osteocyte apoptosis or targeted bone remodeling,[2] the mechanism by which bone repairs diffuse damage remains to be determined.

### Raloxifene Improves the Material Properties of Dead Bone

Raloxifene is a selective estrogen receptor modulator that significantly reduces vertebral fracture risk, with relatively modest changes in bone mineral density. Previous studies in dogs have shown that raloxifene increases bone toughness at the material level, independent of bone size or architecture.[3] Gallant *et al.*[4] performed a series of *in vitro* experiments exposing *post mortem* bone samples to raloxifene, and were able to re-capitulate the increase in toughness observed *in vivo*. Their results show that toughness increased via post-yield energy absorption, and was associated with an increase in bound water. In addition, modification of raloxifene by removing hydroxyl groups negated the effect. In summary, a cell-independent raloxifene–bone interaction enhances bone material properties.

### *SOST*/Sclerostin

Downregulation of *SOST*/sclerostin (an antagonist of Wnt signaling) occurs after mechanical loading *in vivo*, and is likely an important mechanism for loading-induced increases in bone formation. Delgado-Calle *et al.*[5] examined a possible role for nitric oxide (NO) in the mechanoregulation of *SOST*. After subjecting human osteoblasts to pulsatile fluid flow (PFF), they found increased NO synthesis and decreased *SOST* expression, as expected. However, the addition of a NO synthase inhibitor blocked the decrease in *SOST* expression under PFF conditions, whereas the addition of NO to static cells downregulated *SOST* (mimicking the effects of PFF). Thus, a possible connection was identified between two factors (NO and sclerostin) known to have important roles in bone mechanoresponsiveness.

The development of sclerostin antibodies as osteoanabolic agents may soon provide clinicians with new options for treatment of osteoporosis. Because mechanical loading exerts its anabolic effects on bone at least in part via reducing the expression of *SOST*/sclerostin, there is a question if the anabolic effects of sclerostin antibodies take place only under normal mechanical loading. Bouxsein *et al.*[6] addressed this question in a study of mice exposed to microgravity during a 13-day mission on the US Space Shuttle Atlantis. Young (9-week), female C57Bl/6 mice received 100 mg kg$^{-1}$ sclerostin antibody (SclAb, Amgen, Thousand Oaks, CA, USA) or vehicle (Veh) 1 day prior to launch; spaceflight groups (FL) were compared to ground-control (GR) groups. Spaceflight caused the expected reductions in bone mass, microstructure and strength, but these deleterious changes were mitigated by SclAb treatment. In fact, FL-SclAb mice had similar or better bone properties than GR-Veh mice. Additionally, bone histomorphometry and serum markers indicated that SclAb increased bone formation parameters similarly in both GR and FL groups, that is, independent of the loading condition. Thus, the anabolic effects of SclAb occurred in the absence of mechanical loading.

Pflanz *et al.*[7] considered this issue from the other side of the loading spectrum, using *in vivo* compressive loading of mouse tibiae with and without SclAb treatment. Loading ($-1200\,\mu\varepsilon$, 432 cycles per day, 4 Hz) was applied to the left leg of old (78-week), female C57Bl/6 mice 5 days per week for 2 weeks. Some mice were treated with SclAb (25 mg kg$^{-1}$, twice weekly). Both loading and SclAb increased trabecular and cortical bone mass when compared to non-loaded and non-treated conditions, respectively. However, loading had no effect in mice receiving SclAb. Thus, the combination of mechanical loading and SclAb treatment did not produce additive effects, consistent with evidence that they act through a common pathway.

### Tibial Loading in Mice: Effects of Aging

There were numerous studies using axial tibial compression in mice, with several focused on the influence of age in C57Bl/6 mice. Holguin *et al.*[8] reported that young-adult (5-month), middle-aged (12-month) and old (22-month) female mice all exhibited anabolic cortical responses to 2 weeks of daily tibial compression ($-2200$ and $-3000\,\mu\varepsilon$; 1200 cycles per day, 4 Hz). Loaded tibias from each age group had increased periosteal bone formation and increased total area, although 5-month-old mice were significantly more responsive on the *periosteal* surface than the two older age groups. In contrast, there was no effect of age on the *endocortical* response. Lastly, increases in trabecular bone volume were only observed in the 5-month-old group. Castillo *et al.*[9] examined young-adult (4-month) and middle-aged (12-month) female mice subjected to tibial compression on alternate days for 2 weeks (60 cycles per day, 2 Hz). They also reported that older mice had diminished *periosteal* responses, but that the *endocortical* response was actually greater in older mice. Lastly, Meakin *et al.*[10] examined the dose–response between applied strain and increased cortical area in young-adult (4-month) and old (19-month) female and male mice. They found that in females, the strain threshold for a bone response did not differ between young and old mice, although the slope of the response vs strain curve was lower in old female mice (that is, less responsive). In contrast, in males, the strain threshold was greater in old mice than young mice, but the slope of the response–strain curves did not differ. Based on *in vitro* experiments on primary osteoblasts, the authors observed age-related reductions in strain-activated cell proliferation, and suggested that this may explain the differences in responses to loading *in vivo*. In summary, three research groups reported that bones from old mice are mechanoresponsive, although periosteal responses are diminished in old mice compared to young adults. The mechanisms underlying the age effects remain to be determined, although osteoblast proliferation may play a role.

### Muscle–Bone Interactions: *In Vitro* Mechanical Stimulation of Myoblasts

In addition to its anabolic effects, mechanical loading can influence bone resorption, either by inhibiting resorption (following mild overloading) or by increasing resorption (following damaging overloading). Using an *in vitro* approach, Juffer *et al.*[11] examined a possible role for muscle in this phenomenon. C2C12 myotubes were cyclically strained (0–15%, 1 Hz, 1 h) or kept under static conditions; conditioned media (CM) from myotube cultures was added to mouse primary bone marrow cells (in the presence of M-CSF and RANKL). CM from *static* conditions decreased the formation of TRAP-positive multinucleated cells (that is, osteoclasts) compared to non-CM, whereas CM from *cyclic* conditions increased osteoclast formation. Cyclic strain increased the expression of interleukin (IL)-6 by myotubes, while an IL-6 antibody nullified the effect that cyclic-CM had on osteoclast formation. Thus, myotubes secrete soluble factors that inhibit osteoclastogenesis, while cyclic loading of myotubes can cause them to enhance osteoclastogenesis via IL-6.

### Microindentation

Minimally invasive reference-point microindentation is a relatively new, unproven method for assessing bone properties at the material level. Abstracts utilizing this technology were presented in a variety of areas, from laboratory validation to clinical studies. Karim *et al.*[12] compared microindentation measures (for example, creep and total indentation distance, CID and TID) to the traditional mechanical properties obtained from tests of intact and notched rat radii. CID was positively correlated with fracture toughness parameters, while TID was correlated positively with whole-bone bending stiffness and negatively with work-to-failure, that is, bone that allowed greater indentation distance also required less energy to failure. CID and TID were not correlated with bone size (polar moment of inertia, pMOI) or tissue mineral density. Multiple regression analysis showed that pMOI and TID independently contributed to stiffness and failure load ($r^2 = 0.54$ and 0.65, respectively), suggesting that total indentation distance may help explain whole-bone mechanical properties. In another animal study, Ammann *et al.*[13] compared femurs from rats fed normal or low-protein diets. As anticipated, bones from the low-protein group had reduced whole-bone bending stiffness, failure load and plastic energy. These differences were associated with reductions in the microindentation properties indentation distance increase (IDI) and average energy dissipation (AED). Importantly, plastic energy by whole-bone bending was positively correlated with AED by microindentation. In summary, both these studies found moderately strong correlations between traditional biomechanical and newer microindentation properties, although they did not highlight the same microindentation parameters. Thus, a generalized approach to the interpretation of microindentation parameters remains to emerge.

Lastly, an *in vivo*, clinical study using microindentation was reported by Fernandez *et al.*[14] Four groups of patients were studied: atypical femoral fracture (AFFx), typical osteoporotic fracture, long-term bisphosphonate-treated (LTB) without fracture and normal. The duration of bisphosphonate treatment was similar in AFFx and LTB groups (both $\sim$5.5 years). Notably, TID and IDI were significantly greater in both typical and atypical fracture groups compared to normal and non-fracture LTB groups, indicating that microindentation properties differentiated fracture and non-fracture groups. However, there was no evidence presented that microindentation properties could discriminate between typical and atypical fracture groups.

### Conflict of Interest

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