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NEWS

The muscle–bone connection

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Recent *IBMS BoneKEy* webinar focused on the influence of myokines on the skeleton

Muscle influences bone, but what are the specific cellular and molecular mechanisms that link the two tissues? That question was at the heart of New Directions In Muscle–Bone Interactions, a recent *IBMS BoneKEy* webinar presented by Mark Hamrick (Georgia Regents University, Augusta, GA, USA). Focusing on the role of muscle-secreted factors known as myokines, Dr Hamrick described research his group and others have performed revealing that insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (FGF-2), and myostatin (GDF-8) each act as myokines that affect bone metabolism *in vitro* and *in vivo*. After the presentation, a distinguished panel including Ted Gross (University of Washington, Seattle, WA, USA), Thomas Lang (University of California, San Francisco, CA, USA) and Mark Johnson (University of Missouri, Kansas City, MO, USA) expressed optimism that the muscle–bone axis could be targeted for treatment of osteoporosis and sarcopenia. The panel discussion was moderated by Serge Ferrari (Geneva University Hospital, Geneva, Switzerland), *BoneKEy* editor in chief.

The webinar is available for viewing at: http://www.nature.com/bonekey/webinars/index.html?key=webinar27

Muscle Affects Bone, But How?

At the outset of his presentation, Dr Hamrick noted that orthopedic surgeons have long observed a relationship between muscle and bone in the clinic. 'Muscle flaps are commonly used to cover fractures and enhance bone healing, and areas that lack muscle coverage, such as the tibia, are often associated with a very high incidence of fracture nonunion,' he said. Animal studies have also revealed the importance of muscle coverage in bone healing. For instance, fracture callus bone volume is higher in mice when osteotomies are covered with a muscle flap, compared with when they are covered with skin.[1] Dr Hamrick stressed that tissue vascularity does not appear to explain that phenomenon—a mouse study found that fasciocutaneous tissue in fact exhibits higher vascularity than muscle[2]—suggesting that the positive effects of muscle on bone repair cannot be solely due to tissue blood supply.

One possible mechanism by which muscle exerts a positive influence on bone is through the secretion of factors that affect skeletal tissue, and it was to his and others' work supporting a role for these 'myokines,' a term first coined in 2011 by Bente Pedersen,[3] that Dr Hamrick then turned. That muscle cells secrete myokines, with ensuing effects on bone, is suggested, Dr Hamrick noted, from recent *in vitro* work by Lynda Bonewald, panelist Mark Johnson and colleagues.[4] Those investigators found that medium from cultured primary muscle cells protected both osteocytes and osteoblasts from apoptosis when those bone cells were exposed to dexamethasone (which normally induces cell death).

Which specific myokines may have an impact on bone (for review, see Hamrick[5])? One candidate is IGF-1, which experiments in people have shown to be present at high levels in wound exudates from muscle flaps that have been applied to bone.[6] Dr Hamrick and co-investigators[7] have found that in mice *in vivo*, both IGF-1 and the IGF-1 receptor (IGF-1R) are localized at the muscle–bone interface, as assessed by immunohistochemistry;[7] IGF-1 is found in muscle next to the periosteum of cortical bone, while IGF-1R is found in the periosteum. Furthermore, others have found that, in a hindlimb-unloaded rat model, the expression of IGF-1 mRNA in the gastrocnemius (calf) muscle was increased after the leg received electrically stimulated resistance exercise.[8] Meanwhile, human studies have documented increased circulating serum levels of IGF-1 protein in healthy people who performed knee extension resistance exercise.[9] 'Both local and systemic IGF-1 are elevated with muscle contraction, and this may be one mechanism by which physical activity can be linked with increases in bone mass,' Dr Hamrick said.

Like IGF-1 and its receptor, another myokine, FGF-2, and its receptor, FGF-R2, are also localized at the muscle–bone interface.[7] However, Dr Hamrick said that in conditioned medium from primary muscle cells, levels of FGF-2 are relatively lower than levels of IGF-1, which he said is not surprising. 'It's well known that FGF-2 lacks the conventional signal sequence for export out of the cell via the classic exocytotic pathway,' he noted. That raises the question of how FGF-2 is released from muscle. Here, Dr Hamrick pointed to *in vitro* and *in vivo* research showing that FGF-2 can be released from muscle cells through nonlethal, repairable disruption in the muscle cell plasma membrane. These cell membrane disturbances result from mechanical loading, of the sort seen with lengthening (eccentric) muscle contraction characteristic, for instance, of running downhill.[10,11]

The expression of IGF-1 and FGF-2, Dr Ferrari noted during the panel discussion, raises an intriguing question about the

nature and extent of myokine action on bone. 'That IGF-1 and FGF-2 are produced by muscle cells in very close vicinity to bone cells at the periosteum that express the receptors suggests those factors regulate bone modeling at the periosteum,' said Dr Ferrari. 'But to what extent does muscle have a more profound effect on bone—can it also regulate bone remodeling?' The panel agreed that the potential for muscle to influence bone at a deeper level is great, though the specifics are for future research to determine.

In the last part of his presentation, Dr Hamrick turned to myostatin, a myokine that he has investigated in a number of studies. Myostatin levels can increase during cancer and other settings characterized by infection and inflammation, but also after traumatic musculoskeletal injury. Dr Hamrick presented his recent data from a mouse fibula osteotomy model, which includes damage to overlying muscle tissue, showing that expression of myostatin in muscle rises both 12 and 24 h after surgery.[12] Other work from Dr Hamrick's group has found recently that mice lacking myostatin exhibit an increase in proliferation of bone marrow stromal cells (BMSCs); conversely, treating those cells with myostatin suppressed BMSC proliferation.[13] In addition, Dr Hamrick has found that, in a cell culture model where cartilage formation is induced from BMSCs, myostatin suppresses chondrogenesis.[14] Meanwhile, his work has revealed that myostatin impairs fracture healing *in vivo*.[12] All of this research points to an important role for myostatin in bone repair. Finally, Dr Hamrick noted that muscle contraction may be one mechanism by which myostain expression can be suppressed; in rats subjected to sciatic nerve stimulation, muscle contractions, especially those of the eccentric variety, were found to reduce the expression of myostatin.[15]

Dr Hamrick ended his talk with a discussion of potential therapeutic avenues to target muscle and bone at the same time. He noted that recombinant IGF-1 is already approved to treat pediatric growth abnormalities, and recombinant FGF-2 is now in clinical trials for periodontal regeneration. Also of notable interest are myostatin inhibitors. An antibody against myostatin is currently in a phase I trial in facioscapulohumeral muscular dystrophy in adult patients, while a decoy myostatin receptor, the type IIB activin receptor (ActRIIB), has been found in a phase I trial to increase bone formation markers in postmenopausal women, and has also completed a phase 2 trial in children with Duchenne muscular dystrophy. Dr Hamrick was particularly enthusiastic about some recent *in vivo* animal work showing that a decoy myostatin receptor increased muscle force and bone density in adult mice over the course of a 1-month treatment.[16] 'This seems to be a molecule that can affect both muscle and bone, and I would argue that the effect certainly in bone is likely directly due to the molecule itself, and probably not secondary to the increase in muscle, because of the relatively short treatment period over which the study was conducted,' Dr Hamrick stressed.

A Two-Way Street

Although the focus of the presentation was the influence of muscle on bone, Dr Hamrick was careful to note that the communication between the two tissues is bi-directional, with the skeleton also exerting a significant influence on muscle. That reciprocal relationship between the two tissues was the focus of much of the ensuing panel discussion, particularly with regard to the potential to exploit it to treat both sarcopenia and osteoporosis.

'Pharmaceutically, if we can develop anabolics that improve muscle performance as we age, we should also get an effect on bone. At the same time, I think that if we can develop anabolics that target bone effectively, we would improve muscle…it's not only factors coming from muscle that affect bone, but bone is producing factors that affect muscle,' said panelist Mark Johnson. The specific bone signaling pathways and bone-secreted factors that may influence muscle are uncertain at this time, Dr Johnson stressed, though there are plenty of candidates—Wnt signaling and sclerostin, for instance, to name just two possibilities.

The panel agreed that efforts to target the reciprocal muscle–bone relationship must take into account that the relationship changes over the organism's life. 'I think there is potential for resetting how homeostasis between the two tissues is maintained throughout life. I think it would be naive to think that the homeostasis that is generated early on during development is the same homeostasis one faces in a senescent skeleton.... Identifying how the two tissues adapt over their lifespan would hold potential for some [therapeutic] targets that have not at this point really been explored,' said panelist Ted Gross.

Better imaging techniques could go a long way towards illuminating the reciprocal influences that muscle and bone have on each other, said panelist Thomas Lang. In terms of imaging muscle effects on bone, while peripheral quantitative computed tomography (pQCT) and high resolution pQCT provide information on bone geometry/structure and thus represent a large advance upon imaging techniques that rely solely on bone mineral density measurements, those techniques do not capture very localized changes in the tissue that may be occurring. But the advent of new techniques portends a brighter future. 'It's now becoming possible to use, with three-dimensional CT data, algorithms that have been developed in the brain field and adapted to bone to look at voxel-level detail,' Dr Lang said. Prospects to image bone effects on muscle also look promising. 'There are a whole range of new imaging techniques coming up for muscle,' said Dr Lang, who cited his group's work on estimating skeletal muscle protein synthesis rate as just one example. In addition, the ability of *in vivo* techniques to study the link between anatomy and metabolism, rather than to study each separately, is particularly exciting, Dr Lang said.

Also exciting is the prospect of discovering new myokines. As interesting and important as IGF-1, FGF-2 and myostatin may be, those myokines may only be the tip of the iceberg. 'The next 10 years will tell,' Dr Johnson said.

Conflict of Interest

The author declares no conflict of interest.

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