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MUSCLE AND BONE (L BONEWALD AND M HAMRICK, SECTION EDITORS)

Targeting the Muscle-Bone Unit: Filling Two Needs with One Deed in the Treatment of Duchenne Muscular Dystrophy

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Abstract

Purpose of Review In Duchenne muscular dystrophy (DMD), the progressive skeletal and cardiac muscle dysfunction and degeneration is accompanied by low bone mineral density and bone fragility. Glucocorticoids, which remain the standard of care for patients with DMD, increase the risk of developing osteoporosis. The scope of this review emphasizes the mutual cohesion and common signaling pathways between bone and skeletal muscle in DMD.

Recent Findings The muscle-bone interactions involve bone-derived osteokines, muscle-derived myokines, and dual-origin cytokines that trigger common signaling pathways leading to fibrosis, inflammation, or protein synthesis/degradation. In particular, the triad RANK/RANKL/OPG including receptor activator of NF-kB (RANK), its ligand (RANKL), along with osteoprotegerin (OPG), regulates bone matrix modeling and remodeling pathways and contributes to muscle pathophysiology in DMD.

Summary This review discusses the importance of the muscle-bone unit in DMD and covers recent research aimed at determining the muscle-bone interactions that may eventually lead to the development of multifunctional and effective drugs for treating muscle and bone disorders regardless of the underlying genetic mutations in DMD.

Keywords Duchenne muscular dystrophy · Muscle-bone · Crosstalk · Myokine · Osteokine · Osteoprotegerin

Introduction

Skeletal muscle and bone form a large functional unit that enables locomotion and that contributes to metabolism, homeostasis, and thermogenesis [1, 2]. This muscle-bone unit adapts in synchrony during development and also during periods of modified mechanical loading such as exercise or situations of disuse or disease-like microgravity, long-duration bed rest, aging, spinal cord injury, critical illness, and neuromuscular diseases [3–7]. Duchenne muscular dystrophy (DMD) is one of the best examples of synchronicity where muscle degeneration/atrophy and bone loss occur in concert throughout the progression of the disease [8, 9]. Beyond the mechanostat theory, cumulative evidence also supports the existence of bi-directional muscle-bone molecular interactions [10–13]. Muscle and bone cytokines contribute to lifelong paracrine crosstalk while the underlying biological processes involve common signaling pathways [11–13].

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Muscle and Skeletal Decline in DMD: the Scope of the Problem

DMD is a rare X-linked recessive disorder that occurs in 1:5000 live male births and is caused by loss-of-function mutations in the dystrophin gene [14, 15]. The absence of dystrophin in the cytoskeleton of skeletal muscle cells causes architectural fragility and sarcolemmal permeability, leading to chronic inflammation, fibrosis, and progressive skeletal and cardiac muscle deterioration [16].

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Children with DMD generally display clinical signs of muscle weakness or motor dysfunction by 3–5 years of age, are wheelchair-bound by 12–15 years of age, and manifest cardiorespiratory failure in their late 20s or early 30s [17–22]. Patients with DMD also present with a high prevalence of fractures with a poor prognosis for recovery in the absence of osteoporosis therapy [8]. While long-term glucocorticoids (GCs), the standard of care for patients with DMD, prolong ambulation, cardiorespiratory function, and life expectancy, they are a key risk factor for reduced bone mineral density (BMD) and fractures due to their potent osteotoxicity [23–29]. Studies have shown that 20 to 60% of patients with DMD present low-trauma extremity fractures, while up to 30% have symptomatic vertebral fractures [8, 30–32]. The true prevalence of vertebral fractures is likely higher than this, since spine fractures are frequently asymptomatic and will go undetected in the absence of a routine spine imaging monitoring program [28, 33–35]. While vertebral fractures have been observed to occur on average 2 years following GC initiation, they have been reported as early as 6 months after the start of GC therapy [27]. In patients with DMD, untreated vertebral fractures are linked to chronic back pain and spine deformity, while leg fractures can cause premature, irreversible loss of ambulation and challenges in daily care [27, 32]. To date, osteoporosis management in pediatric DMD is based on standard-of-care principles that are similar to those applied to all chronic pediatric illnesses. Treatment with an intravenous bisphosphonate such as pamidronate or zoledronic acid (preferred over oral bisphosphonate therapy) is reserved for patients with clinically significant bone fragility that is detected in early, as opposed to advanced, stages of development [36, 37•]. The main objectives of osteoporosis therapy instituted at the earliest signs of bone fragility include resolution of back pain, stabilization of vertebral fractures, prevention of new vertebral and non-vertebral fractures, and increases in BMD *Z*-scores [38].

The importance of treating early signs of vertebral fractures in DMD is highlighted by the fact that prevalent vertebral fractures predict new vertebral fractures at subsequent time points, even when the initial vertebral fractures are mild or asymptomatic, a phenomenon known as the vertebral fracture cascade [39]. The importance of bone health in DMD children has also been underscored by a recent study showing that early treatment of osteoporosis may improve survival in DMD [40]. At the present time, there have been no studies which have been undertaken to assess the safety and efficacy of first-fracture prevention in DMD; therefore, the current approach is in line with secondary prevention—to identify and treat early instead of late signs of bone fragility, including timely identification of vertebral fractures through periodic spine imaging [37•, 41].

Muscle-Bone Interactions in Muscular Dystrophy

Our understanding of the mechanisms underlying dystrophic muscle and bone interactions originate predominantly from studies in *mdx* mice, a well-established DMD model. Seven-week-old dystrophic *mdx* mice present an acute onset of muscle weakness associated with a 20% decrease in bone biomechanical properties compared to wild-type mice [42, 43]. Moreover, dystrophin-utrophin double-knockout mice, a more severe phenotype than the *mdx* mouse, exhibit muscle degeneration, spinal deformity, cardiomyopathy, a reduced capacity for bone healing, and spontaneous heterotopic ossification in the hindlimb muscles [44]. Nakagaki et al. found that 21-day-old *mdx* mice present changes in the mechanical and biochemical properties of bone prior to the appearance of significant muscle fiber degeneration, suggesting that the inflammatory environment of dystrophic muscles (release of growth factors, interleukins, or other pro-inflammatory cytokines) may contribute to the uncoupling of osteoclastic and osteoblastic activity, eventually leading to osteopenia and osteoporosis [45, 46]. Nevertheless, not enough studies have been carried out to reach definitive conclusions on how muscle-bone interactions and muscle-derived molecules (myokines) and bone-derived molecules (osteokines) influence the course of muscular dystrophy.

Myokines and Their Effects on Bone Tissue in DMD

Myokines are interleukins, growth factors, or peptides released by skeletal muscles that may influence remote tissues such as bone (Fig. 1). Interleukin-6 (IL-6), which is often classified as a pro-inflammatory cytokine, is a crucial mediator of bone homeostasis and an essential regulator of satellite cell-mediated skeletal muscle hypertrophy [47, 48]. It is secreted by muscle and bone and is present at significant levels in patients with DMD and *mdx* mice compared with age-matched healthy controls [46, 49–51]. It has been shown that IL-6 contributes to GC- and rheumatoid arthritis-induced osteoporosis in mice [52, 53]. Rufo et al. demonstrated that wild-type calvarial bone cultures maintained ex vivo that are supplemented with 10% sera from *mdx* mice have increased osteoclast and bone resorption parameters that are rescued by an IL-6 antibody treatment [46]. Tocilizumab, a monoclonal antibody directed against the IL-6 receptor (IL-6R), is a potentially valuable therapeutic strategy for counteracting necrosis and the consequences of chronic inflammation in muscular dystrophy [53, 54•]. It has been shown that IL-6R blockade results in decreased muscle damage, improved muscle fiber regeneration, increased muscle fiber diameter, and reduced fibrosis, while some mice exhibit an improvement in the

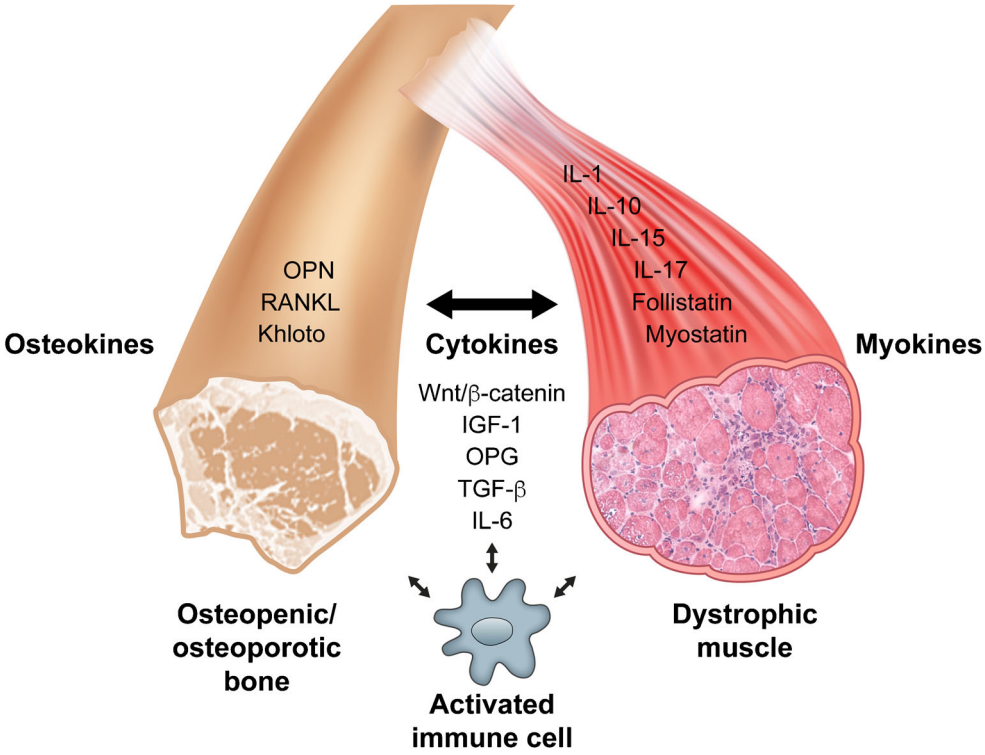

Fig. 1 Myokines, osteokines, and dual-origin cytokines involved in Duchenne muscular dystrophy

kyphosis index [54•]. Interestingly, IL-6 is significantly downregulated in the muscles of 24-week-old *mdx* mice with a mild muscle wasting phenotype, unlike in younger 4-week-old mice that overexpress IL-6 during the most severe peak of muscle degeneration and regeneration [51].

Consistent evidence has also shown that IL-6 has an anti-inflammatory effect and may be involved in mediating the beneficial health effects of exercise by increasing the levels of interleukin-10 (IL-10), an anti-inflammatory cytokine [55–57]. IL-10 plays a central role in regulating the switch of muscle macrophages from the pro-inflammatory M1 to the anti-inflammatory M2 phenotype in injured muscle in vivo, a transition that is necessary for normal muscle growth and regeneration [58]. Levels of IL-10 and its receptor are higher in dystrophic muscles during the acute onset of the pathology and during muscle regeneration. In addition, the ablation of IL-10 expression in *mdx* mice (IL-10 −/− *mdx*) increases muscle damage in vivo and reduces muscle strength in mice with chronic inflammation and severe cardiorespiratory dysfunction [59, 60]. However, in vitro treatments of isolated *mdx* macrophages with IL-10 reduce the activation of the M1 phenotype and promote a shift toward the M2c phenotype [59]. Interestingly, IL-10−/− mice develop the hallmarks of osteoporosis associated with a reduced expression of osteoblast and osteocyte markers [61–63]. Moreover, in vitro bone cells treated with IL-10 exhibit an upregulation of osteoprotegerin (OPG) expression associated with a downregulation of the expression of the receptor activator of NF-κB ligand (RANKL) [64]. The strategy of inhibiting osteoporosis and enhancing the switch to the M2 anti-inflammatory phenotype in isolated *mdx* macrophages may be beneficial for the treatment of DMD.

Furthermore, interleukin-15 (IL-15) is another cytokine that is currently considered a myokine due to the abundant expression of IL-15 mRNA in skeletal muscle [65]. IL-15 induces muscle hypertrophy and protein synthesis in vitro, and IL-15 treatments partially inhibit skeletal muscle wasting in models of cancer cachexia and sepsis [66–68]. It is well established that IL-15 has a stimulatory function on osteoclast differentiation but can also decrease the number of both osteoclasts and osteoblasts in bone marrow cell cultures [69–71]. Interestingly, the release of IL-15 into the circulation by skeletal muscle tissue can modulate remote tissues and increase bone mineral content in vivo [72]. In terms of DMD, the administration of IL-15 improves the pathophysiology of dystrophic muscle, reducing fibrosis and collagen levels in the diaphragmatic muscles of *mdx* mice. However, its effect on bone health remains to be determined in the context of muscular dystrophy [73]. In addition, levels of pro-inflammatory cytokines such as interleukin-17 (IL-17) and interleukin-1 (IL-1), which play key roles in bone homeostasis, have been shown to be elevated in dystrophic muscles, suggesting that other muscle-bone interactions may be in play [74–78]. Further investigations are needed to decipher the role of these ILs in DMD and the suitability of an approach based on IL modulation to treat muscle-bone disorders.

In addition to ILs, transforming growth factor β (TGF-β), a pleiotropic cytokine, plays an important role in muscle

inflammation and fibrosis associated with DMD. It has been shown that TGF-β is activated in patients with DMD and *mdx* mice and induces progressive fibrosis and that treatment with a neutralizing antibody directed against TGF-β1 improves respiratory function and functional performance and decreases fibrosis and serum creatine kinase (CK) levels in *mdx* mice [79–82]. TGF-β also plays an important role in postnatal bone homeostasis. The release of TGF-β from the bone matrix under pathological conditions contributes to muscle weakness by increasing the oxidization of skeletal muscle proteins [83, 84]. Halofuginone, a collagen synthesis inhibitor, is a novel anti-fibrotic agent that prevents estrogen deficiency-induced osteoporosis [85]. In muscle diseases, halofuginone prevents the age-dependent increase in collagen synthesis in the diaphragm (Dia) muscle and the late outcome of dysferlin knockout mice and improves the cardiac muscle function of *mdx* mice [86, 87]. In addition, activin and myostatin are multifunctional growth factors belonging to the TGF-β superfamily. Activin/myostatin pathway antagonism may serve as a new therapeutic approach for countering muscle wasting and bone degeneration in disease. Myostatin null mice have approximately twice the skeletal muscle mass and a greater bone mineral content than wild-type mice [88]. Moreover, treatment with a soluble myostatin decoy receptor (ActRIIB-Fc) increases both muscle and bone mass in a mouse model of osteogenesis imperfecta [89]. A recent study showed that the systemic inhibition of the activin/myostatin pathway in *mdx* mice increases muscle mass, bone volume, and the trabecular number [90•]. Nevertheless, it is not known whether the increase in bone volume following activin/myostatin inhibition is a direct effect or whether it occurs indirectly through an increase in muscle mass. However, recent evidence suggests that activin receptor signaling directly and negatively regulates bone mass by osteoblasts. Indeed, primary osteoblasts express activin signaling components, and the conditional knockout of the activin IIA receptor (ActRIIA) in osteoblasts increases the femoral trabecular bone volume in mice [91]. It has also been shown that soluble ActRIIA-Fc, which binds to circulating ligands such as activin A, decreases bone resorption and increases bone formation in monkeys and postmenopausal women [92]. Since myostatin is a direct regulator of osteoclast differentiation and muscle mass and that there is a GC response element in the myostatin promoter, it is thus doubly important to discuss activin/myostatin in the context of GC-treated patients with DMD [93].

In addition to soluble ActRIIA-Fc, follistatin has emerged as a myostatin antagonist that can increase muscle mass and strength and is considered part of the muscle-bone crosstalk [10, 94]. It is a modulator of bone metabolism and development, possibly acting via activin and myostatin signaling [95]. Recent evidence has confirmed that follistatin has a positive effect on regulating muscle and bone wasting associated with microgravity [94, 96]. In skeletal muscle, follistatin has a positive effect on muscle mass via myostatin and myostatin-independent pathways, increasing muscle mass and enhancing regeneration following injury [97–99]. Interestingly, in dystrophic preclinical and clinical investigations, follistatin gene therapy reduced fibrosis and central nucleation, increased strength, and improved ambulation [100–102]. It is thus clear that myokines contribute to the regulation of bone and muscle mass and that investigating the mechanisms involved in the positive association between bone and muscle is important in the context of muscular dystrophy.

Osteokines and Their Effects on Muscle Tissue in DMD

Like muscle cells, bone cells release osteokines (Fig. 1) such as osteopontin (OPN), which is a well-known inhibitor of bone mineralization [103]. OPN is also expressed by inflammatory cells such as macrophages, and its expression increases significantly during inflammation [104]. Higher serum OPN levels are associated with low BMD in postmenopausal women and are significantly correlated with the phenotypic severity of dystrophic dogs [105, 106]. Interestingly, OPN promotes fibrosis and is the most highly upregulated transcript in dystrophic muscles [107, 108]. The ablation of OPN switches dystrophic macrophages toward a pro-regenerative phenotype, leading to reduced serum CK levels and improved muscle mass and strength based on the results of long-term functional testing [109•]. However, the effects of OPN ablation on the bone quality of *mdx* mice have not been investigated.

Additionally, the canonical Wnt/β-catenin pathway, which interacts with TGF-β, plays a pivotal role in regulating bone homeostasis, myogenesis, and postnatal muscle regeneration [110, 111]. Specifically, Wnt/β-catenin signaling decreases osteoclast differentiation by stimulating the production and secretion of OPG [112]. TGF-β1 stimulates myofibroblast differentiation and the fibrogenic features of satellite cells via the canonical Wnt pathway, potentially increasing fibrosis in dystrophic muscles [113]. However, treating *mdx* mice with Wnt7a efficiently induces satellite cell expansion and myofiber hypertrophy and improves the specific force of the extensor digitorum longus (EDL) muscle [114]. Interestingly, transplanting Wnt3a-pretreated mesenchymal stem cells (MSCs) into *mdx* mice results in long-term improvement in the dystrophic phenotype and restores dystrophin expression in muscles [115]. Sclerostin, which is mainly produced by osteocytes, inhibits the Wnt/β-catenin pathway. The sclerostin antibody (romosozumab) is currently under clinical investigation for the treatment of osteoporosis [116]. With respect to skeletal muscle, pharmacological inhibition of sclerostin does not rescue muscle mass loss in models of spinal cord injury and reduced mechanical loading [117, 118]. In contrast, Wnt

signaling is also antagonized by the senescence-related protein Klotho [119]. Epigenetic silencing of Klotho, a co-receptor for fetal growth factor 23 (FGF23), occurs at the onset of pathology in the *mdx* mouse model of muscular dystrophy [120]. Consistently, Klotho expression is 80% lower in dystrophic muscle tissues from humans and mice during the first peak of muscle degeneration [120, 121•]. In vivo, transgene expression of the *klotho* gene in *mdx* mice reduces TGF-β1 expression and fibrosis in older mice, improves function, and greatly increases the pool of muscle-resident stem cells required for regeneration [120]. Klotho is also a potent regulator of bone formation and bone mass. Klotho deletion in osteocytes leads to a marked increase in bone formation, while the overexpression of Klotho in cultured osteoblastic cells inhibits mineralization and osteogenic activity [122]. Further investigations are thus needed to verify how wnt/β-catenin pathway signaling may mediate muscle-bone crosstalk in DMD.

In concert with cytokines and growth factors, it is well documented that insulin growth factor-1 (IGF-1), a hormone secreted by skeletal muscle and bone tissues, is a crucial factor for the development of the musculoskeletal system [123]. IGF-1 therapy is a useful approach for treating osteoporosis and fractures due to its ability to increase bone mineral density and bone formation [124, 125]. In preclinical studies using *mdx* mice, IGF-1 treatments improved excitation-contraction coupling, reduced fibrosis, and increased force and fatigue resistance [126–129]. It has also been recently shown that IGF-1 enhances the anti-fibrotic effects of losartan, an angiotensin II type 1 receptor blocker clinically investigated in DMD that antagonizes TGF-β signaling [130, 131], and increases locomotor function in merosin-deficient congenital muscular dystrophy type 1A [132]. IGF-1 has been tested in clinical studies for various pathologies, and an open-label trial for patients with myotonic dystrophy type 1 showed that IGF-1 increases lean body mass and improves metabolism, but does not increase muscle strength or function [133, 134]. Similarly, a 6-month trial with IGF-1 in patients with DMD treated with GCs showed that it increased height velocity but had no effect on motor functional outcomes [135]. It remains to be seen how IGF-1 therapy could change the clinical landscape of DMD beyond stature management.

Treating the Bone-Muscle Complex with Single or Combined Drugs in DMD

Bisphosphonates such as pamidronate and zoledronic acid are a family of drugs used to increase bone mineral density and prevent fractures. These molecules bind specifically to calcium and remain sequestered in bone mineral, with a half-life of over 10 years. They inhibit osteoclast activity and osteoclastogenesis. Six-week-old *mdx* mice treated with pamidronate for 8 weeks displayed increased grip strength, improved muscle histology, and markedly reduced the levels of serum CK, a clinical marker for tissue damage [136]. The lack of effect in the Dia muscle suggests that pamidronate may act via a paracrine effect of adjacent bone tissues. Pamidronate also improves the cortical bone architecture and strength of femurs, increasing their resistance to fractures in *mdx* dystrophic mice [136]. Other experiments have confirmed that intravenous pamidronate protects against cortical bone loss in *mdx* femurs during prednisone treatment [137], as is currently a recommended treatment (along with other intravenous bisphosphonate agents) for bone protection in patients with DMD [37•, 41]. Another clinical study showed that a combined treatment with steroids and bisphosphonates significantly increased the lifespan of patients with DMD compared to patients on steroids alone [40]. However, conventional steroid therapy is non-specific and acts on muscles and secondary sexual organs without discrimination and is hepatotoxic. A more targeted approach using non-steroidal androgen receptor (AR) modulators is currently being explored. The AR modulator GTx-026 increases muscle strength and muscle mass, improves cardiopulmonary functions, and reduces fibrosis [138]. AR agonists have a positive effect on growing bones [139, 140]. These results highlight the importance of androgens and a novel, potentially beneficial therapeutic approach using androgen receptor agonists. In addition, selective estrogen receptor modulators such as tamoxifen and raloxifene can be used to treat dystroglycanopathy, a different form of muscular dystrophy, giving additional support for the use of selective steroids for the treatment of muscular dystrophy [141].

Nitric oxide (NO) is another key biological messenger involved in vasodilation and various biological processes. NO is also important for muscle function and integrity and is impaired in dystrophin-deficient mice and humans. NO impairment causes vascular dysfunction and ischemic muscle damage [142–144]. A new therapeutic approach modulates the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling pathway in muscular dystrophy. The inhibition of phosphodiesterase type 5 (PDE5) prolongs the half-life of cGMP and induces an angiogenic response [145]. Treating *mdx* mice with sildenafil or tadalafil, two PDE5 inhibitors, significantly reduces Dia damage, fibrosis, and weakness with no effect on fatigue resistance [146]. Sildenafil also acts on the expression of the pro-fibrotic and pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) [146]. A study involving ten patients with DMD treated with sildenafil or tadalafil showed that sildenafil reduces ischemia and normalizes blood flow in dystrophic skeletal muscle during exercise that is dampened in boys with DMD [147], while tadalafil delays cardiomyopathy in dogs with muscular dystrophy [148]. Several animal studies have reported the positive effects of tadalafil and sildenafil on bone healing following fractures [149, 150]. Tadalafil was tested in a phase 3 randomized placebo-controlled 48-week trial in patients with DMD but the treatment did not delay the

loss of ambulatory ability [151]. However, tadalafil and sildenafil have positive effects on skeletal muscle and bone and can prevent the adverse effects of bisphosphonate treatments in animal models [152]. Additional studies are required to determine whether this treatment can improve the health of patients with DMD and slow the progression of the disease.

Nuclear factor-kappa B (NF-κB) is a key transcriptional factor that plays a central role in muscle degeneration, muscle atrophy, and osteolysis [153–155]. Targeting the NF-κB pathway is thus a potential avenue for managing the muscle-bone complex in DMD. Vamorolone (VBP15) is a new glucocorticoid-derived molecule that has been optimized to inhibit NF-κB. In vitro, VBP15 protects muscle cells against damage and stimulates membrane repair, while in dystrophic *mdx* mice, it enhances strength, improves the phenotype, and limits GC-related adverse effects [156]. Unlike GCs, VBP 15 maintains bone growth and density and reduces heart fibrosis in dystrophic mice [156]. Recent first-in-human phase I clinical trials in healthy adults indicated that ascending doses of vamorolone are well tolerated, as supported by bone and metabolic and immune biomarkers studies [157]. Edasalonexent (CAT-1004), another NF-kB inhibitor, improves the activity, muscle mass, and function of dystrophic mice while reducing fibrosis and cardiac dysfunction [158]. A recent phase II clinical trial showed that edasalonexent reduces muscle edema and circulating CK levels and significantly improves functional performance [159]. The inhibition of NF-κB is thus an important and promising target for the treatment of DMD.

RANK/RANKL/OPG and Muscular Dystrophy

Our most recent publications also support the hypothesis that the muscle-bone unit may be treatable with a single drug in DMD. The discovery of receptor activator of NF-kB (RANK) and the RANK/RANKL/OPG triad, which is part of the TNF superfamily, was a major breakthrough in bone biology 20 years ago [160]. RANKL is secreted by osteoblasts while RANK, its receptor, is located on pre-osteoclastic cells. The RANKL/RANK interaction induces the formation of multinucleated mature osteoclasts, ultimately leading to bone resorption and remodeling [161]. The third contributor, OPG, is also produced by osteoblasts and binds to RANKL, inhibiting the RANKL/RANK interaction and subsequent osteoclastogenesis [162]. The fact that OPG-null mice suffer from osteoporosis and that the overexpression of OPG or the injection of high doses of exogenous OPG induce osteopetrosis-like changes highlights the physiological relevance of OPG [163–165]. OPG also serves as a decoy receptor for the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and increases cell survival by blocking the pro-apoptotic effects of the RANKL/RANK interaction [166]. OPG is thus a very efficient anti-resorptive and anti-apoptotic agent.

Additionally, RANK, RANKL, and OPG mRNAs are present in skeletal muscle, and RANKL/OPG proteins are found in the myoplasm [167–169]. We showed that RANK is expressed in sarcolemmal membranes and may thus potentially interact with bone-derived RANKL [170]. In addition, we showed that fully differentiated myotubes secrete OPG, supporting bi-directional signaling between bone and muscle [171]. In osteoclasts, the RANKL/RANK interaction activates the Ca^{2+}-dependent and TNF receptor-associated factor (TRAF) TRAF/NF-kB signaling pathways, which are dysregulated in DMD [158, 160, 172–178].

Using muscle-specific RANK receptor deletion, we showed that muscle RANK is a regulator of Ca^{2+} storage and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity and function in fast-twitch EDL skeletal muscles [170]. Furthermore, muscle-specific RANK deletion has inotropic effects in denervated EDL muscles, increasing the maximum specific force production while inducing slight muscle atrophy [170]. As the RANK/RANKL pathway is important in Ca^{2+} regulation, and as *mdx* dystrophic mice present a dysregulation of Ca^{2+} homeostasis, we treated dystrophic mice with full-length OPG linked to an Fc fragment (FL-OPG-Fc), the natural inhibitor of RANKL. We showed that the FL-OPG-Fc treatment greatly reduces the inflammation, restores the integrity, and improves the function of dystrophic EDL muscles during the first and most important phase of muscle degeneration [171]. FL-OPG-Fc also significantly improves the function of slow-twitch soleus (Sol) and Dia dystrophic muscles, albeit to a lesser extent [171]. Interestingly, FL-OPG-Fc does not enhance the force of healthy wild-type skeletal muscles, suggesting that, like muscle-specific RANK deletion, an underlying pathology or dysfunction is required to exert its beneficial effect [170, 171].

We next dissected out the contribution of RANK/RANKL/OPG in dystrophic muscles using genetic and pharmacological approaches and showed that RANK mRNA levels are fivefold higher in dystrophic EDL muscles. A recent study showed that the levels of several members of the TNF receptor family are significantly elevated in *mdx* mice serum, including the RANK protein, suggesting that it may be involved in muscular dystrophy [179•, 180]. To examine the involvement of RANK in dystrophic skeletal muscle, we generated *mdx* mice with a muscle-specific RANK deletion. The deletion of muscle RANK significantly improves the force of dystrophic EDL muscles but has no protective effects against eccentric contraction-induced muscle dysfunction. These data indicate that the RANK/RANKL/OPG pathway may play a role in dystrophic muscle pathophysiology.

Alternatively, daily FL-OPG-Fc injections for 10 days increase the maximal specific force of dystrophic EDL muscles, markedly protect against eccentric contraction-induced muscle dysfunction ex vivo, and significantly improve functional performance on an eccentric downhill treadmill and on

traveling distance post-exercise [179•]. Since OPG serves as a soluble receptor for RANKL and as a decoy receptor for TRAIL, we treated *mdx* mice with anti-RANKL and anti-TRAIL antibodies and showed that they significantly increase the force of dystrophic EDL muscles, but to a much lesser extent than FL-OPG-Fc [179•]. Truncated OPG-Fc, which only contains RANKL domains, produced modest but significant gains of force, suggesting that RANK-independent mechanisms are also in play [179•]. In dystrophic muscles, SERCA overexpression reduces susceptibility to eccentric contraction-induced muscle damage, while intrinsic laryngeal muscles that overexpress SERCA are spared from muscular dystrophy [181, 182]. In *mdx* muscles, an FL-OPG-Fc treatment, but not muscle-specific RANK deletion, almost completely restores SERCA activity, providing evidence that FL-OPG-Fc may rescue Ca^{2+} cycling/homeostasis through a SERCA-dependent mechanism [179•]. To confirm that FL-OPG-Fc also acts independently of the RANK/RANKL pathway, *mdx* mice with a muscle-specific RANK deletion were treated with FL-OPG-Fc and exhibited a significant gain in force, indicating that the effect of FL-OPG-Fc is in part independent of the RANKL/RANK interaction [179•]. Investigations are currently underway to understand the RANKL-independent mechanisms of action of FL-OPG-Fc. Since FL-OPG-Fc may protect skeletal muscles and bones simultaneously, it may be a promising therapeutic candidate alone or in combination with the current standard of care for DMD. Although anti-RANKL does not protect against eccentric contractions, our data point to a role for RANK/RANKL in muscular dystrophy. Thus, denosumab, an anti-RANKL antibody that is already prescribed for osteoporosis, GC-induced osteoporosis, and bone metastases, may be of benefit for patients with DMD [183], as shown in a recent case report where 18 months of denosumab therapy improved lumbar bone mineral density and bone turnover markers in a GC-treated boy with DMD [184].

Conclusion

In addition to muscle dysfunctions, low bone mineral density and bone fragility have been documented in various muscular dystrophies, including DMD, with debilitating comorbidities [5, 19, 36, 185, 186]. The bone weakness observed in DMD is partly caused by the decline in locomotion, the chronic use of GCs, and the changes in muscle-bone bi-directional molecular interactions highlighted in the present review. These muscle-bone crosstalks involve bone-derived osteokines, muscle-derived myokines, and dual-origin cytokines that act on common signaling pathways, including inflammation, fibrosis, catabolism, anabolism, angiogenesis, and calcium homeostasis. Given the delays in developing genetic approaches to restore dystrophin expression and function, strategies to target common signaling pathways involved in muscle and bone diseases are an important short-term approach for treating DMD. These novel drugs can be explored on their own, to target the dystrophinopathy with the goal to also provide benefit to bone, or as a complementary adjunct to muscle-targeted therapies in order to counteract the negative effects of GCs on bone. Lastly, further investigations are obviously needed to validate muscle-bone interactions and to focus on crosstalk-based approaches that can protect both bone and skeletal muscle, with the ultimate goal of improving quality of life, and life expectancy in DMD.

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Compliance with Ethical Standards

Conflict of Interest Leanne Ward reports participating in clinical trials with AMGEN.

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Abbreviations ActRIIA, activin IIA receptor; ActRIIB-Fc, soluble myostatin decoy receptor; AR, androgen receptor; BMD, bone mineral density; CK, creatine kinase; DMD, Duchenne muscular dystrophy; EDL, extensor digitorum longus; FGF-23, fibroblast growth factor 23; FL-OPG-Fc, full-length osteoprotegerin linked to a Fc fragment; GC (s), glucocorticoid (s); IGF-1, insulin growth factor 1; IL-1, interleukin-1; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; IL-10, interleukin-10; IL-10 −/− *mdx*, ablation of IL-10 expression in *mdx* mice; IL-15, interleukin-15; IL-17, interleukin-17; MSCs, mesenchymal stem cells; NO, nitric oxide; NO-cGMP, nitric oxide-cyclic guanosine monophosphate; OPG, osteoprotegerin; OPN, osteopontin; PDE-5, phosphodiesterase type 5; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; Sol, *soleus*; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; TRAF, TNF receptor-associated factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VBP15, vamorolone

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