Published in final edited form as:

Exerc Sport Sci Rev. 2011 January ; 39(1): 43–47. doi:10.1097/JES.0b013e318201f601.

A Role for Myokines in Muscle-Bone Interactions

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Abstract

This review presents the hypothesis that muscle is a source of secreted factors (myokines) that can influence bone mass in both positive and negative ways. Growth factor secretion by muscle may therefore be one pathway through which mechanical signals are transduced biologically.

Keywords

IGF-1; FGF-2; Myostatin; periosteum; muscle injury

Introduction: Basic Mechanisms of Muscle-Bone Interactions

Bone mass and strength are known to be highly correlated with body weight, and it is often noted that lean (muscle) mass shows a particularly strong association with estimates of bone strength such as bone mineral content and area moment of inertia (30). Influential concepts in bone biology such as Wolff's Law of Bone Transformation, Harold Frost's Mechanostat Model, and Melvin Moss' Functional Matrix hypothesis all highlight the potential of bone tissue to adapt to the mechanical stresses and strains that it regularly experiences. Muscle is a primary source of mechanical stimuli for bone, providing not only the peak loads that generate the highest bone strains, but muscle also generates low magnitude stimuli in the high frequency domain (18). Although recent studies point to a novel role for bone as an endocrine organ that plays an important role in energy balance (22), bone has historically been viewed primarily as either a reservoir for mineral storage or a structural organ whose function is primarily to move and support body weight. Hence, the cross-sectional geometry of bone and its microstructural organization are expected to generally reflect the mechanical forces that surrounding soft tissues (muscle and fat) apply to the bone, either due to contractile forces in the case of muscle or gravitational loads associated with body weight in the case of fat.

Our understanding of the relationship between body weight and bone mass has changed with the recognition that adipose tissue is a rich source of secreted factors generally referred to as "adipokines" that may have important effects on bone formation and bone resorption (33). These factors include leptin and adiponectin, as well as inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (11, 12). Despite the fact that muscle is the largest internal organ in the body, and muscle also secretes a variety of different cytokines and growth factors collectively referred to as "myokines", a role for muscle-derived factors in health and disease is only recently being appreciated (28, 34). In fact, a PubMed search for "myokines" yields less than 30 publications, whereas a search for "adipokines" produces over 15,000 references. We have identified several myokines that are

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localized to the muscle-bone interface and are known to play a role in bone formation. The muscle-bone interface is defined here as a site of fleshy muscle fiber insertion into periosteum, and excludes tendinous or aponeurotic attachments. This article summarizes the evidence in support of our hypothesis that muscle is a source of secreted factors (myokines) that can significantly influence bone repair and bone metabolism. Hence, muscle may not only regulate bone mass through its effects on mechanotransduction, but also via paracrine and endocrine signaling pathways. This hypothesis suggests that large molecules (e.g., myostatin inhibitors, androgen receptor modulators, or vitamin D receptor agonists) that can selectively enhance muscle mass and increase secretion of osteogenic myokines are also likely to improve bone strength.

Osteogenic Myokines at the Muscle-Bone Interface

One of the more common clinical observations suggesting that muscle may secrete osteogenic factors is the fact that bone fractures covered with muscle flaps heal more quickly than those where muscle coverage is not available or where the muscle is damaged (31). Additional studies have shown that if porous barriers are placed between muscle and bone, healing is enhanced when the pores are large enough for growth factors and other peptides to pass (14, 19). These findings would seem to implicate muscle-derived factors directly in the bone repair process. Several of the myokines identified to date (28, 34) whose secretion appears to be altered with exercise and muscle contraction are known to affect bone metabolism. For example, interleukin 6 (IL-6) is associated with increased osteoclast activity and bone resorption, and leukemia inhibitory factor (LIF) can stimulate bone formation *in vivo*. Other myokines, however, such as fibroblast growth factor 21 (FGF21) and follistatin-like 1 (Fstl-1) are not yet demonstrated to have any effects on either bone formation or bone resorption.

We have recently shown that two well-known osteogenic factors, IGF-1 and FGF-2, are localized to the muscle-bone interface *in vivo*, are abundant in homogenized muscle tissue, and are secreted from cultured myotubes *in vitro* (13). In addition, we have shown that receptors for these growth factors are localized to periosteum at the muscle-bone interface (13), and it has been shown previously that IGF-1 and FGF-2 will stimulate bone formation *in vitro* and *in vivo* (24, 35; Fig. 1). Thus, we propose that IGF-1 and FGF-2 are important myokines for bone, in addition to previously documented muscle-derived factors such as IL-6 and LIF. IGF-1 is abundant in wound exudates of skeletal muscle flaps (33), and both IGF-1 and FGF-2 are present in extracts from crushed muscle (13, 15). Since both IGF-1 and FGF-2 are known to be anabolic for bone, this raises the question of what conditions might favor and/or increase the secretion of these osteogenic myokines. In the case of IGF-1, muscle hypertrophy with growth hormone treatment is associated not only with an increase in liver-derived IGF-1 but also with an increase in skeletal muscle IGF-1 (32). IGF-1 is a potent myoanabolic factor, and it is likely that increased IGF-1 expression with muscle hypertrophy also increases IGF-1 secretion and the local abundance of IGF-1 at the muscle-bone interface (Fig. 1). We believe that muscle hypertrophy and bone anabolism are, in this way, coupled through an IGF-1 mediated paracrine signaling mechanism.

There is now good evidence that FGF-2 is released with myocyte wounding and injury (4; Fig. 1). We have documented this *in vitro*, showing that scraping cultured myotubes increases levels of FGF-2 in conditioned medium (13), and others have demonstrated that muscle injury with eccentric contraction during exercise or with dystrophin deficiency increases local FGF-2 release as well as circulating levels of FGF-2 (5). Plasma membrane disruption occurs not only as a result of tissue trauma, but also in nature under physiological conditions. Plasma membrane disruption injury is now demonstrated to be a widespread, common (especially in muscle) and normal cell event that scales with mechanical load (27).

This process is well described in cardiac muscle, where cardiac muscle myocytes exhibit growth responses to FGF-1 and -2 *in vitro*. If paced electrically *in vitro*, these cells are subject to more frequent plasma membrane disruption injury (self-induced from the contractile activity), grow at a greater rate than non-paced controls, and release more FGF-2 (27). These studies suggest that mechanically induced cell wounding stimulates the release of FGF-2 from injured myocytes, and that this in turn leads to hypertrophy in adjacent muscle cells.

We have proposed that a similar phenomenon exists in skeletal muscle, and that exercise-induced plasma membrane disruption in myofibers is a potent bone anabolic stimulus mediated by FGF-2 signaling (13). The potential osteogenic effects of muscle injury are, we believe, most significant for increasing periosteal bone formation, since osteoprogenitor cells residing in periosteum are those most likely to be affected by growth factors secreted by neighboring myocytes (Fig. 1). Furthermore, periosteal capillaries are known to be confluent with venules of attached muscle, providing a potential route for exchange of muscle- and bone-derived growth factors. This pathway may in part contribute to the well-documented relationship between increased physical activity and the accretion of both muscle and bone in young people. Together, these *in vitro* and *in vivo* studies suggest that activities which induce non-lethal injury to myocytes are likely to enhance bone formation and bone mass. Future studies should be directed at determining the effects of resistance exercise, mild muscle injury, and/or eccentric contraction on the release of these muscle-derived growth factors and the resulting osteogenic response.

We have identified IGF-1 and FGF-2 as two osteogenic myokines, but others also appear to exist (Table 1; 1, 3). For example, a recent study found that proteins such as matrix metalloproteinase-2 (MMP-2), secreted protein rich in cysteine protein (SPARC, or osteonectin), and insulin-like growth factor binding protein-5 (IGFBP5) are secreted by C2C12 myotubes *in vitro* (Table 1; 1, 3, 16). Each of these factors is known to play a role in bone formation and bone metabolism (13). Myokines are also likely to have anabolic effects on other connective tissues besides bone, such as ligaments and cartilage. In fact, a recent publication has shown that cartilage cells co-cultured with muscle cells in 2D or 3D conditions showed a marked increase in cartilage extracellular matrix production (2). Furthermore, chondrocytes treated with conditioned medium from cultured muscle cells showed increased extracellular matrix formation, suggesting that muscle cells secrete pro-chondrogenic factors (2).

Myokines: A Link between Muscle and Bone Atrophy?

We also propose that if bone receives anabolic stimuli from muscle in the form of paracrine signals, then it is likely that catabolic changes in muscle should produce catabolic or anti-osteogenic changes in bone. Such a relationship has been observed using BoTox injections in skeletal muscle, which produce a rapid degradation of bone (8). It is certainly likely that such rapid and localized catabolism of bone is a result of alterations in neuronal connectivity. We have, however, also suggested that conditions favoring muscle atrophy, such as disuse with bedrest, exposure to microgravity, or inflammatory events such as infection, burns, or trauma will inhibit bone formation by increasing local and circulating levels of the muscle-derived factor myostatin (GDF-8) (6; Fig. 2). Myostatin (GDF-8), a member of the transforming growth factor- β superfamily of secreted growth and differentiation factors, is a negative regulator of skeletal muscle growth. Absence of myostatin or myostatin deficiency significantly increases muscle mass in mice, cows, and humans, whereas treatment with myostatin causes muscle wasting (23). Myostatin levels are elevated with disuse atrophy, cancer and AIDS-related cachexia (6, 23), and myostatin levels have been shown to decrease with aerobic exercise (17).

Cloning and sequencing of the myostatin promoter region has demonstrated that the myostatin gene has a glucocorticoid (GC) response element. This has been validated both in vitro and in vivo, where GC treatment increases myostatin expression in C2C12 myoblasts and in skeletal muscle of rats treated with GCs (26). It has also been found that loss of myostatin function ablates GC-induced muscle atrophy in mice (7). Together, these studies indicate that myostatin is a target of GCs in muscle cells. It is known that glucocorticoid levels are elevated in stressful conditions that induce muscle wasting, suggesting that GC-mediated stimulation of myostatin induces muscle atrophy (21; Fig. 2). We and others have shown that myostatin deficiency increases bone density, the osteogenic differentiation of bone-marrow derived stem cells, and bone repair (6, 9-10, 20). Muscle is the primary source of myostatin in the body, and we believe that conditions favoring muscle atrophy and increased myostatin secretion will in turn suppress bone formation and bone mass through myostatin's anti-osteogenic effects (Fig. 2). It is well established that conditions associated with muscle atrophy such as aging, unloading, and paralysis, are also associated with bone loss. The most common explanation for these observations is that a marked decrease in muscle-derived mechanical stimuli leads to increased bone resorption and bone loss. This is likely to be true, however it is also likely that neuronal signals linking muscle and bone are also involved as are paracrine and endocrine signals derived from muscle. Myostatin appears to be a key factor linking muscle atrophy with bone loss, and as such represents a myokine with anti-osteogenic effects on the skeleton.

Summary

A large number of studies have established what is now a relatively well accepted phenomenon, namely that muscle mass and bone mass are closely linked across growth, development, and aging. A significant body of research also exists linking mechanical stimuli derived from muscle with bone anabolism and osteogenesis. We have proposed that endocrine and paracrine signals emanating from muscle are also likely to play a role in modulating bone metabolism. These muscle-derived endo- and paracrine signals are likely to be altered with resistance exercise, eccentric contraction, and muscle hypertrophy. Hence, mechanical and biological stimuli may function synergistically with one another, where growth factor secretion by muscle is one of several possible ways that mechanical signals are transduced biologically (13). A role for myokines in bone metabolism may also explain cases where muscle effects are sometimes observed at distant skeletal sites. For example, total lean mass is a robust predictor of cortical bone size of the non-dominant radius (25), yet the radius is a non-weight bearing bone and the non-dominant forearm normally experiences relative low bone strains. The correlation between cortical bone size and lean mass at such a site might be due to either local, paracrine effects of myokines on the radius or to systemic endocrine effects of myokines on the whole skeleton.

Clinical and laboratory studies have also demonstrated that muscle atrophy is associated with bone loss. Again, changes in mechanotransduction with declining muscle strength have frequently been implicated in these catabolic changes. We suggest that muscle wasting and muscle atrophy are also associated with secretion of anti-osteogenic myokines, such as myostatin, that couple reduction in muscle mass with decline in bone mass. The identification of molecules involved in muscle atrophy and bone loss presents a unique therapeutic opportunity, and not surprisingly various myostatin inhibitors, androgen receptor modulators, and vitamin D receptor agonists are currently being studied for their potential to treat a variety of muscle wasting conditions including age-associated muscle loss (sarcopenia). The paracrine cross-talk between muscle and bone described in this review further suggests that such therapies might also attenuate bone loss with neuromuscular diseases, aging, and muscle atrophy. In addition, if muscle-derived factors can enhance extracellular matrix production by chondrocytes in articular cartilage, or the proliferation

and survival of chondrocytes, then myoanabolic factors may also inhibit the development or progression of osteoarthritis by altering myokine secretion. As noted in the introduction, the field of myokine research is in its infancy. Future research should be directed at better understanding the regulation of myokine secretion, and the changes that occur in myokine-bone interactions with exercise, growth, and aging.

Acknowledgments

Funding Disclosure: Funding for this work was provided by the National Institutes of Health (AR049717) and the Office of Naval Research (N000140810197).

References

1. Bortoluzzi S, Scanapieco P, Castaro A, Danieli G, Schiaffino. Computational reconstruction of the human skeletal muscle secretome. *Proteins*. 2006; 62:776–92. [PubMed: 16342272]
2. Cairns DM, Lee P, Uchimura T, Seufert CR, Kwon H, Zeng L. The role of muscle cells in regulating cartilage matrix production. *J Orthop Res*. 2010; 28:529–536. [PubMed: 19813241]
3. Chan X, McDermott J, Siu K. Identification of secreted proteins during skeletal muscle development. *J Proteome Res*. 2007; 6:698–710. [PubMed: 17269726]
4. Clarke M, Khakee R, McNeil PL. Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. *J Cell Sci*. 1993; 106:121–133. [PubMed: 8270618]
5. D'Amore PA, Brown R, Ku P, Hoffman E, Watanabe H, Arahata K, Ishihara T. Elevated basic fibroblast growth factor in the serum of patients with Duchenne muscular dystrophy. *Ann Neurol*. 1994; 35:362–5. [PubMed: 8122890]
6. Elkasrawy M, Hamrick MW. Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. *J Musculoskelet Neuronal Interact*. 2010; 10:56–63. [PubMed: 20190380]
7. Giston H, Schakman O, Combaret L, Lause P, Grobet L, Attaix D, Ketelslegers J, Thissen J. Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. *Endocrinology*. 2007; 148:452–460.
8. Gross T, Poliachik S, Prasad J, Bain SD. The effect of muscle dysfunction on bone mass and morphology. *J Musculoskelet Neuronal Interact*. 2010; 10:25–34. [PubMed: 20190377]
9. Hamrick MW, Samaddar T, Pennington C, McCormick J. Increased muscle mass with myostatin deficiency improves gains in bone strength with exercise. *J Bone Miner Res*. 2006; 21:477–483. [PubMed: 16491296]
10. Hamrick MW, Shi X, Zhang W, Pennington C, Kang B, Thakore H, Haque M, Isaacs CM, Fulzele S, Wenger K. Loss of myostatin function increases osteogenic differentiation of bone marrow-derived mesenchymal stem cells but the osteogenic effect is ablated with unloading. *Bone*. 2007; 40:1544–1553. [PubMed: 17383950]
11. Hamrick MW, Ferrari SL. Leptin and the sympathetic connection of fat to bone. *Osteoporos Intl*. 2008; 19:905–12.
12. Hamrick MW, Della-Fera MA, Baile CA, Pollock NK, Lewis RD. Body fat as a regulator of bone mass: experimental evidence from animal models. *Clin Revs Bone Miner Metab*. 2009; 7:224–229.
13. Hamrick MW, McNeil PL, Patterson SL. Role of muscle-derived growth factors in bone formation. *J Musculoskelet Neuronal Interact*. 2010; 10:64–70. [PubMed: 20190381]
14. Harry L, Sandison A, Paleolog E, Hansen U, Pearse M, Nanchahal J. Comparison of the healing of open tibial fractures covered with either muscle or fasciocutaneous tissue in a murine model. *J Orthop Res*. 2008; 26:1238–1244. [PubMed: 18404722]
15. Haugk K, Roeder R, Garber M, Schelling G. Regulation of muscle cell proliferation by extracts from crushed muscle. *J Anim Sci*. 1995; 73:1972–1981. [PubMed: 7592081]
16. Hittell DS, Berggren J, Shearer J, Boyle K, Houmard J. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes*. 2009; 58:30–38. [PubMed: 18835929]

17. Hittel DS, Axelson M, Sarna N, Shearer J, Huffman KM, Kraus WE. Myostatin decreases with aerobic exercise and associates with insulin resistance. *Med Sci Sports Exerc*. Epub ahead of print April 8, 2010.
18. Judex S, Rubin C. Is bone formation induced by high-frequency mechanical signals modulated by muscle activity? *J Musculoskelet Neuronal Interact*. 2010; 10:3–11. [PubMed: 20190375]
19. Kaufman H, Reznick A, Stein H, Barak M, Maor G. The biological basis of the bone-muscle inter-relationship in the algorithm of fracture healing. *Orthopedics*. 2008; 31:751. [PubMed: 19292424]
20. Kellum E, Starr H, Immel D, Arounleut P, Fulzele S, Wenger K, Hamrick MW. Myostatin (GDF-8) deficiency increases fracture callus size, Sox-5 expression, and callus bone volume. *Bone*. 2009; 44:17–23. [PubMed: 18852073]
21. Lang CH, Silvis C, Nystrom G, Frost AR. Regulation of myostatin by glucocorticoids after thermal injury. *FASEB J*. 2001; 15:1807–09. [PubMed: 11481237]
22. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn J, Confavreux C, Dacquin R, Mee P, McKee M, Jung D, Zhang Z, Kim J, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007; 130:456–69. [PubMed: 17693256]
23. Lee SJ. Regulation of muscle mass by myostatin. *Annual Rev Cell Dev Biol*. 2004; 20:61–86. [PubMed: 15473835]
24. Liang H, Pun S, Wronski TJ. Bone anabolic effects of basic fibroblast growth factor in ovariectomized rats. *Endocrinology*. 1999; 140:5780–8. [PubMed: 10579344]
25. Lorentzon M, Landin K, Mellstrom D, Ohlsson C. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. *J Bone Miner Res*. 2006; 21:1871–78. [PubMed: 17002557]
26. Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, Arias J, Salehian B. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *Am J Physiol Endocrinol Metab*. 2003; 285:E363–E371. [PubMed: 12721153]
27. Miyake K, McNeil PL. Mechanical injury and repair of cells. *Crit Care Med*. 2003; 31:S496–501. [PubMed: 12907878]
28. Pedersen B. Muscle as an endocrine organ: IL-6 and other myokines. *J Appl Physiol*. 2009; 107:1006–1014. [PubMed: 19696361]
29. Pollock N, Laing E, Baile C, Hamrick MW, Hall DB, Lewis R. Is adiposity advantageous for bone strength? A peripheral and quantitative computed tomography study in late adolescent females. *Am J Clin Nutr*. 2007; 86:1530–38. [PubMed: 17991669]
30. Reid IR. Relationships between fat and bone. *Osteoporos Int*. 2008; 19:595–606. [PubMed: 17965817]
31. Stein AH, Perren SM, Cordey J, Kenwright J, Mosheiff R, Francis MJ. The muscle bed—a crucial factor in fracture healing: a physiological concept. *Orthopedics*. 2002; 25:1379–1383. [PubMed: 12502201]
32. Turner JD, Rotwein P, Novakofski J, Bechtel P. Induction of mRNA for IGF-1 and IGF-II during growth-hormone stimulated muscle hypertrophy. *Am J Physiol*. 1988; 255:E513–7. [PubMed: 3177637]
33. Vogt P, Boorboor P, Vaske B, Topsakal E, Schneider M, Muehlberger T. Significant angiogenic potential is present in the microenvironment of muscle flaps in humans. *J Reconstructive Microsurgery*. 2005; 21:517–523.
34. Walsh K. Adipokines, myokines, and cardiovascular disease. *Circ J*. 2009; 73:13–18. [PubMed: 19043226]
35. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi G, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-1 directly regulate bone growth and density. *J Clin Invest*. 2002; 110:771–81. [PubMed: 12235108]

Figure 1.

A. Cross-section of a proximal limb segment showing skeletal muscle surrounding bone. Muscle injury with exercise releases FGF-2 from wounded myocytes into the circulation, and skeletal myocytes also secrete IGF-1 into the bloodstream in addition to liver-derived IGF-1. Myofiber hypertrophy is thought to increase IGF-1 expression and secretion. B. Enlarged section of the muscle-bone interface, showing osteoprogenitor cells lining the periosteal and endosteal surfaces of cortical bone. Circulating muscle-derived FGF-2 and IGF-1 is delivered to endosteal bone-forming cells lining the marrow cavity, cells that express receptors for FGF-2 (FGF-R2) and IGF-1 (IGF-1R). Periosteal bone-forming cells directly adjacent to myocytes also express these receptors and also receive local, muscle-derived IGF-1 and FGF-2. Hence, muscle-derived growth factors can stimulate their receptors in osteoprogenitor cells and osteoblasts through both endocrine and paracrine pathways.

Figure 2.

Muscle is also a source of the anti-osteogenic factor myostatin (Mstn, or GDF-8). Conditions such as disuse atrophy due to weightlessness, bedrest, and unloading, or conditions associated with stress such as infection, trauma, or burns, elevate circulating glucocorticoids. These glucocorticoids stimulate myostatin expression and myostatin secretion in muscle, which in turn contributes to muscle wasting but also acts to inhibit bone formation directly. Myostatin inhibitors can enhance muscle mass and also increase bone formation in vivo.

Table 1

Osteogenic factors known to be secreted from myotubes.

Factor	Abbreviation	Reference
Insulin-like growth factor 1	IGF-1	1, 13, 16, 32
Insulin-like growth factor binding protein 5	IGFBP5	1
Basic fibroblast growth factor	FGF-2	4, 13
Osteonectin	SPARC	1, 4, 18
Transforming growth-factor beta 1	TGFβ1	1
Matrix metalloproteinase 2	MMP-2	1, 3
Leukemia inhibitory factor	LIF	28