Citation: BoneKEy Reports **1,** Article number: 60 (2012) | doi:10.1038/bonekey.2012.60

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REVIEW

The skeletal muscle secretome: an emerging player in muscle–bone crosstalk

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***In vitro* and *in vivo* studies provide evidence that a variety of growth factors and cytokines are actively secreted by muscle tissue. Muscle can therefore function as an endocrine and paracrine organ. These peptides characterize the muscle secretome, and many muscle-derived factors such as insulin-like growth factor-1, basic fibroblast growth factor, interleukin-15, myostatin and secreted protein acidic and rich in cysteine (osteonectin) are also known to have significant effects on bone metabolism. The factors secreted by muscle may vary according to muscle activity, in that muscle contraction, muscle atrophy or traumatic muscle injury can alter the type and relative abundance of particular factors released from muscle cells. The molecular and cellular pathways by which muscle-derived factors affect different types of bone cells (for example, osteoblasts, osteoclasts and osteocytes) are, however, poorly understood. Nevertheless, these findings further underscore the complex nature of muscle–bone interactions, and highlight the importance of integrating muscle biology and physiology into our understanding of bone growth, development and aging.**

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Introduction

Evidence from numerous studies has revealed for decades, if not centuries, that a close functional and developmental relationship exists between muscle and bone mass. Embryonic muscle paralysis and abnormal myogenesis lead to bones that are poorly mineralized and lack normal curvature,[1] and muscular dystrophies are associated with relatively low bone density and an increased incidence of bone fractures.[2] Loss of muscle mass with age, and the gradual infiltration of muscle with adipose tissue (myosteatosis), have been implicated in age-related bone loss and an increased risk of falls and fractures.[3] Moreover, muscle paralysis using agents such as botulinum toxin induces bone loss[4] and impairs fracture healing.[5] On the other hand, significant increases in muscle mass with myostatin deficiency are associated with larger muscle attachment sites and increases in bone cross-sectional area.[6,7] Historically, a number of different biomechanical and physiological mechanisms have been presented to explain the underlying relationship(s) between muscle function and bone metabolism. These range from mechanical models linking loading rates and strain history with bone adaptation,[8,9] to others linking muscle contraction with changes in fluid flow within bone tissue, which can in turn regulate bone formation.[10]

The majority of models, including those cited above, that link changes in muscle mass with alterations in bone formation and strength appropriately emphasize the important role of muscle contraction in generating mechanical stimuli for bone. Hence, the muscle–bone relationship is in large part a mechanical one, with muscle being the key driver in this relationship via the contractile forces that it imposes upon bone tissue. Indeed, it is clear from numerous studies that mechanical stimulation is important for bone health, and that exercise-induced muscle contraction may enhance bone mass during growth. There is, however, evidence that muscle tissue itself may have positive effects on bone formation and bone repair independent of mechanical stimulation. For example, it is well established in the clinical literature that covering bone fractures with muscle flaps improves fracture healing in cases of traumatic orthopaedic injury,[11–13] and that implants of muscle alongside periosteum can stimulate new bone formation directly.[14] Similarly, muscle damage and trauma to muscle surrounding bone defects, can impair and delay bone healing.[15,16] These findings provide additional support for the concept that health and viability of the muscle bed are key for normal bone formation and bone repair.[17–19]

The fact that muscle flaps alone can enhance bone formation suggests that muscle may serve as an important collateral source of blood for adjacent bone tissue,[16] and perhaps as a source of trophic factors.[14,17] This review focuses on the

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Received 16 January 2012; accepted 6 March 2012; published online 11 April 2012

latter hypothesis for the following reasons. First, it is clear that intact muscle flaps are a rich source of secreted growth factors.[20] Second, although muscle does provide important vascular support for bone, skin has a higher vascular density than muscle, yet fasciocutaneous skin flaps do not have the same anabolic effects on healing bone as muscle flaps.[21] Third, it is clear that conditioned medium from cultured muscle cells has positive effects (for example, increasing extracellular matrix synthesis) on cultured chondocytes,[22] revealing that muscle is a source of secreted growth factors both *in vivo* and *in vitro*. Finally, the muscle secretome has become increasingly well characterized.[23–27] We now know that muscle secretes a wide variety of growth factors, cytokines and molecules involved in extracellular matrix remodeling. Moreover, different laboratories have independently identified the same factors secreted from muscle, further validating the existence of a well-defined muscle secretome. Some of these factors have been referred to as 'myokines' by different authors;[28–30] however, several muscle-derived factors (for example, IGF-1, FGF-2, IL-6) are secreted in abundance by other tissues, so the term myokine should not be taken to imply that they are muscle-specific.

Our growing knowledge of the muscle secretome has important implications for bone biology, as it presents new opportunities for targeting muscle in order to better develop the therapeutic program for aging and healing bone. This review seeks to highlight the muscle-derived factors that may impact bone metabolism, and also propose future directions for research aimed at advancing our current understanding of muscle–bone crosstalk.

Growth Factors Actively Secreted by Skeletal Muscle

Insulin-like growth factor-1 (IGF-1). Wound exudates from intact muscle flaps contain high levels of IGF-1,[20] and immunohistochemistry has been used to localize IGF-1 along the muscle-periosteal interface of mouse forelimbs.[25] These *in vivo* findings are consistent with *in vitro* studies in which proteomic approaches have been used to detect IGF-1 in conditioned medium from cultured myotubes.[23,27,31] IGF-1 expression, measured as an increase in IGF-1 mRNA, is increased in skeletal muscle with muscle contraction,[32] and elevated levels of IGF-1 protein are detected in serum following resistance exercise.[33] It is well established that IGF-1 has important osteogenic effects on the skeleton and IGF-1 is also involved in myofiber hypertrophy, suggesting that muscle-derived IGF-1 may couple both muscle and bone anabolism (**Figure 1**).[30]

Basic fibroblast growth factor (FGF-2). FGF-2 lacks the conventional signal sequence for export out of cells via the classic exocytotic pathway, and it has been shown that mechanically induced plasma membrane disruption is one mechanism by which FGF-2 is released from myocytes both *in vivo* and *in vitro*.[34,35] Eccentric, lengthening contractions are particularly effective for releasing FGF-2 stored in the cytosol of muscle cells,[35] and levels of FGF-2 detected in conditioned medium from cultured myotubes are increased with mechanical stretching.[34] It is important to note here that the muscle 'injury' that occurs during eccentric muscle contraction involves plasma membrane disruptions that are followed by membrane repair.[36] These types of mechanical disruptions are argued here to involve a different cascade of molecular signaling events than

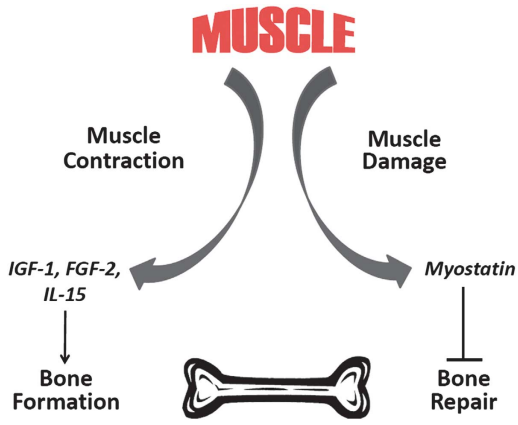

Figure 1 Resistance exercise and eccentric muscle contraction induce the secretion and release of the osteogenic factors IGF-1 and FGF-2 from skeletal muscle. In contrast, traumatic muscle injury and perhaps systemic inflammation and disuse increase the secretion of myostatin from skeletal muscle, which in turns impairs chondrogenesis and bone healing.

traumatic muscle injuries that are associated with muscle regeneration. The latter type of mechanical and structural disruption is much more severe, and involves cleanup of necrotic tissue by macrophages, expression of inflammatory factors and activation of satellite cells. FGF-2 has positive effects on bone formation in estrogen-deficient rodents and is a well-known osteogenic factor.[25,30] Mechanically induced release of FGF-2 following eccentric contraction and plasma membrane disruption is another potential pathway by which physical activity and bone formation may be coupled physiologically (**Figure 1**).[25,30]

Myostatin (GDF-8). Although factors such as IGF-1 and FGF-2 are secreted by a number of tissues in addition to muscle, myostatin is most abundant in muscle tissue and appears to be secreted primarily by muscle. Thus, myostatin can be considered a bona fide myokine. Conditions associated with elevated levels of myostatin expression include disuse atrophy, cancer- and AIDS-related cachexia and increased circulating levels of glucorticoids.[30] Myostatin treatment induces muscle wasting and myostatin deficiency increases muscle mass. We have recently shown that myostatin is highly expressed by injured myofibers following traumatic extremity injury,[37] and that local application of exogenous myostatin increases skeletal muscle fibrosis and inhibits bone repair.[37] These data are consistent with studies referenced above, demonstrating that intact muscle flaps enhance bone repair, whereas coverage of bone injuries with damaged muscle does not have the same positive effects on bone healing. These *in vivo* data are also consistent with our *in vitro* studies showing that myostatin treatment suppresses the proliferation and chondrogenic differentiation of bone marrow-derived stromal (stem) cells.[38] In addition, blocking the myostatin using a recombinant propeptide improves muscle regeneration and fracture healing following orthopaedic trauma.[39] Although severe muscle trauma and muscle damage increase myostatin expression, which in turn impairs bone healing, eccentric muscle contraction and exercise both decrease myostatin expression in skeletal muscle.[40–42] These studies suggest that although IGF-1 and FGF-2 are muscle-derived factors that can have significant, positive effects on bone formation, myostatin is a factor released from muscle during traumatic

Table 1 Growth factors, cytokines and other peptides secreted by muscle, the factors that influence their secretion and their potential effects on bone metabolism

Muscle-derived peptides	*Factors that stimulate peptide secretion*	*Role(s) in bone metabolism*
Growth factors		
IGF-1	Resistance exercise	Stimulates bone formation
FGF-2	Eccentric muscle contraction	Stimulates bone formation
GDF-8	Muscle damage, cachexia, atrophy	Suppresses chondrogenesis and fracture healing
Extracellular matrix molecules		
SPARC	Resistance exercise, muscle regeneration	Promotes bone mineralization
MMP-2	Resistance exercise and re-loading	Fracture callus remodeling, bone formation
BMP-1	Blast trauma to muscle	Cleaving of procollagen and possibly heterotopic ossification
Inflammatory cytokines		
IL-6	Physical activity and muscle contraction	Bone resorption and turnover
IL-7	Physical activity and muscle contraction	Bone resorption
IL-15	Resistance exercise	Increase bone mass, decrease adiposity

Abbreviation: IL, interleukin.

and catabolic conditions that may inhibit and suppress bone repair (**Figure 1**).[30]

Factors Involved in Extracellular Matrix Remodeling Secreted by Muscle

Secreted protein acidic and rich in cysteine (SPARC, or osteonectin). SPARC is a glycoprotein that is abundant in the extracellular matrix of various tissues including bone and skeletal muscle and is involved in tissue repair, remodeling of the extracellular matrix, and promoting collagen mineralization by osteoblasts.[43] One of the more surprising aspects of research on the muscle secretome is the consistency with which SPARC is detected as a factor secreted by isolated muscle cells. A number of different research groups using both human and rodent-derived muscle cells have identified SPARC in conditioned medium from cultured myotubes.[23,24,26,31] SPARC secretion is increased following resistance exercise and myotube hypertrophy,[26] but SPARC is also highly expressed following injury and during muscle regeneration.[44] Additional research is needed to determine the role of muscle-derived SPARC in bone formation, but the studies reviewed above suggest that exercise-induced SPARC secretion could potentially have a role in enhancing bone formation and mineralization (**Table 1**).

Matrix metalloproteinase-2 (MMP-2). Mice lacking MMP-2 are known to show bone loss and reduced bone density, and absence of MMP-2 effects later stages of fracture callus remodeling.[45] MMP-2, similar to SPARC, is found in both muscle as well as bone, and studies using cultured myotubes reveal that MMP-2 is actively secreted by myotubes *in vitro*.[23,24] Insulin treatment of rat myotubes increases secretion of MMP-2, and serum levels of MMP-2 are elevated in diabetic patients suffering from hyperinsulinemia.[46] MMP-2 expression increases with exercise[47] and with re-loading following hindlimb suspension,[48] and decreases with injury,[49] but is elevated in skeletal muscle following disuse.[50] These data show that MMP-2, like SPARC, has an important role in muscle tissue remodeling during various repair events. The role of muscle-derived MMP-2 in bone metabolism is also unclear, but muscle-derived MMP-2 could potentially couple both muscle and bone turnover (**Table 1**).

Bone morphogenetic protein-1 (BMP-1). BMP-1 is not a true bone morphogenetic protein but rather is a protease that cleaves the propeptide fragments of procollagens I, II and III.[51] BMP-1 is secreted from cultured primary human myotubes *in vitro*,[31] and its secretion is decreased in rat myotubes exposed to very high contractions of insulin.[46] Recently, high levels of BMP-1 protein and mRNA were detected in muscle biopsies from patients who had experienced blast trauma in the combat setting.[52] This is significant from the perspective of muscle–bone crosstalk because blast trauma is associated with a high incidence of heterotopic ossification, a condition where bone forms within muscle tissue.[52] Additional work is needed to better understand the role(s) of muscle-specific BMP-1 secretion in normal and pathological bone formation, but BMP-1 could represent a potential therapeutic target for the prevention of heterotopic ossification (**Table 1**).

Inflammatory Factors that are Secreted from Muscle During Exercise

Interleukin-6, -7 and -15 (IL-6, IL-7, IL-15). The term 'myokine' was originally coined in reference to IL-6, a factor that Pedersen and colleagues[28,53,54] determined was released from muscle during exercise, and had important effects on other tissues including the liver and adipose depots. Type I (slow-twitch) fibers express high levels of IL-6, and IL-6 levels are increased in serum with exercise.[53] IL-6 can stimulate expression of the anti-inflammatory factor IL-10, and mice lacking IL-6 develop obesity and insulin resistance.[55] IL-6 is often considered a pro-resorptive cytokine for bone, but mice lacking IL-6 do not show an osteopenic phenotype and IL-6 may facilitate bone formation during conditions of high bone turnover.[56,57] IL-7 is also widely considered to be an osteoclastogenic cytokine,[58] and IL-7 is actively secreted by muscle cells.[59] Interestingly, many of the studies cited above (for example, Chan *et al.*[24], Norheim *et al.*[26], Henningsen *et al.*[27], Yoon *et al.*[46]) that utilized *in vitro* cultures of myotubes to characterize the muscle secretome failed to identify IL-6 and -7 in conditioned medium. These observations raise the possibility that there are other myokines left to be identified that may be discovered through novel alternative *in vivo* and *in vitro* approaches. Finally, IL-15 is highly expressed in muscle tissue and is upregulated following resistance exercise.[60] Transgenic mice overexpressing IL-15 in skeletal muscle that show elevated circulating IL-15 levels also show decreased fat mass and increased bone mass.[61] Importantly, these mice did not differ in lean mass or body weight from normal controls, suggesting that the increased bone mass was not due to any alterations in mechanical factors.

Summary and Future Research

A paracrine role for skeletal muscle is not necessarily a new concept, as experiments where skeletal muscle was trans-

planted into cardiac tissue have revealed that skeletal muscle implants are a source of trophic factors supporting the survival of surrounding myocardial cells.[62] The paracrine and endocrine effects of muscle on bone are, however, only now beginning to become more well defined. We are at a very early stage in our understanding of how the muscle secretome impacts bone and other organs, and hence a number of outstanding questions remain. These include questions as to how muscle-derived factors impact particular cell types. For example, how do factors secreted from myocytes during muscle contraction and muscle hypertrophy influence bone resorption by osteoclasts, bone formation by osteoblasts or the adipogenic differentiation of bone marrow-derived stem cells? Similarly, how do various modes of muscle contraction, such as concentric vs eccentric contraction, alter myokine expression or secretion? Finally, how do unloading, microgravity or prolonged bedrest impact the muscle secretome?

These questions all point to the larger issue of how muscle activity and metabolism impact the overall systemic environment to which cells of bone and other tissues are exposed. For example, it is clear that exposing muscle of aged animals to circulating factors from younger animals improves the regenerative capacity of muscle.[63] Alternatively, how do changes in muscle, be they catabolic or anabolic, alter the systemic milieu to affect other organs such as bone? Preserving muscle mass decreases mortality and improves survival in tumor-bearing mice,[64,65] and loss of lean mass is an important predictor of health outcomes following burns and with chronic wounds.[66,67] The positive effects of muscle mass on health outcomes are related at least to the fact that muscle is the primary source of free amino acids in the body;[68,69] however, it is also likely that certain myokines may also have a role. For example, treatment of a tumor cell-line with mouse serum following exercise, or with conditioned medium from myotubes following electrical stimulation, reduces tumor cell proliferation and increases apoptosis.[70] These effects of muscle-derived serum and media on tumor cells were attributed to the fact that myotubes secrete the anti-tumor protein oncostatin M.[71] This finding has significant implications for cancer and bone (for example, osteosarcoma), and future studies might be directed at elucidating the interface between myokines, metastasis of cancer to bone and tumor growth in bone tissue.

Another outstanding question concerns the interactions of muscle-derived factors with organs other than bone. Skeletal muscle hypertrophy and hyperplasia increase serum IGF-1 levels in mice lacking myostatin. The increase in circulating IGF-1 levels is associated with elevated liver-derived IGF-1 in these mice with no change in muscle-specific IGF-1 expression.[71] Thus, heritable variation in muscle mass has the potential to dramatically alter growth factor production by other organs, which is likely to have broad systemic effects on tissues such as bone and cartilage. Exercise has recently been shown to increase secretion of follistatin by hepatocytes,[72] and follistatin is a potent inhibitor of both activin and myostatin, factors that suppress muscle hypertrophy and impair muscle regeneration. Follistatin also increases osteoblast mineralization *in vitro*,[73] raising the possibility that interactions among myokines and hepatokines may influence bone metabolism both directly, through their effects on bone cells and indirectly, by modulating growth factor and cytokine production in other organs. A similar relationship may exist between muscle and fat, where myokines can induce lipolysis,[74] that would presumably affect circulating levels of adipokines such as leptin, which can in turn significantly alter bone formation and resorption.

In conclusion, *in vivo* and *in vitro* studies now demonstrate that muscle can function as an endocrine and paracrine organ. The factors secreted by muscle may vary according to muscle activity, such as concentric and eccentric contraction, disuse or damage in the form of traumatic injury. Factors actively secreted by muscle range from growth factors to inflammatory cytokines, and these peptides have the potential to alter bone metabolism in a variety of ways. Additional research is needed to better define the molecular and cellular pathways through which muscle-derived factors affect different types of bone cells, and anabolic and catabolic processes, in bone tissue. Nevertheless, the studies reviewed here further underscore the complex nature of muscle–bone interactions, and highlight the importance of integrating muscle biology and physiology into our understanding of bone growth, development and aging.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgments

I am grateful to Dr Serge Ferrari and Neil Andrews for the opportunity to prepare this contribution for the *BoneKey*. Many of the topics discussed here were presented in the Symposium on Muscle–Bone Interactions, at the 2011 meeting of the American Society for Bone and Mineral Research, and I appreciate the ASBMR for giving me the opportunity to present these ideas in San Diego. Drs Gordon Warren, Carlos Isales, Norman Pollock and Richard Lewis provided helpful discussions on the topics presented here, and Dr Moataz Elkasrawy, Mr Matthew Bowser and Mr Phonepasong Arounleut provided assistance with many experiments referenced in the paper. Drs James Cray, Norman Pollock and two anonymous reviewers provided helpful reviews of the paper. Funding for this research was provided by the Office of Naval Research, the Congressionally Directed Medical Research Programs (Department of the Army) and the National Institute on Aging.

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