REVIEW

Integrated Therapies for Osteoporosis and Sarcopenia: From Signaling Pathways to Clinical Trials

Christian M. Girgis

Abstract Sarcopenia and osteoporosis are two sides of the same coin. They represent different aspects of the same age-related process of musculoskeletal atrophy and together culminate in falls, fractures, deconditioning, and increased mortality in older individuals. However, the current therapeutic approach to the prevention of minimal trauma fracture is unilateral and focuses solely on bone. In theory, an integrated approach that recognizes the interaction between muscle and bone could break the vicious cycle of their combined involution and more effectively minimize falls/fractures. In this review, signaling pathways and cross-talk mechanisms that integrate bone/muscle, and the emergence of novel therapies that exploit these pathways to target osteoporosis/sarcopenia will be discussed. In broad terms, these agents act on nuclear receptors (e.g., VDR, AR) or transmembrane receptors (e.g., activins, GH/IGF-1) expressed in muscle and bone, and seek to alter biologic responses to musculoskeletal aging, loading, and injury. Challenges in the development of these dual bone–muscle therapies, early clinical trials examining their safety/efficacy, and novel targets that hold promise in the reversal of musculoskeletal aging will be discussed.

Introduction

Musculoskeletal disorders are very common and affect up to 50 % of individuals in western countries [1, 2]. These diseases are also costly and lead to an annual expenditure of approximately $850 billion in the United States (8 % GDP) and $4.5 billion in Australia (10 % GDP). As the world's population ages, the concerning effects of musculoskeletal wasting will be on the rise: falls, fractures, the need for assisted care, and the financial impact of these significant life events [3].

Reduced muscle mass affects balance, increases postural sway and thereby increases the risk of falls. As the subject falls, fractures result due to bone's inability to withstand the loading forces applied to it by muscle and the subsequent trauma [4]. In this regard, the gradual age-related decline in bone and muscle (i.e., osteoporosis and sarcopenia) culminate in acute events with potentially catastrophic effects on morbidity and mortality [5].

For many years, therapies used to prevent minimal trauma fractures have focused *solely* on bone. Muscle and, more specifically, the effects of sarcopenia in compounding the risk of osteoporotic fracture have not received due attention. As we begin to recognize the tight link between sarcopenia and osteoporosis [6] and the effects of muscle mass on fracture risk [7], a paradigm shift in the treatment of these conditions may be underway. Recently, experts have suggested a more inclusive name be given to the combination of sarcopenia and osteoporosis, such as the "dysmobility syndrome", which integrates their pathogenesis and unites them as a single therapeutic target [8].

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There are, however, technical challenges in considering osteoporosis and sarcopenia as a single entity. Osteoporosis has been clearly defined but sarcopenia and its endpoints remain elusive [9]. Although there is consensus that a combination of functional and quantitative parameters of muscle mass are necessary to define sarcopenia [10–12], the correlation of specific muscle tests to clinically meaningful outcomes remains an open question [13].

Another challenge is to better understand the interconnected biology of the musculoskeletal system. We have known for centuries that muscle and bone interact anatomically and through complex biomechanical signals. More recently, a sophisticated network of paracrine and endocrine signals that coordinates bone and muscle has emerged with integrated effects in development, the response to injury and aging [14]. There has been a call for greater research in bone–muscle interactions and the development of therapeutic agents that target sarcopenia and osteoporosis as a single disorder [5]. A holistic approach to these conditions may be a more effective one, directly addressing the mechanisms of osteoporotic fracture via bone and its fundamentally important neighbor, muscle [15]. In this review, the biological basis of bone and muscle interactions, therapies that target muscle and bone as a single unit, and future directions in this exciting field of research will be discussed.

Bone and Muscle Interactions

Throughout life's various stages, the musculoskeletal system operates as a finely coordinated unit. Muscle and bone originate from the mesenchymal progenitor cell during embryonic life and their development is regulated by overlapping genes and humoral factors [16, 17]. Mechanical forces from developing muscles exert critical effects in periosteal bone growth, bone geometry, and density. In demonstrating this integral association between bone and muscle development, children with Duchenne muscular dystrophy (DMD) and cerebral palsy have reduced bone density and a greater fracture risk [18, 19]. Mice with congenital paralysis have severe impairments in bone development and mineralization [20, 21].

Throughout post-natal life, sex steroids, insulin-like growth factor-1 (IGF-1), and growth hormone (GH) coordinate musculoskeletal growth. Muscle seems to possess the "upper hand" in its relationship with bone, driving changes in bone density. This is seen initially in puberty where the accumulation of lean mass precedes gains in bone mass, and muscle mass determines cortical bone area [22, 23]. Conversely, in aging adults, lean mass declines before bone mass, and muscle mass correlates tightly with reductions in bone density [24, 25]. A potential explanation

for muscle's apparent primacy in these interactions is found in the "mechanostat theory". This theory, as recently reviewed in this journal [26], proposes that muscle loading induces a range of biomechanical signals necessary for bone growth and remodeling. Indeed, subjects exposed to a gravity-free environment, such as astronauts or mice suspended in mid-air for prolonged periods, experience dramatic bone loss due to lack of muscle loading [27, 28]. Pathways responsible for mechanotransduction such as stretch-activated cation channels, G protein-coupled receptors, sclerostin, and LRP5 provide a strong molecular basis for this theory and are being targeted for their therapeutic potential [29, 30].

The "mechanostat theory", however, does not fully explain the complex nature of bone–muscle interactions. For example, the correlation between muscle mass and bone cortical thickness is seen *not only* in adjacent tissues but also in muscles and bones *located remotely to one another* [31]. This opens the possibility of cross-talk and the presence of discrete hormonal influences between these tissues. Indeed a number of muscle and bone-derived hormones (i.e., myokines and osteokines) are under active investigation for their integrative effects. Myostatin, fibroblast growth factor 2 (FGF2), interleukin 6 (IL6), and matrix metalloproteinase 2 (MMP2) are myokines with potential effects in bone [32–34]. Conversely, humoral factors made in bone, including FGF21, undercaboxylated osteocalcin, and sclerostin exert potential effects in skeletal muscle [35, 36]. There are also common pathways such as GH/IGF-1, sex steroids, and Wnt signaling that may centrally govern the bone–muscle unit, its adaptation to mechanical stimuli and injury [31, 37].

Further support for bone–muscle cross-talk is seen in the response to musculoskeletal injury. The use of muscle flaps in the surgical correction of open fractures improves bone healing in both rodents and humans [38, 39]. Conversely, the incidence of nonunion is significantly higher in fractures associated with muscle damage, as seen in compartment syndrome [40]. Therefore, healthy muscle is a positive factor for fracture healing and muscle may represent a sort of 'outerperiosteum', providing morphogens and growth factors to assist bone repair.

Thus, an intricate network of biomechanical and endocrine signals connects muscle and bone with integrative effects in musculoskeletal mass and function throughout life. A number of common pathways, such as the GH/IGF1, vitamin D signaling, and androgens have been investigated for their therapeutic potential in musculoskeletal diseases. Other pathways, such as myostatin/activin signaling and bone-derived factors such as undercarboxylated osteocalcin, are also being investigated for their systemic effects. Examining these individual pathways for their specific effects and therapeutic potential poses significant

experimental challenges. However, a thorough understanding of the endocrine network that connects bone and muscle is critical for the discovery of therapeutic targets that may lead to a more holistic approach to the combined syndrome of osteoporosis and sarcopenia.

Vitamin D

Although history gave vitamin D the misnomer "vitamin", the biologically active molecule (1,25(OH)$_2$D) is a *bona fide* hormone with potent effects in calcium/phosphate handling, bone homeostasis, tissue development, and immunomodulation [41]. Although its function in bone mineralization and growth plate physiology is established, our understanding of its role in muscle physiology is emerging.

Vitamin D's capacity to integrate muscle and bone is suggested by the clinical syndrome of vitamin D deficiency/rickets. As described four centuries ago, children with rickets display the combination of "flexible, waxy" bones and "flabby, toneless" muscles [42]. Likewise, adults with vitamin D deficiency display concurrent defects in bone and muscle, characterized by osteomalacia (i.e., reduced bone mineral) and type 2 muscle fiber atrophy [43]. These conditions are also seen in subjects with mutations of the vitamin D receptor (VDR), implying local genomic effects of vitamin D in the muscle–bone unit.

However, the precise mechanism of these effects has been debated. Although vitamin D may *directly* alter these tissues, its musculoskeletal effects are predominantly *indirect* and relate to systemic calcium/phosphate homeostasis [44, 45]. To demonstrate this, subjects with rickets develop muscle wasting and bone deformity *after* weaning, concurrent with declining levels of calcium and phosphate (Li et al. 1997; Yoshizawa et al. 1997). This is despite the early presence of the vitamin D receptor (VDR) within embryonic mesoderm [46], the tissue from which bone and muscle arise.

In bone, the VDR is expressed mainly in osteoblasts and osteocytes, where it has been shown to directly regulate mineral formation and bone mass [47]. The presence of the VDR in muscle has been controversial for many years. However, recent studies have reported its expression in mouse and human skeletal muscle and its upregulation following vitamin D supplementation in older women [48, 49]. In studies of cultured muscle cells, vitamin D treatment leads to doubling in the size of myotubes and downregulation of myostatin, a TGF-β which negatively regulates muscle mass [50]. The potential for vitamin D to exert anabolic muscle effects was supported by a recent pilot study. In 21 older women receiving vitamin D (4,000 IU day, 4 months), there was a 30 % increase in muscle fiber size and activation of myonuclear VDR [51]. However these changes did not translate to clinically meaningful effects in muscle strength.

Vitamin D deficiency is common in the elderly, due to nutritional deficits, reduced capacity of the skin to synthesize vitamin D, and lack of sun exposure, and has been associated with sarcopenia and osteoporosis [52, 53]. At a basic level, vitamin D deficiency mimics effects of aging with increased adipose tissue infiltration, activation of muscle proteolytic pathways, osteoclastogenesis, and increased bone turnover [54]. Elderly subjects may be more vulnerable to vitamin D deficiency due to lower expression of VDR in muscle and bone [55].

Subjects living in institutions are at particular risk of vitamin D deficiency and have been shown to benefit from vitamin D supplementation with a reduction in falls and fractures [56, 57]. However, effects of vitamin D supplementation are less clear among community dwellers. Vitamin D supplementation may increase bone mineral density in such individuals but this effect is small, limited to the femoral neck, and not associated with clear reduction in fracture risk [58, 59]. Clear effects in muscle function have been difficult to establish without standardized endpoints for strength or performance-based indices in these trials [43].

The definition of vitamin D deficiency and recommended daily intakes of vitamin D remain contentious. There is also evidence of a U-shaped curve in the musculoskeletal response to vitamin D. Individuals with high and low serum vitamin D have the greatest risk of fracture and frailty compared to those with intermediate levels [60, 61]. However, defining the intermediate range at which vitamin D effects are *most* beneficial has been elusive. While the Institute of Medicine (IOM) recommends 25(OH)D target levels >50 nmol/l [62], the US Endocrine society advocates a higher serum target level >75 nmol/l [63]. This uncertainty is compounded by reports of a greater incidence of kidney stones and increased falls and fractures in subjects receiving megadoses of vitamin D [58, 64]. Thus, at a certain point, the dose-limiting calcemic effects of vitamin D may outweigh its potential musculoskeletal benefits.

Therapeutic VDR agonists with low calcemic potency are being used in Japan to treat osteoporosis. One such agent, eldecalcitol, has been shown to increase bone mineral density, reduce the risk of falls/fractures, and improve lower limb strength in older individuals [65, 66]. Preclinical trials support these effects, demonstrating alterations in bone microstructure, bone turnover, and locomotive ability in rodents receiving eldecalcitol [67, 68]. These effects may result from tissue-specific activation of VDR [51] and/or direct effects on calcium flux within bone and muscle cells [67]. Although promising, the drug discovery process

for VDR agonists faces potential hurdles and may hamper their widespread use. To achieve tissue selectivity, a better understanding of VDR's molecular mechanisms, its downstream responses, particularly in muscle, and effects of polymorphisms are necessary.

In summary, musculoskeletal levels of VDR decline with age concurrent with the physiological involution of muscle and bone. Evidence suggests that musculoskeletal aging responds to vitamin D at a cellular level and in human clinical studies. Therapeutic VDR agonists provide hope in reversing the effects of musculoskeletal aging and in circumventing vitamin D's dose-limiting calcemic effects in order to directly target muscle and bone.

Myostatin and Activin-Based Therapies

Discovered in 1997, myostatin is a member of the TGF-β superfamily and a muscle-derived hormone which is a potent negative regulator of muscle mass [69]. Mutations in the myostatin gene lead to pronounced increases in muscle mass in mammalian species [69, 70]. Conversely, increases in myostatin lead to muscle wasting in chronic diseases such as HIV [71], renal failure [72], and chronic obstructive pulmonary disease [73]. To produce these effects, myostatin binds to a transmembrane receptor (i.e., activin receptor IIB, ActRIIB), activates Smad family proteins and regulates downstream signals, such as the ubiquitin–proteasome system, that govern muscle protein turnover.

Myostatin also affects bone. Myostatin polymorphisms correlate with peak bone density [74] and myostatin knockout mice display increased bone mass and mineral [32, 75] with larger callus size following osteotomy [76]. These effects on bone are predominantly indirect, related to the increased loading forces from muscle. However, direct effects are possible due to the local expression of activin receptors in bone marrow stromal cells and osteoblasts (ActRIIA) which can weakly bind myostatin [77, 78]. In vitro studies demonstrate that directly targeting activin receptors alter bone cell differentiation [79, 80].

Activin-based therapies have the potential to target both osteoporosis and sarcopenia, given the evidence that activin signaling and myostatin regulate bone and muscle mass. Several activin-based therapies have already been developed and broadly include soluble activin receptors, recombinant follistatin (i.e., an endogenous inhibitor which bioneutralizes myostatin), derivatives of follistatin and myostatin inhibitors (i.e., propeptides and neutralizing antibodies) [78]. Rodents administered with such agents demonstrate substantial increases in muscle mass [34, 79] and reversal of muscle wasting in models of cancer cachexia [81], aging [82], and androgen deficiency [83]. Animal studies also demonstrate beneficial effects of

soluble activin receptors (i.e., ActRIIB-Fc and ActRIIA) on bone mass via dual anabolic–antiresorptive effects and direct influences on osteoblast activity [79, 82, 84]. However, effects differ according to the particular agent. While soluble decoy receptors exert dual effects in muscle and bone, effects of myostatin inhibitors are specific to muscle [85, 86]. This highlights differences in tissue-specific activin signaling and may allow different therapeutic approaches depending on the predominance of muscle or bone wasting.

Human clinical trials have reported promising effects of activin-based therapies. In a phase I trial, stamulumab, a recombinant human myostatin antibody (MYO-029), was generally safe in healthy adults and patients with muscular dystrophy [87]. Although stamulumab improved single muscle fiber contraction [88], anabolic effects on muscle mass were not statistically significant. Another myostatin inhibitor (AMG-745) was shown to significantly increase lean body mass in men receiving androgen deprivation therapy for prostate cancer [89]. Activin decoy receptors have also been studied for their dual effects in muscle and bone. ACE-031 (ActRIIB decoy receptor) was shown to significantly increase lean mass (3.3 %) and thigh muscle volume (5.1 %) in a double-blind, placebo-controlled study of post-menopausal women [90]. As an indication of beneficial effects on bone, ACE-031 also significantly increased bone-specific ALP and decreased C-telopeptide, as did another soluble decoy receptor (ACE-011) [90, 91]. A significant increase in bone mineral density (3.4 %) was also reported at ∼100 days in a trial of 60 post-menopausal women on ACE-031 but this data appeared only on the company website (www.acceleron.pharma.com) and has not been published or peer reviewed.

A number of adverse effects have hampered the development of activin-based therapies. Reports of nosebleeds and skin telangiectasia in two trials of ACE-031 raised concerns about unrecognized, systemic side effects of inhibiting activin signaling (NCT01099761, clinicaltrials.gov) [90]. Sudden drops in serum FSH levels after ACE-031 and ACE-011, probably related to suppression of GnRH signaling [90, 91], also raised concerns and the need for larger and longer trials to examine the safety of these therapies.

In summary, activin signaling is an attractive therapeutic target in treating osteoporosis and sarcopenia. Preclinical trials have demonstrated anabolic musculoskeletal effects of activin-based therapies and preliminary human trials confirm these effects, particularly in muscle mass. However the clinical trials are generally small, inadequately powered to detect changes in muscle strength, and too short to show convincing effects on bone density. Thus, longer and larger studies are needed to assess well-defined functional endpoints of activin-based therapies and to address concerns about their long-term safety.

Growth Hormone (GH), Insulin-Like Growth Factor 1 (IGF-1), and GH secretagogues

The GH/IGF1 axis plays a central role in musculoskeletal growth and maturation during childhood and puberty. Throughout life, it exerts system-wide effects on lipid and glucose homeostasis [92], body composition, and bone mineralization [93]. GH is a polypeptide hormone secreted in a pulsatile fashion by the anterior pituitary gland under the control of central (GHRH) and peripheral signals (IGF-1) [94]. GH acts via specific growth hormone receptors in peripheral tissues or indirectly through the induction of IGF-1 [94]. Circulating IGF-1 is synthesized mainly in the liver but IGF-1 is also expressed in numerous tissues, suggesting that local paracrine effects may be responsible for growth. At a molecular level, GH/IGF-1 signaling relies on JAK/STAT, PI3 K, and ERK pathways, and tissue-specific effects of GH versus IGF-1 illustrate the complexity of this system [95, 96]. In muscle, for example, GH determines muscle cell proliferation, fiber size, and insulin sensitivity while IGF-1 exerts effects on fiber type and is locally induced following exercise and injury [94]. In bone, GH and IGF-1 synergistically promote osteoblast activity, inhibit osteoclast-mediated bone resorption, and regulate the renal synthesis of $1,25(OH)_2D$ and phosphate reabsorption [97–100].

GH deficiency and mutations of the GH receptor (i.e., Laron syndrome) are characterized by failure of longitudinal bone growth with short stature, failure of growth plate fusion, and reduced muscle mass, all of which respond to GH or IGF-1 replacement [101]. During aging, serum levels of GH and IGF-1 decline, correlating with reductions in muscle/bone mass and a greater risk of osteoporotic fracture [102]. In muscle, aging leads to attenuation of growth hormone receptor (GHR) activity and of exercise-mediated IGF-1 signals by the local dysregulation of miRNAs [103, 104]. Similarly, bone's sensitivity to the effects of IGF-1 diminishes with age [105]. Given these associations and the vital importance of GH/IGF-1 in promoting musculoskeletal growth, this pathway presents an attractive therapeutic target in addressing age-related osteoporosis and sarcopenia. Animal studies support this notion, showing a reversal in sarcopenia in aged rodents in response to GH treatment via effects in muscle protein synthesis, mitochondrial function, and oxidative stress [106].

However, the use of recombinant human growth hormone (rhGH) to reverse musculoskeletal aging is highly contentious. Twenty-five years ago, a famous study by Rudman and colleagues in older men treated with rhGH for 6 months demonstrated improvements in lean mass (8.8 %) and lumbar bone density (1.6 %) but no change in femoral neck density [107]. Despite the small number of participants ($n = 21$) and the lack of a control group, this study provoked intense interest in the 'anti-aging' potential of rhGH. Subsequent studies and a recent meta-analysis have tempered this excitement by demonstrating inconsistent effects of rhGH on bone density and physical function in older adults and concerning side effects including diabetes, edema, arthralgias, and entrapment neuropathy [108–110]. These studies are also limited by variable dosing regimens of rhGH, short treatment duration and follow-up periods (~ 6 months), and a lack of long-term safety data. In addition, there is the theoretical possibility that rhGH may *increase* mortality. This is suggested by a greater incidence of cancer and cardiovascular disease in patients with acromegaly (GH-secretory pituitary adenoma). Conversely, reduced GH/IGF-1 signaling has been shown to prolong lifespan in worms, insects, and mice [111]. In humans, GH deficiency and resistance are also associated with advanced longevity [101], and within the same population, short individuals have a statistical advantage in longevity versus tall individuals [112].

In spite of equivocal benefits of rhGH on musculoskeletal mass, its reported side effects and concerns of increased mortality, anti-aging clinics across the US have spawned a lucrative industry in the off-label use of rhGH as an "elixir of youth". This raises serious concerns. In vitro studies report increased proliferation of cancer cells in response to rhGH [113]. In mice, rhGH enhances the growth of hepatic carcinoma xenografts [114]. There is also a published report of an elderly male with Crohn's disease who developed IGF-1 receptor-expressing metastatic colon cancer 7 years after commencing rhGH [115]. Therefore longer-term and larger clinical trials are needed to determine the risks and benefits of rhGH in older subjects together with functional outcomes in osteoporosis and sarcopenia.

In principle, GH secretagogues are a more physiological alternative to rhGH. GH secretagogues result in pulsatile rather than prolonged increases in GH and respond to negative feedback by IGF-1. There have been small studies of GH secretagogues (GHRH-1,44-amide and ghrelin mimetic MK-677) which have demonstrated improvements in lean mass, unclear effects in physical function, and no change in bone mineral density [116, 117]. The largest randomized clinical trial of a GH secretagogue (capromorelin) involved 395 elderly subjects and had a planned duration of 2 years [118]. The trial was ceased prematurely due to significant increases in weight gain (1.4 kg at 6 months), arising from the appetite-stimulatory effect of this drug's ghrelin mimetic action. On a positive note, capromorelin was associated with improvements in lean mass, tandem walking, and stair climbing [118]. However, subjects in this trial had mild functional decline and it therefore remains unclear whether GH secretagogues may

have similar functional effects or improvements in bone density in frail subjects. There are also a number of downstream targets of GH/IGF-1, such as SOCS proteins, Grb10 [119], and IGF binding proteins (IGFBP) that may present future therapeutic targets which could potentially circumvent undesirable systemic effects of GH therapy.

Androgens and Selective Androgen Receptor Modulators (SARMs)

In addition to established effects in the reproductive system, androgens regulate growth by exerting anabolic effects in the musculoskeletal system. These effects are complex, relying on the activation of the androgen receptor (AR) at these sites, and the accrual of muscle tissue via an increase in protein synthesis, muscle fiber hypertrophy, and an increase in satellite cell number [120]. In bone, androgen-mediated increases in cortical and trabecular mass rely on the local conversion of testosterone to estrogen and the activation of estrogen receptors (ER) in osteoblasts [121].

Muscle wasting and osteoporosis are cardinal features of male hypogonadism and are amenable to testosterone therapy [122]. Similarly, men with muscle wasting due to HIV or chronic glucocorticoid therapy display increased muscle mass and strength in response to testosterone therapy [123]. The potential for testosterone therapy to reverse musculoskeletal aging has been suggested by a number of observations. Elderly men with reduced serum testosterone have a higher risk of muscle and bone loss and low-energy fracture [124]. Following testosterone therapy, older men display significant increases in bone density and lean mass [125, 126]. Studies suggest that testosterone therapy leads to improvements in strength in older men, related to muscle fiber type-specific effects and increased muscle mass [126, 127]. However, effects in bone were limited to the lumbar spine and the incidence of hard outcomes such as falls/fractures in those receiving testosterone therapy have not been established.

Safety concerns have been raised regarding the long-term use of testosterone therapy in older subjects. In particular, cardiovascular events and prostate cancer have been raised as potential risks, generating significant controversy in this field. In 2010, a study of community-dwelling older men was terminated due to a significantly higher incidence of cardiovascular incidents following the use of transdermal testosterone gel [128]. Larger studies have failed to clearly demonstrate the greater risk of cardiovascular events and in a statement this year, the FDA reported that the evidence was inconclusive [129]. However, a large meta-analysis of non-industry funded trials and a US-based epidemiologic study showed a two-fold increase in the risk of cardiovascular events with

testosterone [130, 131]. Despite these concerns, prescription sales for testosterone in the US increased by 25 % each year from 1993 to 2002 [132].

Tissue-specific agents that exploit anabolic effects of androgens in muscle/bone, while circumventing troublesome effects in other tissues, have been studied for decades [133]. Selective androgen receptor modulators (SARMs) achieve tissue selectivity by differences in tissue distribution of AR isoforms and local interactions with 5-alpha reductase and aromatase [123]. Non-steroidal SARMs (e.g., quinolones, aryl propionamides) have greater oral bioavailability, AR specificity, and tissue selectivity than steroidal SARMs (e.g., 19-nortestosterone, 17-alpha-methyl-testosterone) and their development has therefore progressed further. Andarine (also known as S-4) is considered the ideal SARM due to its high oral bioavailability, long biological half-life allowing once-daily dosing, and preclinical data demonstrating consistent anabolic effects in muscle and bone [134]. A related compound, Ostarine (GTx-024, enobosarm), has been studied in phase I–III clinical trials and shown to increase lean mass and physical function in elderly men, post-menopausal women, and cancer patients [135–137]. Effects in bone density, however, have not been demonstrated possibly due to relatively short study periods of up to 3 months [135]. The agent LGD-4033 increased muscle mass and strength in healthy males in 3 weeks [138], and according to the company website (www.ligand.com), had beneficial effects in bone mass in preclinical studies. A phase II trial for this agent is planned for disorders of muscle wasting (e.g., cancer, fracture). In a recent mouse study, GLPG0492 reduced muscle fiber atrophy in immobilized muscles by modulating atrophy and tissue pleiotropic pathways involving MuRF1, myogenin, and FOXO1 [139]. Other agents such as BMS-564929 and LGD-2941 have shown similar results and are currently in phase I trials for age-related functional decline.

SARMs, therefore, hold great promise as anabolic agents that may reverse effects of musculoskeletal aging and by their tissue selectivity, circumvent concerns arising from off-target effects of AR activation. However, functional outcomes and long-term side effects of these agents need to be further explored.

Exercise and Nutrition

For decades, we have been instructing our patients with osteoporosis to participate in weight-bearing exercise. Enhanced muscle tone and balance, reduced falls, and bone loss, particularly of the femoral neck, are beneficial effects of regular exercise [140, 141]. Mechanical forces activate diverse signaling pathways involved in bone formation and

muscle protein turnover, thereby forming a plausible basis for the beneficial effects of regular exercise [142]. Indeed, a recent extension study demonstrated long-term benefits of exercise in improving gait, parameters of physical function, and reducing falls in older individuals [143]. However these effects rely on continued participation in exercise. Positive effects in muscle strength and bone density were lost within 6 months of ceasing regular exercise in a group of post-menopausal women [144]. There is no consensus on the type, duration, or intensity of exercise that is most effective. Some studies advocate the benefits of regular walking [140] while others suggest music-based programs and resistance exercises [143, 145].

Malnutrition is rife in the elderly, affecting up to 40 % of those living in institutions [146]. It is also estimated that 1 in 5 older individuals in the USA consumes inadequate amounts of protein (i.e., <0.66 g/kg body weight per day) [147]. Malnutrition may exacerbate musculoskeletal aging as nutrients form substrates that are essential for muscle and bone accretion (protein, calcium, magnesium, phosphate) and dietary protein correlates with muscle mass and fractures [139]. However, protein supplements have not shown consistent effects in improving muscle mass and strength [148]. Small studies have suggested some benefit of essential amino acid (EAA) and leucine supplementation in improving muscle function in community-dwelling older individuals [149]. However, these studies were highly variable in terms of quality, supplemental doses, and the inclusion of exercise, and therefore cannot guide practice.

The use of calcium supplements for bone health is contentious. Calcium supplements lead to small increments in bone mineral density but these effects do not persist beyond their duration of use and they do not reduce fractures [150]. On the other hand, meta-analyses have linked calcium supplements with an increased risk of myocardial infarction, raising serious concerns about their widespread use [151, 152]. In general, it appears that potential risks of calcium supplementation, particularly in the elderly and those with renal impairment, outweigh their minimal benefits.

For 25 years, we have known of the association between vitamin K deficiency and osteoporotic fractures [143]. Vitamin K_2 carboxylates osteocalcin, facilitating the incorporation of calcium into hydroxyapatite, increases the production of other matrix Gla proteins and may inhibit osteoclast-mediated bone resorption [145, 153]. However, RCTs of vitamin K supplementation have not shown significant improvements in bone mineral density or fracture risk among the elderly [154]. The use of vitamin K supplements for this group therefore remains an open question.

In summary, exercise and nutritional interventions lead to equivocal and short-lived results in muscle/bone mass in older individuals. The current evidence base is limited by small studies of variable quality and interventional strategies. Larger, standardized trials examining clinically meaningful outcomes in response to exercise/dietary intervention are needed before guidelines can be considered.

Future Directions

In addition to myostatin, there are myriad muscle-secreted factors, known as myokines, that also affect bone and may serve as therapeutic targets in musculoskeletal disorders. These include FGF-2, IL-6, IGF-1, BDNF, FSTL-1, and irisin [33]. As an example, IL6 stimulates bone resorption and IL6-related cytokines, Oncostatin M, and ciliary neurotrophic factor (CNTF), alters osteoblast differentiation and bone strength [155, 156]. Bone-secreted factors may also present important therapeutic targets. Osteocalcin alters muscle mitochondrial function [157] and lower limb strength in older women [36]. Sclerostin regulates the osteogenic response to muscle loading and is already being targeted for treatment in osteoporosis [158], although its effects in muscle mass are unclear. Increased understanding of the interconnected nature of the musculoskeletal system and the vast network of osteokines and myokines will potentially open new avenues of research in bone/muscle therapeutics.

Conclusions

Musculoskeletal aging, defined by the concurrent atrophy of muscle and bone, the infiltration of adipose tissue at these sites, and the attenuation of muscle fibers and bone mineral, is a highly complex and integrated process. Functionally, this progressive involution of muscle and bone leads to postural imbalance, deconditioning, falls and ultimately, fractures with dire consequences on morbidity and mortality. Our current efforts in reducing fracture are geared heavily toward bone and neglect the vastly intricate network of signals that govern and connect bone and muscle mass. An integrated approach that targets the muscle–bone unit *as a whole* maybe more effective in "breaking the vicious cycle" of musculoskeletal aging by exerting concurrent effects that reduce falls and fractures.

There has been significant progress in the development of novel anabolic therapies for muscle and bone, most notably activin pathway inhibitors, SARMs, and non-calcemic VDR agonists. Preclinical data show clear musculoskeletal benefits of these agents but the demonstration of unequivocal effects in falls and fractures are yet to be seen. This will depend on phase III clinical trials which are yet to be conducted for the majority of these therapies.

There are major challenges in the development of dual muscle–bone therapies. The definition of sarcopenia remains unclear and functional endpoints and markers of the 'musculoskeletal unit' are needed to confirm positive outcomes in efficacy trials. There have also been safety concerns with these agents and these relate to off-target effects of manipulating widespread signaling pathways. Activin pathway inhibitors have been hindered by reports of telangiectasia, bleeding, and gonadotropin suppression [90]. Similar issues have been overcome for therapies targeting other pathways. To circumvent undesirable effects of androgens, SARMs have been developed to specifically target muscle and bone. GH secretagogues preserve IGF-1-mediated negative feedback of GH and avoid the effects of supraphysiological levels of GH due to GH therapy [123]. Finally, VDR agonists may specifically target muscle and bone without dose-limiting systemic effects on calcium and phosphate homeostasis.

In recognizing the integrated biology of the musculoskeletal system and the complex signals involved in bone/muscle aging, the time has come to reconsider our approach to the prevention of osteoporotic fracture. Collaborative efforts by basic scientists, physicians, and the pharmaceutical industry are needed to address these complicated issues and ignite the development of novel therapies for the combined treatment of osteoporosis and sarcopenia.

Conflict of interest Christian M. Girgis has no disclosures to report.

Human and Animal Rights and Informed Consent All research discussed in this review has been subject to ethics approval and informed consent has been obtained in human studies.

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