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MUSCLE AND BONE (A BONETTO AND M BROTTO, SECTION EDITORS)

Myokines and Osteokines in the Pathogenesis of Muscle and Bone Diseases

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Abstract

Purpose of Review In this review we aim to summarize the latest findings on the network of molecules produced by muscle and bone under physiological and pathological conditions.

Recent Findings The concomitant onset of osteoporosis and sarcopenia is currently one of the main threats that can increase the risk of falling fractures during aging, generating high health care costs due to hospitalization for bone fracture surgery. With the growing emergence of developing innovative therapies to treat these two age-related conditions that often have common onset, a broader understanding of molecular messengers regulating the communication between muscle and bone tissue became imperative.

Summary Recently it has been highlighted that two muscle-derived signals, such as the myokines Irisin and L-BAIBA, positively affect bone tissue. In parallel, there are signals derived from bone that affect either positively the skeletal muscle, such as osteocalcin, or negatively, such as RANKL.

Keywords Irisin · L-BAIBA · Rank-L · Osteocalcin · Osteoporosis · Sarcopenia

Introduction

Given their anatomical proximity, the closely coupled muscle and bone tissues communicate through mechanical interaction and via a finely tuned network of molecules released by both tissues in physiological and pathological conditions [1, 2].

The mechanical load, which is simply applied in an upright posture, reaches its maximum benefit while exercising. Regular physical activity, due to synchronous reinforcement of bone and muscle mass, improves overall health and can prevent or delay several diseases, including osteoporosis, sarcopenia, diabetes, and obesity [3, 4]. Numerous prospective studies have confirmed that mortality can be reduced by 20–40% by walking or running activities [5, 6] slowing down the progression of age-related conditions [3].

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Since it is well documented that increased exercise, hence mechanical loading, has anabolic effects on muscle and bone, as well as states of unloading or immobility cause catabolic responses, the scientific community is currently making efforts to investigate the molecular mediators of the bone–muscle crosstalk.

The first two muscle-secreted factors identified during the last decade of research and termed myokines were myostatin and interleukin 6. It has been shown that myostatin, a suppressor of skeletal muscle mass and development [7], also negatively regulates bone mass. Myostatin knockout mice display improved bone strength following physical activity [8] and increased differentiation of bone marrow mesenchymal cells into osteoblasts [9]. Interleukin 6 (IL-6), highly expressed in skeletal muscle [10], enhances bone resorption, most likely by increasing RANKL gene expression in osteoblasts [11] but, at the same time, increases differentiation of early osteoblasts [12].

First reports showing that bone cells produce factors that affect muscle are instead more recently dated. Among these, prostaglandin E2 and Wnt 3a, two molecules produced by osteocytes in response to shear stress, were found capable of stimulating myogenesis and muscle function [13].

In this review, we will focus on two novel muscle-derived signals, such as the myokines Irisin [14] and L-BAIBA [15••]

that can directly affect bone. We will also discuss very recent evidence regarding signals derived from bone that can affect skeletal muscle with positive effects, such as osteocalcin [16••], and with negative effects, such as RANKL [17••] (Fig. 1).

A better understanding of molecular mechanisms underlying the actions of these messengers would make possible in the future to develop innovative therapies, especially to treat the two twin conditions of aging that commonly affect both tissues, such as osteoporosis and sarcopenia.

Crosstalk from Muscle to Bone

Irisin

The myokine irisin is produced under skeletal muscle contraction in the form of the precursor, the fibronectin type III domain-containing protein 5 (FNDC5), which is then cleaved and released into the bloodstream. Irisin stimulates transdifferentiation of white adipose tissue into a brown adipose tissue-like, by increasing cellular mitochondrial density and expression of uncoupling protein-1 to promote thermogenesis and energy expenditure [18]. Our studies demonstrated that a weekly irisin dose lower than that required for WAT transdifferentiation increases cortical bone mass and strength in mice, thus suggesting that the bone was the primary target organ of the action of Irisin [19].

Both young healthy mice and osteoporotic murine models have been studied to rule out the effects of treatment with exogenous recombinant irisin (rec-irisin). In young mice rec-irisin clearly recapitulated the effects of exercise by improving cortical bone geometry, increasing bending strength and resistance to fracture [19]. In hindlimb unloaded mice, a murine model which mimics adverse effects on musculoskeletal system caused by physical immobility or microgravity [20, 21], intermittent administration of rec-irisin preserved cortical and trabecular bone mineral density, bone volume fraction (BV/TV), and Fractal dimension [22••]. Molecular studies showed that this effect was mainly mediated through inhibition of unloading-induced sclerostin increase and osteoprotegerin decrease [22••]. More recently, we also showed that irisin prevented disuse-induced reduction of viable osteocytes and Caspase-9 and Caspase-3 activations in cortical bone of hindlimb unloaded mice [23]. This murine model is also characterized by muscular atrophy, whose onset was fully prevented by rec-Irisin treatment which preserved muscle integrity, fiber size and the expression of myosin Type II (MyHC II), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (TFAM) [22••].

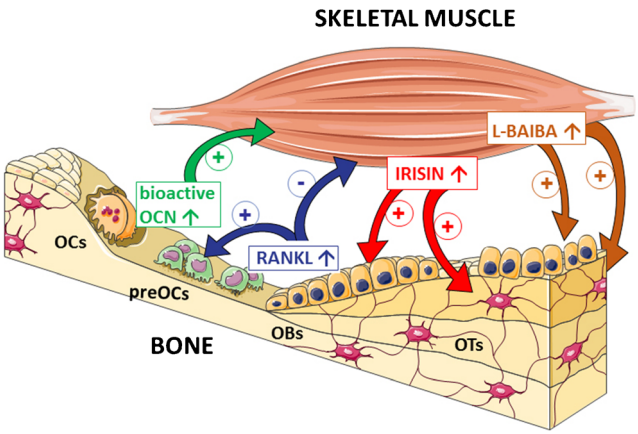

Fig. 1 Schematic representation of the network of molecules produced by skeletal muscle and bone tissues. Some muscle-derived signals, such as the myokines Irisin and L-BAIBA, which positively (+) affect bone tissue by increasing the activity of osteoblasts (OBs) and preventing the apoptosis of osteocytes (OTs). In parallel, there are signals derived from bone that affect either positively the skeletal muscle, such as osteocalcin, which improves muscle functions during exercise, or negatively (−), such as RANKL, which reduces muscle function and strength, and glucose uptake, *We thank Servier Medical Art (https://smart.servier.com/) for providing free image software to build the figure*

We have previously shown that the highest expression of the Irisin precursor, FNDC5, was detectable in muscle tissue and that mice treated with irisin showed a higher number of FNDC5 positive fibers than control mice, suggesting that its autocrine action may amplify its synthesis [19]. The autocrine action of irisin has been also studied in vitro on C2C12 myotubes, in which the a 24-h treatment with rec-irisin significantly increased the expression of the proliferator-activator peroxisome γ receptor-activator-1α (PGC-1α), NRF1, and TFAM, leading to an increase in mitochondrial content and oxygen consumption [24]. Other in vitro studies have shown that an enhancement of FNDC5 mRNA expression and irisin secretion occurs during myogenic differentiation and that treatment with irisin increased insulin-like growth factor 1 (IGF-1) and decreased myostatin gene expression via the ERK pathway [25].

In humans, we and other authors observed a positive correlation between irisin and bone mineral density in young athletes [26, 27]. In soccer players, we found higher linear association at the lumbar vertebrae, right arm, and head than at the femurs. As these bone segments have a lower impact from mechanical loading in soccer, the surprising result indicated that the effect of irisin on bone might be systemic, rather than strictly connected to bone sites where load is applied [27]. We also found in 96 children diagnosed with childhood type 1 diabetes mellitus under insulin replacement therapy that irisin serum levels were positively associated with bone quality and the improvement of glycemic control [28]. Soininen et al. showed that irisin was one of the determinants of bone mineral density in a population of 6–8 years old children [29] In line with this, we found in healthy children that irisin positively correlated with circulating osteocalcin, and negatively with DKK1, one of the bone anabolic inhibitors of the WNT pathway [30]. Additionally, multivariate regression analysis

showed that Irisin was a greater determinant of bone mineral status than bone alkaline phosphatase [31]. In adults, irisin was negatively associated with serum sclerostin levels in patients with prediabetes [32] and with vertebral fragility fractures [33, 34] and sarcopenia [35] in post-menopausal women. We also observed reduced irisin levels in osteoporotic women with hyperparathyroidism compared with controls [36••]. In vitro data further supported this observation showing that Teriparatide (1–34 PTH) treatment decreased the expression of FNDC5 in C2C12 myotubes by acting on PTH receptor, which in turn activates Erk1/2 phosphorylation, most likely through the increase of intracellular cAMP [36••]. On the other hand, rec-irisin reduced PTH receptor expression on osteoblasts by 50% compared with untreated cells, suggesting that irisin can exert its anabolic effect on bone, not only by stimulating osteoblast activity but also by inhibiting the catabolic action of PTH on these cells [36••].

Despite debates and controversies since its discovery, overall the scientific consensus has now unanimously recognized that irisin plays a crucial role in bone metabolism; therefore, Irisin could be a possible serum marker of bone status and a therapeutic option to treat bone diseases in the future.

L-BAIBA

In 2014, Roberts et al. described BAIBA as a small molecule (103.6-Da) produced by skeletal muscle during exercise. BAIBA may be present in the form of two enantiomers: L-BAIBA and D-BAIBA. Using amino-acid L-valine as an energy source, L-BAIBA is produced through the control of the transcriptional co-activator PGC-1α. It has been demonstrated that L-BAIBA is a mediator of the beneficial effect of exercise from skeletal muscle to other organs, especially bone, in an endocrine manner [37].

Experimental evidence showed that BAIBA is involved in the β-oxidation pathway of fatty acids in the liver, is implicated in the browning of white adipose tissue [37], prevents diet-induced obesity [38], plays a role in protecting against metabolic disorders in type 2 diabetes [39], and increases insulin resistance and inflammation of skeletal muscle [40].

In a recent study, L-BAIBA has been described as a molecule that protects osteocytes from cell death. Kitase et al. have shown that only the enantiomer L-BAIBA is produced by contracted muscle independently of skeletal muscle type, sex, and age. In this study, the authors demonstrated that treatment with L-BAIBA on osteocytes in vitro protects against apoptosis induced by exposure to reactive oxygen species [15••]. In hindlimb unloaded mice, in which bone loss is characterized by osteocyte apoptosis [41], it has been observed a reduction in trabecular bone volume and increased osteocyte apoptosis when mice received normal drinking water, whereas in mice receiving drinking water supplemented with L-BAIBA (100 mg/kg/day), the bone volume and viability of osteocytes were preserved [15••]. Osteoblasts appear to be the main mediators of the molecule effect on bone volume, whereas no changes in the number of osteoclasts and their activity as well as changes in the levels of the nuclear factor receptor activator κB (RANK)-ligand have been observed in male or female mice following treatment with L-BAIBA.

L-BAIBA has been shown to bind to glycine receptors and to the MRGPRD, which is also known to be a receptor for β-alanine and gamma-Aminobutyric acid (GABA). Kitase et al. found that MU6840, an antagonist of MRGPRD, blocked the beneficial effect of L-BAIBA on ROS-induced cell death [42]. Potentially L-BAIBA may have a direct effect on osteoblasts, but since osteocytes express much higher levels of MRGPRD, the effects on osteoblasts may be indirect and mediated by osteocytes [15••]. Furthermore, the MRGPRD receptor is strongly expressed on osteocytes in young mice, but its expression is reduced in old mice, implying that osteocytes lose their response to BAIBA with aging, despite muscles of young and old mice both produce L-BAIBA under contraction [15••]. Data currently suggest that L-BAIBA-mediated effects of exercise can increase bone mass and strength in younger subjects, but this positive effect on bones would be significantly reduced with age.

L-BAIBA also plays autocrine functions on skeletal muscle. It has been reported that this myokine mitigates insulin resistance and inflammation and stimulates fatty acid oxidation via the AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor δ (PPARδ) signaling pathway in skeletal muscle [40]. It has been also shown that L-BAIBA increased the muscle contractile strength of the extensor digitorum longus and soleus muscles in male mice but not in females. Possible gender differences in response to L-BAIBA have been hypothesized, as musculoskeletal responses to exercise can change between males and females [43].

In summary, L-BAIBA is a novel muscle-derived molecule that can exert both direct and indirect effects on bone cells, also preserving muscle strength especially in male mice [15••].

Crosstalk from Bone to Muscle

RANKL

The increased levels of the nuclear factor kappa-B ligand receptor activator (RANKL) in menopausal women play a primary role in the development of osteoporosis [44], as this molecule, by binding to its receptor (RANK), activates the differentiation, activity, and survival of bone-resorbing cells, the osteoclasts [45]. On the other hand, osteoprotegerin (OPG), a soluble receptor for RANKL, prevents its binding to RANK, thus inhibiting osteoclastogenesis. Denosumab

(Dmab), a RANKL-blocking antibody mimicking the OPG action, has been shown to reduce the risk of fracture and is widely used for the treatment of osteoporosis [46]. Recently, it has been observed that the fall rate was also reduced in patients receiving Dmab compared with placebo, but this effect has remained for a long time only an unexplained observation [47, 48].

The receptor for RANKL is also expressed in skeletal muscle and its activation mainly inhibits myogenic differentiation, resulting in skeletal muscle dysfunction [49, 50]. In turn, exogenous OPG administration has been shown to reduce inflammation and to restore skeletal muscle function in mdx mice, a mouse model of Duchenne muscular dystrophy [51, 52].

A recent study showed that in post-menopausal osteoporotic women treated with Dmab or Bisphosphonates, both treatment increased bone mineral density at lumbar spine; however, only Dmab significantly increased appendicular lean mass and improved handgrip strength. In transgenic mice over-expressing RANKL, bone loss was associated with impairment of muscle function and strength, and glucose absorption [17••]. These alterations were coupled with increased expression of factors inhibiting muscle growth and function, such as myostatin [17••]. In line with these data, other findings showed that conditional RANK deletion in muscle prevented muscle atrophy and dysfunction induced by denervation in mice [53]. Taken together, these data established that the RANK/RANKL/OPG system also plays a crucial role in muscle metabolism and open new opportunities to further investigate benefits of RANKL inhibition for the treatment of sarcopenia.

OSTEOCALCIN

Osteocalcin is a protein secreted in a carboxylated form solely by osteoblasts [54]. It is a key factor responsible for the mineralization of extracellular matrix and its serum levels are clinically used as marker of osteoblastic bone formation [55].

In 1996, Ducy et al. observed that mice carrying deletion of the osteocalcin gene (osteocalcin knockout mice) had more visceral fat, were poor breeders, and generated litters with significantly fewer pups than wild-type littermates. To date, several roles of osteocalcin have been showed by both in vitro and in vivo studies revealing that the undercarboxylated (bioactive) form of osteocalcin controls numerous physiological processes in an endocrine fashion [56]. Karsenty's group observed that bioactive osteocalcin induces insulin production in pancreatic islets as well as adiponectin expression in adipocytes [57–59]. Moreover, it has been observed that deletion in osteoblasts of the insulin receptor mimics the phenotype observed in osteocalcin knockout mice. These findings suggested that the insulin-mediated osteocalcin expression in osteoblasts could further stimulate insulin secretion in pancreatic islet cells, thus forming an endocrine pancreatic bone loop [60]. In line with this, it has been demonstrated that glucose tolerance in mice on a high-fat diet was restored by daily injections of osteocalcin [61, 62].

In 2016, the scientific evidence that osteocalcin levels increased in mice and humans during physical activity and at the same time its levels decreased during aging led to the discovery of a remarkable function of this bone-derived hormone [16]. Mera et al. showed that treatment with exogenous osteocalcin, whether administered acutely or chronically, increased the exercise capacity of 3-month-old mice and restored the exercise capacity of 9-, 12- and 15-month-old mice. The authors showed that osteocalcin promotes the expression of fatty acid transporters, stimulates β-oxidation, and the translocation of the GLUT4 glucose transporter to the plasma membrane by promoting the absorption of glucose and stimulating catabolism in the skeletal muscle [63]. Osteocalcin also stimulates the synthesis of IL-6 [1], a myokine whose circulating levels increase during exercise, which in turn promotes adaptation to exercise by stimulating the production of osteocalcin in bone [63].

More recently, it has been observed that osteocalcin is necessary for brain development and functions. Its absence makes the brain, particularly the hippocampus, smaller and less developed and, in addition, mice with osteocalcin deficiency are less active and suffer from anxiety and memory impairment compared with wild-type mice [64]. The regulation of cognitive functions by osteocalcin, together with the observation that its circulating levels decrease in mid-life compared with adolescence in all tested species, suggested that osteocalcin may be an anti-geronic hormone effective in preventing age-related cognitive decline [64]. The numerous functions that osteocalcin regulates in the brain during development and after childbirth have long required identification of its receptor, the G protein-coupled receptor 158, which has recently been discovered [65]. Remarkably, the same group hypothesized that bone evolved, in part, to improve the ability of bone vertebrates to escape dangerous situations. In 2019, it has been reported that in rodents and humans exposed to various types of stress, there is a rapid wave of circulating bioactive osteocalcin which mediated acute stress response by inhibiting parasympathetic tone [66]. More specifically, the authors reported that when the amygdala in brain perceives danger, it transmits a signal to osteoblasts to secrete bioactive osteocalcin in the bloodstream. In turn osteocalcin reduces the activity of the nerve fibers of the parasympathetic nervous system, triggers the body's stress response and the release of adrenaline resulting in surges in both heart rate and breathing, as reaction to the threat. These results would explain why in adrenalectomized rodents, lacking both adrenal steroid hormones and adrenal-derived catecholamine, and in glucocorticoid-deficient

patients, the acute stress response to dangerous situation is still observed [66].

Although the many functions of osteocalcin seem unrelated, its ability to improve muscle function during exercise, promote memory and facilitate the acute response to stress, suggest that this hormone of exclusive bone derivation gives a survival advantage which, from a broader point of view, could result in a slowdown in the onset of age-related diseases.

Conclusion

Throughout life, from embryogenesis to aging, bone and muscle tissue cooperate mechanically and biochemically as a single unit. During aging, it is known that osteoporosis often coexists with sarcopenia, creating a negative loop between muscle and bone responsible for reducing quality of life and increasing mortality. However, it is not yet clear if the pathological conditions develop at the same time or if one precedes the other. From a mechanical point of view, it is more likely that the decline in muscle function, resulting in decreased load on the skeleton, is responsible for the loss of bone mass. However, there are osteoporotic patients who have not been diagnosed with sarcopenia. The most likely hypothesis currently under investigation is that age-dependent reduction in regenerative capacity of both tissues could be a shared mechanism for sarcopenia and osteoporosis. The decrease of regenerative capacity of both tissues implies a parallel dysregulation of their biochemical communication through the musculoskeletal secretome. Hopefully a better understanding of these molecular entities involved in the crosstalk between these tissues can shift the paradigm for the treatment of osteoporosis and sarcopenia simultaneously.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this paper. All authors approved the final version of the submitted manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Human and Animal Rights and Informed Consent All studies by the authors involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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