Endocrine Crosstalk Between Muscle and Bone

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Abstract The musculoskeletal system is a complex organ comprised of the skeletal bones, skeletal muscles, tendons, ligaments, cartilage, joints, and other connective tissue that physically and mechanically interact to provide animals and humans with the essential ability of locomotion. This mechanical interaction is undoubtedly essential for much of the diverse shape and forms observed in vertebrates and even in invertebrates with rudimentary musculoskeletal systems such as fish. It makes sense from a historical point of view that the mechanical theories of musculoskeletal development have had tremendous influence of our understanding of biology, because these relationships are clear and palpable. Less visible to the naked eye or even to the microscope is the biochemical interaction among the individual players of the musculoskeletal system. It was only in recent years that we have begun to appreciate that beyond this mechanical coupling of muscle and bones, these 2 tissues function at a higher level through crosstalk signaling mechanisms that are important for the function of the concomitant tissue. Our brief review attempts to present some of the key concepts of these new concepts and is outline to present muscles and bones as secretory/endocrine organs, the evidence for mutual genetic and tissue interactions, pathophysiological examples of crosstalk, and the exciting new directions for this promising field of research aimed at understanding the biochemical/molecular coupling of these 2 intimately associated tissues.

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Introduction

The musculoskeletal system is a complex organ comprised of the skeletal bones, skeletal muscles, tendons, ligaments, cartilage, joints, and other connective tissue. Although not considered in detail in this review, nervous system innervation of both bone and muscle is another important element of musculoskeletal anatomy and regulation. The coupling of skeletal muscles and bones has long been considered primarily to be a mechanical one in which bone provides an attachment site for muscles and muscles apply load to bone. This coupling is necessary to support the locomotion and the shape/form of animals. In recent years we have begun to appreciate that beyond this mechanical coupling of muscle and bones, these two tissues function at a higher level through crosstalk signaling mechanisms that are important for the function of the concomitant tissue.

The tight coupling between skeletal muscle and bone in animals begins during embryonic development with the formation of the paraxial mesoderm and subsequently the somites that gives rise to these tissues [1]. As the skeleton develops it has been postulated that muscle contraction in the developing fetus even contribute to skeletal growth and development and that skeletal adaptations in early postnatal life are driven by changing mechanical forces [2, 3]. Clearly peak bone mass accrual during prepubertal growth is dramatically affected by exercise (physical activity) and to a lesser extent thereafter, although exercise across all ages has benefits [4]. Human females begin to lose bone mass with a rapid loss phase occurring 2–3 years before menopause and continuing for 3–4 years after the last menses [5] and then a more gradual steady decline thereafter, while males experience only the

more gradual steady decline in bone mass [6]. Individuals with reduced bone mass as seen in osteoporosis often also develop the reduced muscle mass and function, a condition known as sarcopenia. However, declines in bone mass do not fully explain sarcopenia, nor does muscle atrophy fully explain the totality of osteoporosis. In part this may be due to the use of bone mass and muscle mass as the measures for osteoporosis and sarcopenia, when bone quality and muscle function assessments might better reflect the physiological basis for these diseases, but these metrics have not been fully established to date.

The mechanical coupling of skeletal muscle and bone is easily appreciated. Bone is known to adjust its mass and architecture to changes in mechanical load and as contraction of skeletal muscle is essential for locomotion it is self-evident that these contractions apply load to the bone. This mechanical perspective implies that as muscle function declines, this would result in decreased loading of the skeleton and would lead to a decrease in bone mass. However, as noted above, the inability to fully account for osteoporosis based upon the presence of sarcopenia (and vice versa) based solely on mass measures implies that beyond the mechanical coupling their might also be a biochemical coupling.

As we will discuss in this review, it is now fully evident that muscle and bone produce factors that circulate and can/do act on distant tissues, the classical definition of endocrine action. What is most remarkable, however, is the fact that despite considerable evidence for the actions of these factors on various tissues in the body, it has only been with the past few years that potential actions of these muscle and bone derived factors on these two most intimately associated tissues has been examined. Perhaps this oversight has been driven by the bias of mechanical coupling, but understanding this apparent endocrine crosstalk and biochemical coupling is an exciting new avenue of research.

Muscle as an Endocrine Organ

The full recognition of skeletal muscles for their secretory and endocrine capacities has only occurred during the last decade of research [7]. It is interesting to note that Myostatin was the first myokine identified. Myostatin is one of the most potent inhibitors of skeletal muscle cell proliferation and growth ever identified. The discovery of myostatin occurred in 1997 in Se-Jin Lee's laboratory [8, 9••]. Because myostatin is an inhibitor of muscle growth, it is its downregulation or inactivation that can lead to potentiation of muscle growth. In fact, it has been demonstrated in animals and humans that strength training and aerobic exercise attenuate myostatin, which seems to potentiate the beneficial effects of exercise on metabolism [10]. Although myostatin might have been the first identified myokine, the term itself was first coined by Pedersen and

colleagues when they suggested a link between IL6 and exercise [11] that has been recently confirmed by other research groups [12, 13]. Certainly the list of myokines is not limited to IL-6 anymore, which now includes:

(1) IL-5, being studied for a potential role in the crosstalk of the adipose and muscle tissues [14];
(2) IL-7, might have specific effects on satellite cells during the process of myogenic differentiation [14];
(3) IL-8, stimulation of angiogenesis [15];
(4) Brain-derived neutrophic factor (BDNF) [16];
(5) Irisin, a new myokine that is a key regulator of the conversion of white fat into brown fat [17];
(6) IL-15, a myokine implicated in the reduction of adiposity, and intriguingly, mice that overexpress IL-15 concomitantly display higher bone mineral density, further reinforcing that tissue crosstalk might be critical for body composition [18•].

Intriguing studies have recently suggested a direct correlation between irisin and the augmented risk of metabolic syndrome and cardiovascular diseases [19]. Furthermore, it is very interesting that some of these muscle myokines might be an intrinsic part of the muscle regenerative process. IL-6 itself and also another myokine termed LIF are thought to assist muscle regeneration during injury, while TGFα and TGFβ1 have apposite effects, in that, they seem to act negatively on myoblast proliferation and differentiation [7, 17], perhaps as a part of the muscle homeostasis. It will be important to see how the balance among these myokines shifts with exercise, diet, and aging. In addition, as the muscle secretome becomes further elucidated [20, 21], new insights and discoveries will be added to the role and therapeutic potential of myokines.

Bone as an Endocrine Organ

The vertebrate skeleton has long been recognized to have important roles such as a structural support essential for locomotion, provide protection for the internal organs, serve as a reservoir for calcium and phosphorus and being the site of adult hematopoiesis. The skeleton has also been long recognized to be an endocrine target tissue, responding to hormones such as PTH and sex steroids. However, the concept of the skeleton being an endocrine organ is relatively new, with the bulk of the supporting data emerging in the literature since the year 2000. Evidence from several groups has shown that bone (osteoblasts and osteocytes) functions in an endocrine fashion by the production and secretion of at least 2 circulating factors, FGF23 and osteocalcin, which are capable of altering distant tissue function. Several excellent reviews have discussed the important endocrine functions of bone [22–32]. At present the

best studied endocrine roles of bone involve mineral and energy metabolism.

Mutations in *FGF23* as the underlying cause of Autosomal Dominant Hypophosphatemic Rickets (ADHR) suggested that this protein might be the "phosphatonin" hypothesized as the phosphaturic factor in tumor induced osteomalacia (TIO) [33], which was later shown to be the case [34]. With the development of an Fgf-23 knockout mouse, in which an *eGFP* gene was inserted into the *Fgf23* locus, it became clear that Fgf-23 was produced mainly by osteocytes in bone [35]. Several human diseases of phosphate metabolism have also been described that lead to altered levels of FGF23 resulting from proteins produced by the osteocyte. For example, X-linked hypophosphatemia is caused by mutations in the *PHEX* gene [31, 36–38], while mutations in DMP1 have been shown to be causal in autosomal recessive hypophosphatemic rickets. FGF-23 is a key player in the regulation of phosphate and Vitamin D levels in the circulation through its endocrine actions on the kidney to suppress 1,25-dihydroxyvitamin D production [6, 29, 30]. Vitamin D acts at the level of bone to suppress FGF-23 expression. A somewhat controversial aspect of FGF-23 action is whether there also exists an FGF-23/PTH endocrine loop [29]. In addition to the normal physiological actions of FGF-23, elevated levels may play an important role in other pathologic conditions such as cardiac hypertrophy [39, 40], suggesting more widespread sites of action.

Osteocalcin, produced by osteoblasts, is another bone derived endocrine factor that seems to play an important role in energy metabolism [22, 23]. Lee et al. [22] using a series of genetic mouse models demonstrated that deletion of the *Esp* gene in osteoblasts increase β–cell proliferation along with increased insulin secretion and insulin sensitivity. Mice lacking osteocalcin in the osteoblast lineage display decreased cell proliferation and insulin secretion and increased adiposity. The $Esp^{-/-}$ mouse phenotype was corrected by deletion of a single allele of osteocalcin in $Esp^{-/-}$ mice. As a molecular explanation for this finding, these investigators demonstrated that uncarboxylated osteocalcin induced expression of adiponectin in adipocytes, which acts to increase insulin sensitivity. These data lead to a model in which bone plays an endocrine function in energy metabolism through the production of osteoblast-produced osteocalcin [22, 23, 28].

In addition to the aforementioned Fgf-23, Phex and Dmp-1 produced by osteocytes' other molecules such as sclerostin, RANKL, and OPG are known to have local paracrine effects on bone [32]. What will be of interest is whether any of these and other osteocyte produced factors have endocrine effects on other tissues. In this regard, sclerostin is particularly of interest as it is a negative regulator of the Wnt/β-catenin signaling pathway [41], which is active in a number of tissues. Whether osteocyte derived, circulating sclerostin exerts effects on these tissues is an unanswered question at this point in time. Regardless, it is clear that bone functions as an endocrine organ just as muscle does and as we shall discuss next, endocrine crosstalk between bone and muscle is emerging as another key mechanism involved in the regulation of these tissues.

Evidence of Muscle-Bone Crosstalk

The mechanostat theory posits that bone adjusts its mass and architecture so that the strains experienced operate within a physiological window [42]. Strains above this window induce bone formation, while strains below this window result in bone resorption. This physiologic strain window within which bone normally exists is a composite of low-magnitude strains that dominate as a result of daily physical activity and the generally short duration, high magnitude strains that result from rarer levels of activity, such as jumping or more intense forms of exercise. Of course, persistent higher magnitude events should induce an adaptive response by the skeleton. Underlying the environment cues that drive skeletal mass and other properties are a complex set of genetic factors. Heritability studies have estimated that between 40 %–80 % of the major skeletal phenotypes that are routinely assessed are due to genetics. Similar estimates of muscle traits have also been reported [43–46]. Given the high degree of genetic influences underlying both bone and muscle traits and their coupled development, growth, and physiological relationships, it seems highly likely that there would be some degree of shared genetic components underlying some of their phenotypes. The identification of these pleiotropic genetic factors holds great potential for identifying the molecular/biochemical coupling that may exist between muscle and bone.

Genetic Studies

Genome Wide Association Studies

Over the past 10–15 years Genome Wide Association Studies (GWAS) have produced a litany of candidate gene regions that show association with variations in a number of different human bone phenotypes and muscle traits. We note just a small sampling of the bone phenotype GWAS [47–55] and muscle phenotype GWAS [56–64] studies reported in the past 5 years. More recently GWAS has been used to identify pleiotropic candidate genes/SNPs/regions associated with traits in both bone and muscle [62, 65–69]. These later GWAS based studies that included both bone and muscle phenotypes have produced a short list of several novel potential candidate genes for further biological validation such as *PRKCH* and *SCNN1B* [67]; *HK2*, *UMOD*, and 2 microRNAs; *MIR873* and *MIR876* [68]; *HTR1E*, *COL4A2*, *AKAP6*, *SLC2A11*, *RYR3* and *MEF2C* [69]; and *GLYAT* [62]. The *MEF2C* gene encodes a transcription factor (myocyte enhancer factor 2C) that was

originally shown to be involved in cardiac and skeletal muscle development and mark myogenic cells in the somites [70]. Recently, mouse *Mef2C* deletion in the osteocyte has been shown to result in increased bone density through a complex mechanism involving reduced Sost expression, increased OPG expression resulting in a reduced RANKL/OPG ratio, and reduced osteoclastogenesis [71]. Overall, these findings suggest an important role for MEF2C in both skeletal muscle development and adult bone mass regulation and support the concept that shared genetic determinants are operational in both muscle and bone growth and development.

Single Gene Disorders

A large number of candidate genes have been assembled that demonstrate pleiotropic actions in muscle and bone [66]. Of these single gene traits the deletion and/or mutations in myostatin, which result in muscle hypertrophy or "double muscling" in animals [72–77] and humans [78], is a prime example of how a mutation presumably restricted to 1 tissue can lead to altered properties in the other. Myostatin (*MSTN*) or growth and differentiation factor 8 (*GDF8*) is a member of the TGF-β superfamily and is a secreted myokines that circulates in the blood, making it an attractive candidate to be involved in muscle-bone endocrine signaling [79]. The loss of myostatin also leads to a generalized increase in bone density and strength [80]. The major mechanistic question is how or does myostatin exert its effects on bone? Possible explanations include direct effects of mechanical loading of bone due to the increased muscle mass, indirect action by regulating hepatic production of IGF-1 [81], or some other unknown mechanism. The IGF1 (and GH) axis is a particularly appealing mechanism that has known effects on age-related changes in bone and skeletal muscle [82].

Fracture Healing

An intriguing and well documented observation that cannot be ignored anymore in the context of bone-muscle interactions is the fact that in open fractures if muscle injury is also extensive or if muscle atrophy develops, healing of the fracture is significantly impaired [83–86].

Rodent models of fracture have also supported this concept that muscle secretory activity aids in the process of fracture healing. For example, a significant difference (ie, lesser healing) was found in rats with fractured femurs when their quadriceps muscles had also been paralyzed by botulin injections. In mice with tibial open fracture, Harry et al. reported that when the fracture area had been covered with muscle flaps, bone repair was significantly improved [87]. The clinical significance of these findings cannot be overstated since these findings have also been confirmed in humans with open tibial fractures [88]. Furthermore,

using a mouse model of deep penetrating bone fracture and muscle injury the Hamrick and coworkers found that exogenous administration of recombinant myostatin significantly reduced bone callus formation, while increasing fibrous tissue in skeletal muscle. They suggested an early intervention in these types of injuries to inhibit myostatin could be beneficial for healing of both tissues [89•].

Taken together, these studies strongly suggest that even under conditions where substantial mechanical forces are not being produced, muscles have the intrinsic biochemical capacity to secrete factors that stimulate growth and repair, almost as if muscles could function as a second periosteum layer as recently proposed by Little and colleagues [90, 91]. Yet another line evidence derives from the documented observation in humans that fracture healing is improved upon the stimulation of the affected bone with pulsed electromagnetic stimulation [92]. Our groups have recently demonstrated that PEMS enhances myogenesis of C2C12 myoblasts [93]. Therefore, it is possible that the effects of PEMS on bone might be attributable to direct effects on bone cells and indirectly through muscle cells secretory effects on bone.

Disease Conditions with Multiple Tissue Affects

The endocrine interactions that are being discovered through the effects of bone cells secreted factors and myokines seem to go well beyond the musculoskeletal unit. The interconnection of bone, muscle, and adipose tissue has become more evident. The striking rise on chronic diseases such as diabetes, metabolic syndrome, and obesity seem to closely parallel the raise in the prevalence of sarcopenia and osteoporosis, particularly in the elderly population [94]. If we embrace the concept that both bone and muscle produce and secrete a myriad of factors that as outlined in this review article, influence not only each other but multiple organs, and particularly overall body metabolism, it makes sense that when the 2 largest organ systems of the body become less effective during aging that other organs would also be affected. Therefore, if bone cells are secreting less "osteokines" and skeletal muscles is secreting less myokines, fat metabolism could become compromised, as well as kidney function, even testosterone levels could be affected, thereby translating into multiple organ effects that are normally interpreted as "aging consequences". This new view could not only help to explain some of these multiple organ decline of function, but also lead to new therapies to rebalance the secretory actions of bone and muscle.

Future Directions

Great leaps in progress for the musculoskeletal areas of research will largely depend on continued research into both the

mechanical relationship and biochemical crosstalk that exists between bone and muscle. Perhaps, the time is right for highly integrated research that could utilize new tools arising from cell and molecular biology, genetics, and systems biology to propel us forward in a manner that the ideal models will come to fruition. The promise of the field is too great to ignore its potential for the development of new interventions and therapies that could target not only one tissue but the entire system itself. A very specific challenge ahead of us is the integration of cartilage, ligaments, and tendons into what we call the bone-muscle unit, which in fact has been essentially limited to bone and muscle at the most. Furthermore, the in-depth exploration of the relationships between osteoporosis-sarcopenia with a host of other chronic diseases must be pushed as a high priority, since these twin diseases of aging are a true threat for the mankind's dream of healthy aging.

The elegant studies of Conboy [95] and colleagues using a parabiosis model showed that muscles from aged mice can significantly improve their otherwise impaired regeneration when exposed to the circulatory system of young mice. This suggests that at least part of the aging process of the musculoskeletal system might be attributed to a shift or a change of either the amount or the quality of these circulating factors, which further emphasizes the exquisite importance of endocrine crosstalk in musculoskeletal tissues.

While it is clear that both muscle and bone behave as endocrine organs and share some common genetic influences and function as a coordinated system at multiple levels; there are several proof-of-concept questions that need to be answered. For example, is there clear direct evidence for a muscle factor that directly influences bone cell function and vice versa? A step in this direction has been provided by our groups, in which conditioned media from C2C12 myotubes protect in vitro against MLO-Y4 osteocyte dexamethasone induced apoptosis [96]. These studies support the concept that muscle cells produce a soluble factor that can target bone cells, specifically osteocytes. Reciprocal studies are underway in our laboratory examining conditioned media from osteocytes and their alteration of muscle cell function. At present the identity of these factors remains unknown, but once they are identified, then we will be able to test these molecules in vivo to demonstrate crosstalk signaling. Other big questions are, what happens with aging to the production of these factors? How do these factors reach the other tissue; ie, do they circulate as would be expected for an endocrine factor, do they diffuse directly between bone and muscle? Are these factors a new class of agents that can be used to treat osteoporosis and sarcopenia jointly, or will treating bone with one of these factors induce bone to produce its muscle specific factors? These are intriguing questions and the next decade should prove tremendously exciting in terms of our understanding of how muscle and bone crosstalk to each other.

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