PROSPECTS
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Interactions Between Muscle Tissues and Bone Metabolism

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ABSTRACT

Sarcopenia and osteoporosis have recently been noted for their relationship with locomotive syndrome and increased number of older people. Sarcopenia is defined by decreased muscle mass and impaired muscle function, which may be associated with frailty. Several clinical data have indicated that increased muscle mass is related to increased bone mass and reduced fracture risk. Genetic, endocrine and mechanical factors as well as inflammatory and nutritional states concurrently affect muscle tissues and bone metabolism. Several genes, including myostatin and α-actinin 3, have been shown in a genome-wide association study (GWAS) to be associated with both sarcopenia and osteoporosis. Vitamin D, growth hormone and testosterone as well as pathological disorders, such as an excess in glucocorticoid and diabetes, affect both muscle and bone. Basic and clinical research of bone metabolism and muscle biology suggests that bone interacts with skeletal muscle via signaling from local and humoral factors in addition to their musculoskeletal function. However, the physiological and pathological mechanisms related to muscle and bone interactions remain unclear. We found that Tmem119 may play a critical role in the commitment of myoprogenitor cells to the osteoblast lineage. We also reported that osteoglycin and FAM5C might be muscle-derived humoral osteogenic factors. Other factors, including myostatin, osteonectin, insulin-like growth factor I, irisin and osteocalcin, may be associated with the interactions between muscle tissues and bone metabolism. J. Cell. Biochem. 116: 687–695, 2015.

KEY WORDS: MUSCLE; BONE; SARCOPENIA; OSTEOPOROSIS

Sarcopenia and osteoporosis are becoming worldwide health concerns. Gene, endocrine and mechanical factors as well as inflammatory and nutritional states affect both muscle tissues and bone metabolism (Fig. 1). Results from basic and clinical research of bone and muscle suggest that bone interacts with skeletal muscles via local and humoral signaling pathways in addition to their musculoskeletal function. However, the physiological and pathological mechanisms related to the interactions between muscles and bones remain unclear. Developing interest in this field has led to a new understanding of how these tissues integrate and crosstalk in both the normal physiological and disease states [Kaji, 2014]. This prospect describes the various viewpoints concerning the interactions between muscles and bones.

SARCOPENIA AND BONE

Osteoporosis is a disease characterized by reduced bone density, increased bone porosity and a high risk for fractures. Sarcopenia is defined by decreased muscle mass and impaired muscle function, which may be associated with frailty [Cruz-Jentoft et al., 2010]. Sarcopenia may lead to abnormal physical function, decreased quality of life and increased patient mortality. More than 30% of elderly people (>80 years) suffer from sarcopenia and/or osteoporosis [Kaji, 2013]. It has been widely shown that increased muscle mass, measured as lean body mass, is related to increased bone mineral density (BMD) and a reduction in vertebral fracture risk [Kaji, 2013]. These findings suggest that sarcopenia is related to osteoporosis.

Various factors affect muscle wasting, muscle strength and muscle function differently. Because muscle mass and muscle strength are independently related to bone mass in postmenopausal women, clinicians should consider these parameters separately for the management of patients with sarcopenia and osteoporosis. Although muscle and fat mass change in a similar manner in pre- or peri-menopausal women, muscle mass and fat mass decrease and increase in postmenopausal women, respectively. These findings suggest that aging and estrogen levels are important for changes in body composition.

Which factors are responsible for the effects of aging on both osteopenia and muscle wasting is unknown. Adiposity in bone marrow and muscle as well as in visceral fat tissues progresses with aging, and fat infiltrates nerves and capillaries. Various

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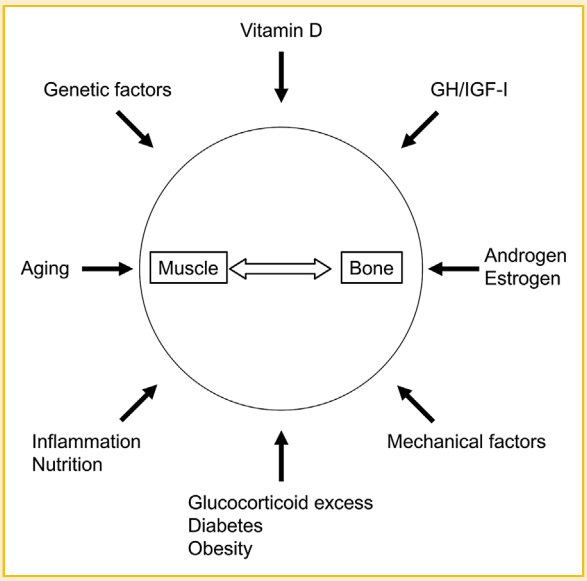

Fig. 1. Factors affecting the interactions between muscle and bone. GH/IGF-I, growth hormone/insulin-like growth factor-I.

adipocytokines, such as leptin, adiponectin and tumor necrosis factor (TNF)-α, produced from adipose tissues may exert negative impacts on muscle and bone. Although debates have been continued as to how fat mass would affect fracture risk, many researchers agree that obesity will negatively affect the risk of non-vertebral fracture. Sarcopenia comorbid with obesity appears to increase the risk of fracture and falls. Recently, the term osteosarcopenic obesity has been used to describe the appearance of obesity in patients with osteoporosis and sarcopenia [Ormsbee et al., 2014]. The increased fat mass may affect interactions between muscle and bone.

LINKAGE BETWEEN MUSCLE AND BONE

Muscle mass is closely related to bone mass during development and growth. Muscle grows more rapidly than bone mass in growing people, suggesting that muscle developed early during growth may enhance the following bone accrual. Moreover, muscle mass is positively correlated with bone mass in the aging population. Calcium, in addition to being central to bone formation, are critical ions for muscle function. Muscle wasting and osteoporosis are observed in patients with pathological states, such as vitamin D-deficient osteomalacia, glucocorticoid excess state and diabetes. These findings raised the interesting topic of whether there are some linkage between muscle tissues and bone/mineral metabolism.

GENETIC FACTORS AFFECTING MUSCLE AND BONE

Common genetic factors seem to affect muscle tissues and bone metabolism concurrently because both osteogenic and myogenic cells are differentiated from mesenchymal stem cells. The contributions of genetic influence on muscle loss and osteopenia are 60–70% [Karasik and Kiel, 2008]. Gene polymorphisms, such as those in the genes for vitamin D, estrogen and androgen receptors, may affect muscle loss and osteopenia concurrently [Karasik and Kiel, 2008]. A study by Bogl et al. [2011] revealed that genetic factors are more important in the relationship between muscle and bone mass, compared with the relationship between fat and bone mass in a study of young adult twins. High-throughput techniques allow genome-wide association studies (GWAS) of a specific variant(s) to be analyzed. Attracting genes, such as myostatin, α-actinin 3, proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and myocyte enhancer factor 2 C (MEF-2C), are included in GWAS as being related to muscle loss and osteoporosis concurrently [Karasik and Cohen-Zinder, 2012].

Myostatin has been shown to be a negative regulator of muscle mass. α-Actinin 3 has been shown to be highly expressed in fast skeletal muscle fibers and may influence muscle differentiation toward a fast twitch fibers. Yang et al. [2011] showed that a lack of α-actinin 3 may be related to reduced BMD in humans and mice. PGC-1α is critical for the regulation of mitochondrial biogenesis [Baldelli et al., 2014], and a study by Handschin and Spiegelman revealed that PGC-1α induced by physical exercise is crucial for the oxidative metabolism in muscle [Handschin and Spiegelman, 2008]. An et al. showed that mitochondrial biogenesis induced by the increase in PGC-1α levels mediates Wnt signaling-induced osteoblastic differentiation of mouse mesenchymal C3H10T1/2 cells [An et al., 2010]. These findings suggest that PGC-1α plays some roles in the commitment and differentiation of mesenchymal stem cells into osteoblasts. MEF-2C is an important factor that interacts with myogenic regulatory factors, such as MyoD and Myf5. This interaction synergistically activates muscle-specific genes and myogenic differentiation. Mice lacking MEF-2C in osteocytes display reduced levels of sclerostin, which is a humoral factor produced by osteocytes that acts as an inhibitor of the Wnt signaling pathway involved in increased bone formation. These findings indicate that MEF-2C-sclerostin signaling may negatively regulate bone mass through the inhibition of Wnt signaling. Since sclerostin is a humoral factor linking bone to muscle as described below, MEF-2C may regulate the interactions between muscle and bone through sclerostin.

The GLYAT gene was detected in a bivariate GWAS as a pleiotropic gene for bone size and muscle mass [Guo et al., 2013]. The GLYAT gene encodes the glycine N-acyltransferase protein, which contributes to the regulation of glucose and energy metabolism. In a recent bivariate GWAS study, methyltransferase-like 21 C (METTL21C) was identified as a suggestive pleiotropic gene for sarcopenia and osteoporosis [Huang et al., 2014]. METTL21C, which is highly expressed in muscle, belongs to the METTL2 family of the methyltransferase superfamily and has protein-lysine N-methyl-transferase activity. The METTL21 family proteins methylate chaperone proteins. Chaperone proteins can harbor specific mutations that are causal to myopathy. This study indicated that METTL21C has a role in myoblastic differentiation and survival of osteocytes through the modulation of the nuclear factor-κB (NF-κB) [Huang et al., 2014]. NF-κB signaling regulates the transcription of interleukin (IL)-6 and sclerostin, humoral factors affecting both

muscle and bone, in muscle cells and osteocytes, respectively. Qiu et al. [2013] showed that NF-κB signaling regulates the transcription of myostatin in myoblasts under conditions of cirrhosis-induced hyperammonemia. This finding suggests that NF-κB antagonists may be used therapeutically to reverse cirrhosis-induced sarcopenia [Qiu et al., 2013]. These findings also suggest that METTL21C modulation of NF-κB may affect the transcriptions of humoral factors linking muscle and bone. Identifying significant genetic variants in both muscle and bone tissues will provide novel insights into the mechanisms of the interactions between muscle and bone.

ENDOCRINE FACTORS AFFECTING MUSCLE AND BONE

Several important endocrine factors affect muscle and bone concurrently (Fig. 1). Vitamin D receptor is expressed in both osteogenic and myogenic cells. Some patients with vitamin D-deficiency suffer from marked osteopenia and muscle wasting. Type II myofibers are predominantly damaged in osteoporotic patients with vitamin D insufficiency. These clinical findings suggest that vitamin D influences the interactions between muscle and bone. Moreover, vitamin D-deficient rats show muscle atrophy and decreased levels of myogenic marker genes including MyoD, myogenin and Myf-5 [Bhat et al., 2013], suggesting that vitamin D plays a significant role in myoblastic differentiation. Genes for muscle atrophy markers including atrogin-1 and MuRF1 as well as proteasomal subunit genes, such as PSC2 and PSC8, were elevated in vitamin D-deficient rats [Bhat et al., 2013], suggesting that vitamin D deficiency accelerates degradation of muscle protein by stimulating the ubiquitin pathway. Although the molecular mechanisms of vitamin D actions in the linkage between muscle and bone remain unknown, Garcia et al. [2011] reported that active vitamin D promotes the differentiation of myogenic cells through increased levels of insulin-like growth factor (IGF)-II and follistatin expression and decreased levels of myostatin and IGF-I. We recently reported that active vitamin D increases the levels of osteoglycin, then resulting in rescuing the decrease in myotubular differentiation and an increase in osteoblast differentiation through muscle-derived soluble factors [Tanaka et al., 2014]. These findings suggest that vitamin D regulates the levels of IGF-I, myostatin and osteoglycin in myoblasts. Because IGF-I, myostatin and osteoglycin are included in the factors linking muscle to bone, vitamin D may affect the linkage of muscle to bone by regulating the levels of those factors in myoblasts (Fig. 2). Vitamin D insufficiency is common in elderly people. The impaired interactions between muscle and bone caused by vitamin D insufficiency may be involved in age-related sarcopenia and osteoporosis. There is an ongoing debate about whether the effects of vitamin D on muscle are direct or indirect.

Both growth hormone (GH) and IGF-I play some role in muscle and bone growth. GH signaling occurs directly through the activation of specific GH receptors or indirectly via IGF-I signaling. Circulating IGF-I is synthesized primarily in the liver as an endocrine hormone and its production is stimulated in a GH-dependent manner. IGF-I is also produced by specific tissues, including the bone

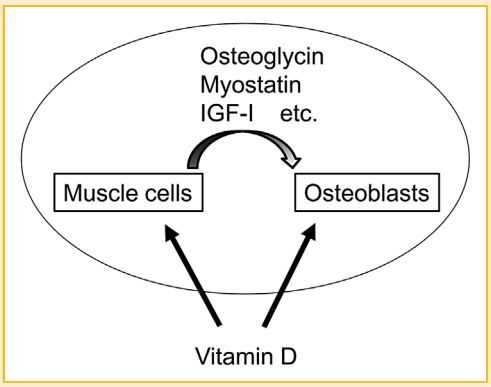

Fig. 2. Vitamin D affects the linkage of muscle to bone.

and muscle, where it is involved in autocrine and paracrine signaling. IGF-I may affect both muscle and bone through systemic and tissue specific signaling pathways (Fig. 3). A study by Terracciano et al. [2013] revealed that osteoporosis is related to muscle atrophy, which correlated with BMD and a reduced the level of Akt, a component of the IGF-I/phosphatidylinositol 3-kinase (PI3-K)/Akt pathway. It appears that abnormalities in GH/IGF-I signaling have a negative effect on muscle and bone in older people. Exercise-induced mechanical loading induces the secretion of IGF-I from muscle. Because it is well known that IGF-I exerts bone anabolic effects, mechanical loading may positively influence bone through the stimulation of IGF-I secretion from muscle. Taken together, these findings indicate that the GH/IGF-I axis may affect both muscle and bone. IGF-I secretion during exercise appears to be a key factor linking muscle to bone. However, the roles of GH/IGF-1 axis in the interactions between muscle and bone are not fully understood.

IGF-I action is regulated by IGF-binding proteins (IGFBPs), which are secreted from muscle and may act on bone. In a cohort study, appendicular skeletal muscle mass correlated with cortical thickness and trabecular BMD [Lebrasseur et al., 2012]. In that study, serum IGFBP-2 concentration was the most robust negative predictor of appendicular skeletal muscle [Lebrasseur et al., 2012]. Thus, IGFBP-2 may have potential as a biomarker for indicating the state of the musculoskeletal system. The data from that study led us to speculate that IGFBP-2 may bind to IGF-I in the circulation and inhibit the beneficial effects of IGF-I on muscle and bone. IGFBPs have both IGF-I–dependent and IGF-I–independent effects. IGFBP-2 has Arg-Gly-Asp (RGD) integrin-binding motifs and interacts with integrins $alpha_5$, $beta_1$ and others. Recently, Xi et al. [2014] revealed that IGFBP-2 facilitates the differentiation of osteoblasts through an interaction of the heparin binding domain-1 with receptor tyrosine phosphatase β. Taken together, IGFBP-specific receptors may mediate the effects of IGFBPs in bone tissues, although further studies are necessary.

Sex hormones also concurrently influence muscle and bone. Androgen deficiency leads to muscle wasting and osteopenia. Moreover, declines in muscle mass, muscle strength and bone mass are observed in men receiving androgen deprivation therapy.

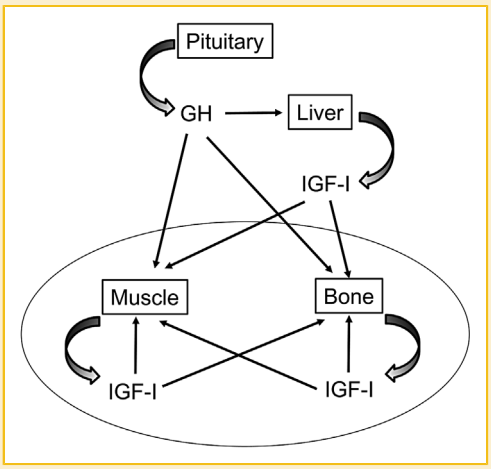

Fig. 3. GH/IGF-I axis in the interactions between muscle and bone. IGF-I affects both muscle and bone in the endocrine as well as autocrine and paracrine manners.

Postmenopausal women show an increased risk of developing sarcopenia and osteoporosis due to lower estrogen levels. In postmenopausal women, a decrease in the bone mass precedes a decrease in muscle mass. Results from a clinical trial with healthy men treated with endogenous testosterone or estrogen inhibitors suggest that androgen deficiency accounted for decreases in muscle mass, size and strength [Finkelstein et al., 2013]. In this trial, estrogen deficiency, however, primarily accounted for increases in body fat [Finkelstein et al., 2013]. Testosterone and estrogen appear to have different effects on muscle, fat and bone.

INFLUENCE OF PATHOLOGICAL ENDOCRINE DISORDERS

It is generally known that muscle loss and osteoporosis are serious problems in glucocorticoid-treated patients. Glucocorticoid excess can cause osteoporosis through decreased proliferation and differentiation of osteoblasts as well as an increase in apoptosis of osteoblasts and osteocytes. Glucocorticoid excess may induce muscle atrophy by inhibiting protein synthesis and stimulating a ubiquitin/proteasome-dependent proteolysis in the skeletal muscle. Results from our preliminary study suggest that plasminogen activator inhibitor-1 may be involved in the decreases in both muscle and bone mass in mice. Therefore, pharmacological doses of glucocorticoids seem to have a negative influence on both muscle and bone.

Type II myofibers are mainly involved in myopathy in diabetic patients. Insulin resistance, lipid accumulation, decreased glycogen synthesis and mitochondrial dysfunction are observed in muscle of patients with type 2 diabetes. In general, the diabetic state is associated with a decrease in muscle mass. It has been recently accepted that diabetes is included in the cause of secondary osteoporosis. In type 1 diabetes, the fracture risk is increased partly through osteopenia. The fracture risk is increased presumably for the decreased bone quality and an increased fall risk partly through muscle wasting in type 2 diabetes. The decreased bone quality might be caused by decreased osteoblastic bone formation and by the negative effects of advanced glycation end-products (AGEs) on collagen quality in bone matrix. Diabetic hyperglycemia increases the levels of AGEs generated from non-enzymatic reactions between reducing sugars and free reactive amino acid residues of proteins. Receptor for AGE (RAGE), a multi-ligand receptor belonging to the immunoglobulin superfamily, mediates the effects of AGEs. Our recent study showed that AGEs suppressed the expression of myogenic genes, such as MyoD and myogenin, as well as osteoglycin in myoblasts [Tanaka et al., 2014]. Moreover, diabetes has been associated with a decrease in muscle mass. These findings suggest that, in diabetes, AGEs are involved in muscle wasting and impairment of osteoglycin-mediated interactions between muscle and bone (Fig. 4).

Chronic inflammatory states have been linked to a loss of muscle mass [Argiles et al., 2014]. In chronic inflammatory states, such as rheumatoid arthritis and inflammatory bowel disease, muscle loss is usually accompanied by bone loss. These pathological conditions lead to elevations in proinflammatory cytokines, such as TNF-α, IL-1β and IL-6. All of these factors facilitate bone loss though an inhibition of osteoblast differentiation and enhanced osteoclastic bone resorption. IL-6 expression in muscle tissues is induced after exercise, then may affect bone metabolism. These factors might facilitate muscle loss through the suppression of myogenic proliferation and differentiation as well as an increase in muscle degradation. These findings suggest that chronic inflammatory states affect the interactions between muscle and bone.

Nutrition has been recognized as an important factor that can influence both muscle and bone [Daly et al., 2014], and nutritional disorders are common among elderly people. Elderly people with a lower protein intake show greater bone loss, suggesting that protein intake is important for maintaining bone mass. Decreased protein

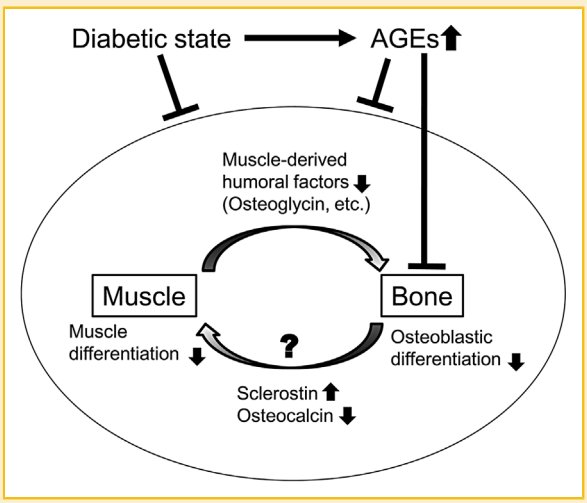

Fig. 4. Effects of the diabetic state on the interactions between muscle and bone. Advanced glycation end-products (AGEs) may be involved in muscle wasting and impairment of osteoglycin-mediated interactions between muscle and bone in diabetic state.

synthesis in muscle seems to be responsible for muscle wasting in patients with inadequate protein intake. These findings suggest that insufficient protein intake might affect the interactions between muscle and bone.

MECHANICAL FACTORS

Mechanical factors play significant roles in both muscle and bone. Mechanical factors may affect the interactions between muscle and bone through an effect on the differentiation of mesenchymal stem cells into osteoblasts and myotubes. Mechanical unloading, where muscles are not used, occurs in situations of long-term bed rest and microgravity in space flight. Mechanical unloading induces muscle atrophy and bone loss. Muscle loss is improved much earlier than osteopenia in astronauts, suggesting that muscle-derived factors related to mechanical loading may be needed to support recovery from bone loss. Exercise causes increase in various factors linking muscle to bone. These factors includes osteoglycin, irisin, osteonectin, fibroblast growth factor (FGF) 2, IL-6, IL-15, IGF-I and osteoactivin. Juffer et al. [2012] revealed that mechanical loading increases the production of various factors, including IGF-I, mechano-growth factor (MGF), the IGF-I gene alternative splicing product, vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in osteocytic MLO-Y4 cells. Humoral factors, such as sclerostin and Wnt3a, are produced by osteocytes, then may affect muscle mass. Therefore, osteocytes may influence muscle mass through the action of various humoral factors as well as via a neural network. Elucidating the role of the mechanostat constituted mainly by osteocytes may lead to a further understanding of the pathogenesis of osteoporosis and sarcopenia.

MUSCLE-DERIVED LOCAL FACTORS AFFECTING OSTEOBLASTIC DIFFERENTIATION

Healing of fractures covered with relatively intact muscle is more rapid than that of fractures covered with more severely damaged muscle, and the application of muscle flaps to autogenous bone grafts is effective for promoting bone reconstruction. Liu et al. [2011] reported that muscle-derived stem cells are important for the recovery of the periosteum from severe injury. These findings suggest that muscle tissues produce local factors that modulate the osteoblastic differentiation of mesenchymal stem cells.

Fibrodysplasia ossificans progressiva (FOP) is a genetic disease with progressive heterotopic ossification in muscle tissues. A heterozygous, constitutively active mutation (c.617G>A; p.R206H) in the glycine-serine domain of bone morphogenetic protein (BMP) type I receptor, activin receptor type I/activin-like kinase 2 (ALK2), is responsible for the pathogenesis in most FOP patients [Shore and Kaplan, 2008]. The mechanism and signaling pathways leading to muscle ossification in FOP still remain unclear. We can speculate that local muscle-specific regulatory factors that enhance or suppress ossification may exist because we cannot observe ossification in muscle tissues in the normal physiological state. FOP may provide some clues for clarifying the local muscle ossification mechanism. In our comprehensive DNA microarray analysis between control and ALK2 (R206H)-transfected myoblastic C2C12 cells [Tanaka et al., 2012a], Tmem119, osteoactivin and Frizzled-3 were included in the genes whose expressions were increased by ALK2 (R206H). Tmem119 interacts with Smad1/5 and Runx2 and is induced by parathyroid hormone, a potent bone formation-stimulating drug [Hisa et al., 2011]. Because Tmem119 enhances the differentiation of myoblasts into osteoblasts, it may be important in muscle ossification in FOP [Tanaka et al., 2012a]. In addition, we reported that matrix metalloproteinase (MMP)-10 and Tmem176b, the factors whose levels are increased by ALK2 (R206H) in C2C12 cells, are related to osteogenic differentiation of myoblasts [Mao et al., 2013; Yano et al., 2014].

Osteoactivin, a type I transmembrane glycoprotein, is involved in the differentiation of osteoblasts and osteoclasts. Nikawa et al. [2014] showed that osteoactivin is among the genes upregulated in skeletal muscle from space-flown rats, suggesting that osteoactivin may be a local regulator of interactions between muscle and bone relate to mechanical loading.

Local muscle-derived factors may be useful for the enhancement of fracture healing as well as the development of clinically efficient bone/cartilage regeneration. This approach may lead to development of a new bone anabolic drugs for osteoporosis.

HUMORAL FACTORS AFFECTING MUSCLE OSSIFICATION

Results from several studies suggest that certain associations between muscle tissue and bone metabolism may be regulated by the release of humoral factors. There are various muscle-derived endocrine factors, such as IGF-I, myostatin, osteoglycin, FAM5C, irisin, osteonectin, FGF2, FGF21, transforming growth factor (TGF)-β, follistatin-like protein 1, leukemia inhibitory factor (LIF), brain-derived neurotrophic factor (BDNF), IL-6, IL-7, IL-15 and MMP-2. These factors exert some biological activity in bone cells (Fig. 5).

Osteoglycin belongs to the small leucine-rich proteoglycan family. In our study, osteoglycin was identified as a molecule whose expression was decreased to <1/4 by the activation of ALK2 signaling, which would activate muscle ossification in myoblasts [Tanaka et al., 2012b]. Osteoglycin as well as the conditioned medium from osteoglycin-overexpressed myoblasts stimulated late osteoblast differentiation, and osteoclycin was detected in human serum [Tanaka et al., 2012b]. These findings suggest that osteoglycin may be included in the humoral bone anabolic factors secreted from muscle tissues.

FAM5C is related to cell proliferation, migration and atherosclerosis. We reported that FAM5C as well as the conditioned medium from FAM5C-overexpressed myoblasts induces late osteoblast differentiation [Tanaka et al., 2012c]. FAM5C may be included in humoral bone anabolic factors secreted from muscle tissues.

Myostatin, a member of the TGF-β superfamily that is expressed mainly in skeletal muscle, has been shown to exert its activity through the activin receptor type IIB (ACVR2B). Binding of myostatin to ACVR2B leads to the phosphorylation and activation of ACVR1 (ALK4 and ALK5) that in turn initiates the intracellular

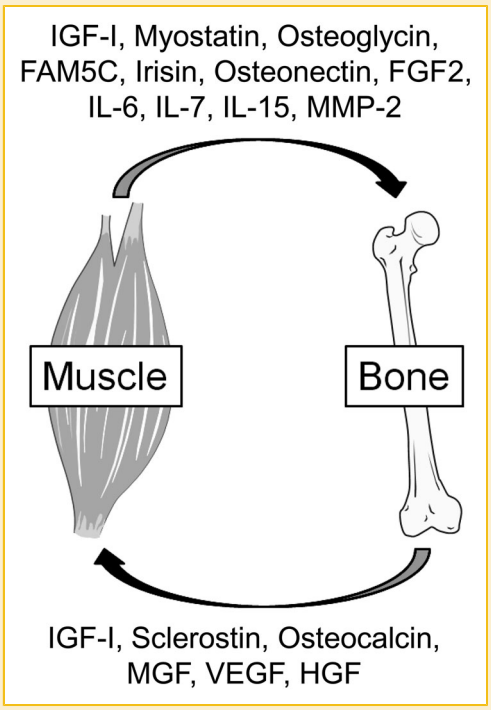

Fig. 5. Humoral factors linking muscle to bone. FGF2, fibroblast growth factor 2; FAM5C, family with sequence similarity 5, member, C; IL, interleukin; MMP-2, matrix metalloproteinase-2; MGF, mechano growth factor; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor.

signaling mediated by the phosphorylation of Smad2 and Smad3. Myostatin gene deficiency appears to induce skeletal muscle hypertrophy [McPherron et al., 1997], whereas overexpression or systemic administration leads to muscle atrophy [Zimmers et al., 2002]. These findings suggest that myostatin is a key negative regulator of skeletal muscle. Kim et al. [2005] revealed that resistance training decreases myostatin levels, suggesting that a negative correlation exists between exercise-induced hypertrophy and myostatin levels. As for bone, Jeong et al. demonstrated that the systemic administration of ACVR2B-Fc, a soluble myostatin decoy receptor, induced gains in hindlimb skeletal muscle weight in *oim/oim* mice (a model of osteogenesis imperfecta) [Jeong et al., 2014]. ACVR2B-Fc enhanced muscle mass and bone formation indices in postmenopausal women. These findings suggest that myostatin exerts an anti-anabolic effect on both muscle and bone through ACVR2B and may be an important humoral factor linking muscle to bone.

Exercise enhances irisin expression in a PGC-1α–dependent manner in muscle [Bostrom et al., 2012]. Anastasilakis et al. reported that circulating irisin levels have been associated with previous osteoporotic fractures in postmenopausal women with low bone mass [Anastasilakis et al., 2014]. Recently, Colaianni et al. revealed that irisin enhances the differentiation of bone marrow stromal cells into osteoblasts [Colaianni et al., 2014]. Moreover, Zhang et al. [2013] showed that irisin facilitates osteoblast differentiation via the Wnt/β-catenin pathway in osteoblastic MC3T3-E1 cells. In that study, irisin inhibits osteoclast differentiation via suppression of the receptor activator of nuclear factor-kappa B ligand (RANKL)/nuclear factor of activated T cells (NFAT) c1 in Raw264.7 cells [Zhang et al., 2013]. These findings suggest that irisin may be a muscle-derived bone anabolic factor. Park et al. [2013] revealed that circulating irisin is related to insulin resistance and an increased risk of metabolic syndrome, cardio-metabolic variables and cardiovascular disease in humans. This suggests that lower circulating irisin levels may reflect the metabolic status of patients suffering from metabolic disorders, including type 2 diabetes. Irisin may be a useful muscle-derived biomarker for osteoporotic fracture risk in patients with type 2 diabetes.

Osteonectin, a 43-kDa matricellular glycoprotein, is involved in cell differentiation, vessel formation and growth factor binding. Aoi et al. reported that the levels of osteonectin were elevated in muscle and plasma of mice and humans undergoing the exercise [Aoi et al., 2013]. The levels of osteonectin gradually returned to the pre-exercise levels within 6 h after exercise [Aoi et al., 2013]. These findings indicate that exercise induces osteonectin secretion from muscle. Osteonectin is one of the most abundant non-collagenous extracellular matrix proteins and exerts anabolic effects in bone.

FGFs have many cellular functions related to processes such as proliferation, differentiation and survival. FGFs bind to FGF receptors and activate downstream signaling pathways, including the Ras/mitogen-activated protein kinase (MAPK) pathway. FGF2 is released from the muscle after injury and extreme exercise. Fei et al. [2011] revealed that FGF2 promotes nuclear accumulation of β-catenin and induces an inhibition of glycogen synthase kinase (GSK)-3β in osteoblasts. These findings suggest that FGF2 facilitates bone formation mediated by the modulation of the Wnt/β-catenin signaling pathway. In addition, mechanical factors may positively influence bone mass through FGF2 release from the muscle. Xiao et al. showed that overexpression of the low-molecular weight/18 kDa FGF2 isoform in osteoblasts and osteocytes enhances the repair of critical size calvarial bone defects in mice [Xiao et al., 2014]. These results suggest that muscle-derived FGF2 may facilitate bone formation during bone repair and regeneration.

LINKAGE OF BONE TO MUSCLE

Recent studies have indicated that bone also demonstrates endocrine function. Osteocytes are recently considered as endocrine cells, which may transfer some signals to distant organs in response to mechanical stress. Osteoblast- or osteocyte-specific factors including osteocalcin, sclerostin and FGF23 may act as endocrine factors linking bone to muscle. Mera et al. [2014] showed that osteocalcin-deficient mice exhibited a decrease in muscle mass and function, suggesting that osteocalcin might exert muscle anabolic effects. Moreover, in primary myotubes, osteocalcin activates AMP-activated protein kinase (AMPK)/mTOR/S6 kinase axis via GPRC6A, a G-protein–coupled receptor [Mera et al., 2014]. These recent findings suggest that osteocalcin may be a humoral factor released from bone tissues and may be involved in the regulation of muscle mass and function through GPRC6A/AMPK/mTOR/S6 kinase. Osteocalcin affects glucose and energy metabolism during adulthood as well as male fertility. The effects of osteocalcin are mediated

by GPRC6A and appear to regulate insulin secretion in β-cells and stimulate testosterone secretion in Leydig cells. Because insulin and testosterone both affect muscle, the effects of osteocalcin on muscle may be due to either direct effects on muscle or indirect effects via the increasing levels of insulin and testosterone (Fig. 6).

Sclerostin is a secreted glycoprotein that is mainly produced by osteocytes and acts as an antagonist of bone formation through the canonical Wnt/β-catenin signaling pathway. Osteocytes and their cellular network are considered as the sensor for mechanical loading, although the process of bone mechanosensation has not been clearly defined. Robling et al. [2008] revealed that the levels of sclerostin were decreased under loading conditions in rodent ulna, particularly in areas of greatest stress. Krause et al. [2014] revealed that sclerostin-deficient mice displayed greater trabecular bone volume and lower muscle mass relative to wild-type mice, suggesting that long-term sclerostin deficiency may have deleterious effects on muscle. Canonical Wnt signaling is activated during muscle regeneration in vivo, and the ligand Wnt proteins, including Wnt3a and Wnt4 enhance myoblast differentiation in vitro. Canonical Wnt signaling induces the switch from myoblast proliferation to differentiation. These findings suggest that osteocyte-derived sclerostin may affect the differentiation and proliferation of myoblasts. Further studies are necessary for elucidating the roles of sclerostin in muscle-bone associations.

FGF23 is predominantly produced in osteocytes. The primary physiological functions of FGF23 appear to be the regulation of phosphorus metabolism by the downregulation of the expression of sodium-phosphate cotransporters and the regulation of vitamin D 1α-hydroxylase in the renal proximal tubule. This occurs through binding to FGF receptor and klotho, its co-receptor. Faul et al. [2011] showed that FGF23 induced left ventricular hypertrophy via FGF receptor-dependent activation of the calcineurin/NFAT signaling pathway, suggesting that FGF23 may affect myocardial cells in an endocrine manner. These findings raise the possibility that FGF23 is one of the several endocrine factors involved in the linkage of bone to muscle. However, the effects of FGF23 on skeletal muscle remain unknown.

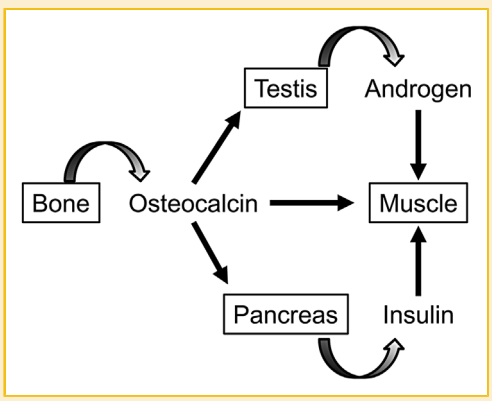

Fig. 6. Osteocalcin affects muscle through either direct effects on muscle or indirect effects by increasing the levels of insulin and androgen.

FUTURE PROSPECTS

The interactions between muscle and bone have recently been established as a new area of research. New findings about muscle and bone interactions are accumulating, although conclusions about the mechanisms involved in these interactions remain unclear. Genetic factors, aging, endocrine factors, pathological condition and mechanical factors all appear to affect the interactions between muscle and bone. The sympathetic nervous system is involved in glucose and energy metabolism in skeletal muscle and bone metabolism. Neurological regulation therefore may influence the interactions between muscle and bone. However, how the nervous system regulates the interactions between muscle and bone is currently unknown.

A variety of humoral factors are considered to participate in the interactions between muscle and bone. The most important factors are unknown. Numerous factors, including IGF-I, FGF2 and TGF-β, are abundantly expressed in many tissues including bone. The physiological and pathological significance of those factors in the muscle-bone interactions will be difficult to clarify. Exercise increases osteoglycin, irisin, osteonectin, FGF2, IL-6, IL-15 and IGF-I in skeletal muscle as previously described in this prospect. Humoral factors that specifically affect the bone during exercise are unknown. Because each factor affects bone through its specific receptor, studies using bone-specific, receptor-deleted mice may be helpful in further defining the interactions between muscle and bone.

Both muscle and bone are endocrine organs affecting each other. Johnson et al. showed that muscle-derived ciliary neurotrophic factor (CNTF) inhibited osteoblast differentiation [Johnson et al., 2014]. These results suggest that there might be some negative regulation between muscle and bone. CNTF appears to be a likely candidate as the negative regulator between muscle and bone. However, it is not fully understand how bone and muscle negatively regulate their endocrine function and how one affects the other.

Inflammatory states affect muscle and bone simultaneously. However, the interactions between muscle and bone in inflammatory states are unclear. Tissue resident macrophages change their phenotypes in response to the surrounding microenvironment including the levels of macrophage-activating cytokines, growth factors and metabolic state. The differentiation of monocytes into osteoclasts is facilitated by inflammatory cytokines. These findings suggest a possibility that tissue resident macrophages may mediate the interactions between muscle and bone in pathological conditions including inflammation and diabetes.

Recent progress has been made in clarifying the relationships between several tissues. Muscle mass and bone mass decreases, but fat mass increases in untreated postmenopausal women. Bonnet et al., showed that peroxisome proliferator-activated receptor (PPAR)-β deficiency caused a decrease in bone formation with a concurrent increase in bone marrow fat infiltration and muscle weakness in mice [Bonnet et al., 2014]. These findings suggest that factors from fat tissues may affect the interactions between muscle and bone. Adipocytes are derived from mesenchymal stem cells similar to osteoblasts and myotubes, suggesting that similar genetic factors may influence bone, muscle and fat concurrently. All of these findings suggest that muscle, bone and fat interactions may be important for better understanding of the pathophysiology of sarcopenia and osteoporosis.

The prevalence of geriatric diseases including sarcopenia and osteoporosis is increasing. Sarcopenia and osteoporosis are clinically very important as common pathological age-related states as well as the cause of locomotive syndrome. Elucidation of the interactions between muscle and bone at the cellular and molecular levels may be helpful for the exploitation of drugs and biomarkers for the treatment and diagnosis of sarcopenia and osteoporosis.

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