# Saving Bone

**OREF grant recipient investigates a natural inhibitor of osteoclast formation**
The process of bone remodeling involves a balancing act between two cell types: osteoclasts that resorb bone and osteoblasts that form new bone. Researchers have characterized the molecules primarily responsible for regulating this balance. The mechanism of age-associated bone loss—when osteoclasts outpace the work of osteoblasts—is also generally well defined.

And yet, gaps in our understanding remain. For example, more accurately defining the signaling pathways that regulate osteoclast formation and function—may suggest new targets for therapy. **Charla R. Fischer, MD,** assistant professor of orthopaedic surgery at Columbia University, is studying the role of the calreticulin protein in regulating osteoclast formation and the protein's therapeutic potential to inhibit bone loss in women.


Charla R. Fischer, MD

Dr. Fischer specializes in the surgical treatment of the spine, including interventions for treating osteoporosis-associated vertebral compression fractures.

"I started out with a question based on my patients' needs," Dr. Fischer said. "Now I'm looking at the question in the lab, and ultimately, I would like to use the answers to treat my patients more effectively. I'm particularly interested in how osteoporosis affects spine surgery. If the bone is weak, the results of surgery are not as good. If we can better understand how bone loss occurs, we may improve outcomes."
Dr. Fischer's work was supported by a 2014 Orthopaedic Research and Education Foundation (OREF) New Investigator Grant, a $50,000 award to advance the scientific training of next-generation orthopaedic surgeons by providing seed and start-up funding for promising research projects.
**The basics of osteoclast formation and function**
There are three primary players in regulating osteoclast formation and function:

- Receptor activator of nuclear factor κB (RANK), a receptor on the surface of osteoclasts and macrophages, which are the precursors to osteoclasts

- RANK ligand (RANKL), a signaling protein released by osteoblasts that binds to RANK
- Osteoprotegerin (OPG), a decoy receptor for RANKL, that is also released by osteoblasts

When RANKL binds to RANK receptors on the surface of macrophages, clusters of these cells fuse to form a multinucleated osteoclast. RANKL also binds to RANK on the surface of mature osteoclasts—a signal that sets in motion the cell's bone-resorbing activity. OPG regulates osteoclast activity by intercepting some RANKL proteins.

Estrogen also plays a role by limiting the expression of RANKL by osteoblasts. In women, the decline in estrogen after menopause results in greater RANKL expression and, subsequently, more osteoclast formation and activity.

**Inhibiting RANKL-induced osteoclastogenesis**

In previous research, Dr. Fischer and her colleagues identified the protein calreticulin as a potential regulator of osteoclastogenesis. In subsequent experiments, they observed that calreticulin inhibited the RANKL-induced activation of a master transcription factor of osteoclast formation.

By binding to a segment of DNA, a transcription factor moderates the rate at which that gene's code is transcribed into the messenger-RNA the cell uses to build the protein. By inhibiting a primary transcription factor, calreticulin may block the expression of one or more genes necessary for osteoclastogenesis.

**Investigating calreticulin in an osteoporosis model**

In the OREF-funded study, Dr. Fischer had two goals: to characterize calreticulin's effect on osteoclastogenesis-signaling pathways and to assess calreticulin's therapeutic effect in a mouse model of osteoporosis.

She explained, "If we better understand steps along the way, we can further explore mechanism-based treatments. The results may introduce calreticulin as a new, naturally occurring inhibitor of bone resorption."

In a series of in vitro experiments, the researchers introduced recombinant human calreticulin (rhCRG) to mouse macrophages. They assessed the effect of rhCRG on the expression of genes known to be involved in osteoclastogenesis, as well as its potential interaction with other transcription factors and associated proteins. They also tested whether rhCRG interacts with other transcription factors and inhibits the function of mature osteoclasts.

The in vivo experiments were conducted with 60 adult female mice in three groups:

- In one test group, each subject underwent ovariectomy to model menopause-induced osteoporosis.
- In another test group, each subject underwent ovariectomy and received a subcutaneous injection of a controlled-release hydrogel with rhCRG.
- The control group had a sham surgery with only an abdominal incision.

At six weeks, the researchers harvested the fifth lumbar vertebrae of all subjects. Using a series of tests, they compared the following among the three groups: total bone mass, trabecular bone volume, cortical bone thickness, compressive strength, and energy absorption.

"The results showed that calreticulin inhibits osteoclast formation in the mouse model," stated Dr. Fischer. "Some of the next steps will be taking actual bone marrow–derived cells from patients and looking at calreticulin's effect on osteoclastogenesis in these cells."

**Supporting emerging clinician scientists**

Dr. Fischer credits her mentor and research colleague **Francis Y. Lee, MD, PhD,** for supporting her career as a clinician scientist. "Dr. Lee has been doing research on the osteoclastogenesis pathway for years," she said. "He has not only helped me understand the science but also encouraged me to apply for grants, introduced me to other researchers in the field, and collaborated with me on experiments."

Dr. Fischer also views OREF as a supporting partner in her research career, noting that it "is an advocate for the clinician scientist."

*Jay D. Lenn is a contributing writer for OREF. He can be reached at communications@oref.org*