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Therapies for Musculoskeletal Disease: Can we Treat Two Birds with One Stone?

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Abstract

Musculoskeletal diseases are highly prevalent with staggering annual health care costs across the globe. The combined wasting of muscle (sarcopenia) and bone (osteoporosis)— both in normal aging and pathologic states—can lead to vastly compounded risk for fracture in patients. Until now, our therapeutic approach to the prevention of such fractures has focused solely on bone, but our increasing understanding of the interconnected biology of muscle and bone has begun to shift our treatment paradigm for musculoskeletal disease. Targeting pathways that centrally regulate both bone and muscle (eg, GH/IGF-1, sex steroids, etc.) and newly emerging pathways that might facilitate communication between these 2 tissues (eg, activin/myostatin) might allow a greater therapeutic benefit and/or previously unanticipated means by which to treat these frail patients and prevent fracture. In this review, we will discuss a number of therapies currently under development that aim to treat musculoskeletal disease in precisely such a holistic fashion.

Keywords

Muscle; Bone; Anabolic agents; Osteoporosis; Sarcopenia

Introduction

Musculoskeletal diseases are highly prevalent, affecting up to 1 in every 2 individuals in western countries [1, 2]. Moreover, the annual cost of these diseases is staggering, estimated

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at nearly 8 % GDP in USA ($850 billion) and an even greater proportion of GDP in other countries (eg, 10 % GDP or $4.5 billion in Australia). As the world's population ages, the sequelae of musculoskeletal wasting, falls, and fractures are a highly concerning health problem; not only for their financial impact, but as well for the significant increases in patient morbidity, the need for assisted care, and mortality [3]. Falls/fractures represent the common end-point in the age-related involution of bone (osteoporosis) and muscle (sarcopenia) [4••]. In this regard, the combined wasting of muscle and bone—both in normal aging and pathologic states—can lead to vastly compounded risk for fracture in patients. Reduced muscle mass can lead to poor balance and falls, and these falls are then more likely to result in fractures due to the osteoporotic bone's inability to withstand load.

Until now, our therapeutic approach to the prevention of low-energy fracture has focused solely on bone. While osteoporosis has been clearly defined, sarcopenia and its end-points remain open to debate [5]. Sarcopenia has been provisionally defined on the basis of anthropomorphic parameters (appendicular lean mass relative to height or corrected for body weight/fat mass) [6], performance-based parameters (lower limb strength, timed up and go test, walking speed) or a combination of both (lower limb strength/leg lean mass on DXA) [7]. The unclear relationship between muscle mass and function and sex-specific differences highlight difficulties in reaching a consensus definition that corresponds to clear outcomes.

A paradigm shift may be underway with increasing recognition of the interaction of 2 adjacent tissues, bone and muscle. As we are becoming increasingly aware, these interactions are not merely at their anatomic interface or related to mechanical effects of muscle loading on bone function. Rather, bonemuscle interaction encapsulates an intimate relationship, in which bone and muscle communicate via complex paracrine and endocrine signals to coordinate their growth and development from their earliest embryologic stages to involution, as well as to adapt in response to loading and injury [8••]. Research in bone-muscle interactions opens an immense field of potential therapeutic targets and the possibility of addressing osteoporosis and sarcopenia as a single disorder, rather than parallel pathologies, and may present the possibility of a way to 'treat 2 birds with 1 stone.' In this review, we will discuss factors involved in bone-muscle interactions and their therapeutic implications.

Muscle and Bone Development

The musculoskeletal system grows, functions, and ages as a finely coordinated unit. Muscle and bone are derived from a common mesenchymal progenitor during embryogenesis, and their development is closely coordinated by the action of myriad overlapping genes and growth factors [9, 10•]. In addition to these biochemical cues—and likely intertwined with them—mechanical force from developing muscle drives periosteal bone growth, bone density, and bone geometry; even during embryogenesis. Evidence of this integral association of bone and muscle during development can be observed in various mouse models, in which mice with paralyzed or nonfunctional muscle display severe impairments in bone development and mineralization [11, 12]. Likewise, children with Duchenne muscular dystrophy (DMD) and cerebral palsy are also known to have abnormal bones and a higher fracture risk [13, 14].

The close coordination of bone and muscle development in mammals continues into adult life, driven in large part by sex steroids and the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, which will be discussed in detail later. In puberty, the accumulation of lean mass precedes gains in bone mass, and skeletal muscle area determines cortical bone area [15, 16]. A similar link exists in aging adults, in whom lean mass is lost before bone mass, and again, muscle parameters correlate tightly with loss of bone mineral density [17, 18]. Muscle, therefore, seems to "set the pace" for both bone growth and involution—a point that may be key in considering our approach to treating musculoskeletal disease.

One possible explanation for the apparently dominant role of muscle in coordinating bone mass is that muscle loading induces a cascade of biomechanical signals necessary for bone growth and remodeling. In support of this notion, individuals exposed to a gravity-free environment, such as astronauts, experience dramatic bone loss due to lack of muscle loading [19]. However, this "mechanostat theory," as it is commonly known, presents an incomplete picture of bone-muscle interactions. Importantly, appendicular muscle mass correlates with bone cortical thickness *even at remote sites* and not just adjacent, mechanically loaded bone [20••], suggesting additional paracrine or endocrine cross talk, by which bone and muscle coordinate their mass.

Further support for bone-muscle cross talk can be observed in fracture repair, where it has been repeatedly demonstrated that the presence of healthy muscle tissue is a positive factor for fracture healing. For example, the use of muscle flaps in the treatment of open fractures results in faster rates of bone healing in both mice and humans [21, 22]. In addition, the rate of nonunion is markedly higher in fractures associated with acute compartment syndrome, where muscle viability is compromised [23]. In this regard, skeletal muscle may represent a kind of "second periosteum", providing trophic factors, morphogens, and even cells to aid bone repair. Several myokines with potential effects on bone have been proposed, including myostatin, interleukin 6 (IL6), fibroblast growth factor 2 (FGF2), and matrix metalloproteinase 2 (MMP2), amongst others [24-26]. Communication between bone and muscle is likely bi-directional, and bone may also 'talk back' to muscle via a range of osteokines, such as FGF21 produced by osteocytes and other factors [27••]. Additionally, common pathways such as GH/IGF-1, sex steroids and Wnt signaling can centrally coordinate the bone-muscle unit during development and adaptation to mechanical stimuli [20••, 28].

Thus, a complex interplay of mechanical, endocrine, and paracrine signals exists between muscle and bone that serves to coordinate their mass and function throughout life. In the following sections, we will discuss some of these common pathways that have been, or are currently being investigated, as possible targets to treat musculoskeletal diseases. Unraveling the individual effects of these pathways and stimuli poses significant experimental challenges. However, achieving a more thorough understanding of the biochemical links that intertwine bone and muscle physiology is critical for the discovery of therapeutic targets that may lead to a more holistic approach to musculoskeletal disease.

Growth Hormone (GH) and GH Secretagogues

GH plays a fundamental role in bone and muscle growth during childhood and puberty. It also exerts important effects throughout life in glucose and lipid metabolism [29], body composition and bone mineralization [30]. GH is secreted in a pulsatile manner by the pituitary gland and acts by specific growth hormone receptors (GHR) in peripheral tissues, or indirectly through induction of insulin-like growth factor-1 (IGF-1) [31••]. Circulating IGF-1 is produced mainly in the liver, but it is also produced locally in numerous peripheral tissues, including muscle during exercise [32] and regeneration [33]. GH/IGF-1 signaling is complex and tissue-specific, involving JAK/STAT, PI3K, and ERK pathways [34, 35]. Effects of GH in muscle cell proliferation, fiber size and fiber type depend on IGF-1, whilst effects on insulin sensitivity are IGF-1-independent [31••]. In bone, GH/IGF-1 promotes osteoblast proliferation and differentiation, inhibits osteoclast activity, and modulates renal 1α-hydroxylase, (which activates 25-OH-Vitamin D) and phosphate reabsorption [36-39].

Patients with GH deficiency or congenital mutations of GH signaling display short stature, impaired muscle development, and failure of epiphyseal fusion, which respond to GH or IGF-1 replacement, respectively [40]. Even in healthy, GH-replete patients, serum GH and IGF-1 levels decline during aging and are correlated with losses in muscle, bone, and an increased risk of osteoporotic fracture [41]. Furthermore, muscle levels of growth hormone receptor (GHR) drop in proportion to reduced muscle fiber size in older adults [42], and bone responsiveness to IGF-1 also decreases with age [43]. Given these correlates, its central role in postnatal growth, and examples of effective treatment in pathologic states, GH would seem a logical therapeutic for musculoskeletal disease.

However, treatment of older adults with recombinant human growth hormone (rhGH) to reverse age-related changes in muscle, bone, and fat is controversial. In the landmark study by Rudman and colleagues, 12 older men treated with rhGH for 6 months showed increases in lean mass (8.8 %) and lumbar bone density (1.6 %), reduced fat mass (14.4 %), and no change in femoral neck bone density [44]. These results were consistent with effects of GH treatment in adults with hypopituitarism [45] and sparked intense interest in GH as an ‘anti-aging’ therapy. However, subsequent studies and a metaanalysis of 18 randomized controlled trials reported more modest changes in lean mass, inconsistent effects in bone density and physical function, and a number of side effects of rhGH treatment in older patients, including arthralgias, edema, carpal tunnel syndrome, and diabetes [46-48]. It should be noted that these studies were generally small, the treatment duration was short (~ 6 months) and follow-up times and rhGH dosing were variable.

Additional concerns surround the possibility that GH therapy might increase mortality. Reduced GH/IGF-1 signaling has been demonstrated to increase lifespan in worms, flies, and rodents [49]. A similar observation can be made in humans, where GH deficiency and resistance are associated with advanced longevity [40], and short individuals are more likely to live longer than tall individuals from the same population [50]. Conversely, acromegaly (GH-secretory pituitary adenoma) leads to increased mortality due to cardiovascular disease and cancer. The question of whether GH therapy increases mortality has yet to be adequately addressed.

Despite this uncertainty, equivocal effects in body composition, and reported side effects (eg, edema, diabetes), a multibillion dollar industry based on the off-label use of rhGH as anti-aging therapy has emerged in the US. The case of an 86 year-old male with Crohn's disease who developed metastatic colon cancer 7 years after commencing rhGH for antiaging is concerning [51]. The tumor showed greater expression of IGF-1 receptor, suggesting a direct link with rhGH. Larger and longer-term studies are needed to determine the risk: benefit ratio of rhGH in elderly patients, its functional effects in osteoporosis and sarcopenia, and address long-term safety concerns. Proteins involved in tissue-specific GH/IGF-1 signaling in muscle and bone, such as Grb10 [52], SOCS proteins, and local isoforms of IGF and IGF binding proteins (IGFBP) [53] may provide future therapeutic targets that could circumvent undesirable side effects of GH therapy.

Another alternative to rhGH therapy is the use of GH secretagogues. In principle, these agents are "more physiological" than administration of rhGH, as they result in pulsatile—rather than prolonged—elevation of GH and preserve negative feedback by IGF-1. Small studies of GH secretagogues (including GHRH-1,44-amide and ghrelin mimetic MK-677) confirmed increases in GH and IGF-1 levels, showed improvements in lean mass, no change in bone density, and inconsistent effects in physical function [54, 55]. In the largest clinical trial of a GH secretagogue, 395 older individuals were randomized to capromorelin or placebo for a planned 2-year period [56]. The trial was ceased prematurely as significant increases in weight gain (1.4 kg at 6 months) offset improvements in lean body mass. This probably resulted from an appetite-stimulating effect of this drug, a ghrelin mimetic. Interestingly, 2 of 6 functional parameters improved significantly by 12 months, namely tandem walking and stair climbing [56], but older patients in this trial were healthy with mild functional decline. It remains to be seen whether GH secretagogues demonstrate similar functional effects, or improvements in bone parameters, in a more frail population.

Androgens

Sex steroids are another critical player in regulating growth that might serve as a potential bone-muscle therapeutic, in particular, androgens. Apart from their established effects in the reproductive system, androgens exert anabolic effects in muscle and bone—the former being quite easy to appreciate in professional bodybuilders. The mechanisms by which androgens exert their anabolic actions in muscle and bone are complex and extend beyond simply androgen receptor (AR) activation in these tissues. In bone, testosterone must first be converted to estrogen (aromatization) to exert effects on osteoclast activity via estrogen receptors [57]. In muscle, testosterone stimulates protein synthesis, leads to muscle fiber hypertrophy, and increases myonuclei and satellite cell number, suggesting effects on pluripotent precursors [58].

Clinically, men with classic hypogonadism develop muscle wasting and osteoporosis that are reversible with testosterone therapy [59••]. HIV-positive men and glucocorticoid-treated men also display increases in lean mass and muscle strength following testosterone supplementation [60]. Elderly males with reduced testosterone levels are more likely to have muscle/bone loss and a higher fracture risk [61], but testoster-one replacement is controversial in this group. Studies demonstrate significant increases in lumbar BMD in

older men receiving testosterone [62, 63]. This effect was more pronounced in those receiving intramuscular rather than transdermal formulations, and in general, there was no improvement in femoral neck BMD. Despite increases in lean mass, effects of testosterone on muscle strength are heterogeneous with a tendency to improved leg/knee extension and handgrip strength [63]. In 1 randomized trial of frail, older men, transdermal testosterone led to improved physical function and increased fat-free mass after 6 months [64]. However, no clinical trials have evaluated the effects of testosterone on hard outcomes such as falls or fractures.

There are also safety concerns about long-term use of testosterone in vulnerable, older patients. In particular, data on cardiovascular events and prostate cancer are limited; trials are also not sufficiently powered to assess such effects [65•]. The risk of obstructive sleep apnea and polycythemia in individuals using testosterone is also higher. In 2003, the Institute of Medicine (IOM) reported that the existing evidence-base was so equivocal that it could not even recommend large-scale clinical trials without better short-term evidence [66]. However, the US Endocrine Society advocates an individualized approach in the consideration of testosterone therapy in older men [59••]. Despite the uncertainty, prescription sales of testosterone in the US have grown by about 25 % annually between 1993 and 2002, suggesting that increasing proportions of older males are using these medications [66].

Selective Androgen Receptor Modulators (SARMs)

The ‘holy grail’ of decades of preclinical research has been a highly tissue selective and safe agent that does not inhibit gonadotropins [67•]. Selective Androgen Receptor Modulators (SARMs) have been developed to produce anabolic effects in muscle and bone without the dose-limiting androgenic effects associated with testosterone (eg, prostate growth, acne, oily skin). These compounds achieve tissue selectivity by differences in gene regulation, tissue distribution, and local interactions with aromatase and 5-alpha-reductase [60]. In general, nonsteroidal SARMs (eg, aryl propionamides, quinolines) have greater AR specificity, oral bioavailability, and tissue selectivity than their steroidal counterparts (eg, 17-alpha-methyl-testosterone, 19-nortestosterone) and have, therefore, progressed further. Andarine (also known as 8 or S-4) has been described as the ideal SARM due to single daily dosing, complete oral bioavailability and a wealth of preclinical data reporting anabolic muscle and bone effects [68]. Early clinical data were also encouraging, and a related compound, Ostarine (GTx-024, enobosarm), showed increases in lean mass and physical function in elderly men, postmenopausal women, and cancer patients in randomized controlled trials [69, 70•]. There was no improvement in BMD, but this may have been due to the relatively short study period of 3 months [69]. A phase III trial is currently underway for Ostarine, focusing on cancer cachexia in particular. Another agent, LGD-4033, increased lean mass and strength in healthy males after 3 weeks [71], and according to the company, increased bone mass in preclinical studies (www.ligand.com/). A phase II trial for this agent is currently in development for disorders associated with muscle wasting (eg, cancer, fracture). Other nonsteroidal SARMs such as BMS-564929 and LGD-2941 are currently in phase I trials for age-related functional decline.

The first steroidal SARM to enter clinical trials, MK-0773, showed increases in lean mass but no change in physical function or bone mineral content over 6 months in women aged >65 years [72•]. It has now entered a phase II trial for sarcopenia. Clinical data on the efficacy and safety of SARMs continues to emerge, and they hold great promise as anabolic and function-promoting agents in a range of musculoskeletal conditions. However, functional outcomes and long-term side effects of these agents remain to be seen.

Vitamin D

In addition to sex steroids, a number of other hormone pathways impinge on bone and muscle development and may present viable therapeutic targets to treat musculoskeletal diseases. Vitamin D is one such hormone, and while its importance in bone physiology is quite well established, our understanding of its involvement in muscle physiology and function is only emerging. The biologically active form of vitamin D, $1,25(OH)_2D$, is a bona fide hormone that binds to a nuclear receptor (VDR), regulates gene expression, and exerts effects on mineral homeostasis, tissue development, and cell cycle [73•]. Effects of vitamin D in bone and muscle are mainly *indirect*, resulting from effects on calcium and phosphate homeostasis [74, 75]. In bone, direct effects of vitamin D are also possible, as both osteoblasts and osteocytes express VDR [76•]. Osteoblast VDR inhibits bone mineralization to preserve normal serum calcium levels [77] and consistent with this, osteoblast-specific VDR knockout mice display increased bone density [78]. Conversely, VDR overexpression in osteoblasts and osteocytes protects against the bone effects of vitamin D deficiency [79]. By contrast, whether the VDR is expressed in muscle remains controversial, but studies in cultured muscle cells and VDR knockout mice suggest that vitamin D signaling does play a role in muscle differentiation and fiber size regulation [80, 81].

In humans, severe vitamin D deficiency leads to osteomalacia and muscle weakness due to type II muscle fiber atrophy [73•]. Vitamin D deficiency is common in the elderly, owing to both nutritional deficits and lack of sun exposure, and has been associated with falls, sarcopenia, and osteoporosis [82, 83]. One study even observed a reduction in the levels of VDR in muscle with age, suggesting an even greater vulnerability of older individuals to low vitamin D levels [84].

Randomized trials have demonstrated that vitamin D supplementation reduces the risk of falls and fractures in older, institutionalized individuals [85, 86]. However, the effects of vitamin D supplementation are less clear amongst those living in the community. Although vitamin D supplementation may increase femoral neck and hip BMD in such individuals, this effect is small and not associated with reduction in fracture risk [87, 88]. Interestingly, vitamin D supplementation may increase muscle fiber size in frail, older patients [89•], confirming effects demonstrated at a cellular level [81]. Whether these effects on fiber size translate into any functional benefit (eg, muscle strength or improved physical performance measures) is not clear without standardized end points for muscle function in these trials [73•, 89•].

While generally well tolerated, a greater incidence of kidney stones and increased falls and fractures have been reported in individuals receiving mega-doses of vitamin D [87, 90•].

Such reports have raised questions and vigorous debate about what precisely constitutes vitamin D sufficiency, and safe doses to achieve positive benefit from vitamin D. Indeed, discord persists regarding recommendations for vitamin D. For example, the IOM recommends 25 OHD target levels of 50 nmol/L and daily vitamin D doses of 800 IU in older adults (> 70 years) [91•]. The US Endocrine society advocates higher serum target level of 75 nmol/L and daily doses of at least 1500-2000 IU in this age-group [92•]. Perhaps most attractive for treating musculoskeletal disease because of its availability and ease of use, the ongoing uncertainty regarding risks and benefits of vitamin D supplementation, together with continued controversy regarding optimal serum levels, point to a need for further study; especially in the context of its potential effects on skeletal muscle.

Exercise and Nutrition

Perhaps the simplest of all possible therapies to treat—or in this case, even more importantly, prevent—musculoskeletal disease is also one of the most difficult to implement. For many years, health professionals have been advising patients with osteoporosis to engage in weight-bearing exercise. The benefits of exercise in elderly patients are quite clear: improved muscle tone and balance to prevent falls and attenuation of bone loss, particularly at the femoral neck [93, 94]. Sufferers of chronic diseases, such as breast cancer, may also prevent muscle and bone loss by regular strength training and exercise [95]. Unfortunately, the positive effects of exercise on bone and muscle can only be maintained through continued engagement in the activity; a fact with which many of us who sit at desks and write papers about musculoskeletal therapies are all too familiar. For example, a study of premenopausal women demonstrated that 6 months after ceasing regular exercise, positive effects in muscle strength and BMD were lost [96]. An additional confounder in recommending exercise, there is no clear consensus on the type, intensity, or duration of exercise that is most effective. However, regular walking has shown positive effects on muscle and bone in elderly individuals [93]. Even low magnitude mechanical signals have been demonstrated to have positive effects on bone and muscle [97], providing an encouraging prospect for those who have restricted mobility due to prior injury or concomitant disease. Electrical muscle stimulation may also prevent muscle and bone loss, as demonstrated in patients with spinal cord injury [98].

Nutrition provides substrates necessary for bone matrix and mineral (protein, calcium, magnesium, phosphate) and muscle accretion (protein). Nutrition is of particular concern in the elderly, where malnutrition affects up to 40 % of those living in institutions. Moreover, 20 % of older individuals in the USA consume inadequate protein, as defined by <0.66 g/kg/ actual body weight per day [99, 100]. Although an association between dietary protein intake and lean mass exists [101], the use of protein supplementation to reduce sarcopenia is controversial. Small trials suggest that 25–30 g of high-quality protein is necessary to maximize skeletal muscle protein synthesis [102]. However, a meta-analysis of 62 trials found no improvement in physical function in elderly patients on highenergy protein supplements [103].

Another concern has been the co-occurrence of muscle wasting and visceral adiposity, known as “sarcopenic obesity”. This is associated with functional disability and

osteoporosis, possibly related to adipocyte infiltration in bone and muscle and subsequent pro-inflammatory state [8••]. The addition of exercise training to energy restriction preserves muscle mass during periods of weight loss in older adults [104]. Activin signaling inhibitors, discussed below, show promising results in the reduction of fat mass whilst increasing lean mass [105].

The use of calcium supplements and their benefit in bone health is similarly controversial. Calcium supplements may lead to small benefits in bone mineral density, but they do not clearly reduce fracture risk and their effects do not persist beyond their duration of use [106]. A potentially increased risk of myocardial infarcts with calcium supplements have also called the benefits of calcium supplementation into question [107]. Taken together, exercise, and dietary interventions would seem to produce equivocal results, at best, in elderly patients with existing osteoporosis and sarcopenia. However, their value as both preventative and concurrent approaches to help maintain bone and muscle mass should not be overlooked, especially given the additional health benefits of exercise and proper nutrition in other organ systems (eg, cardiovascular).

Activin Signaling Inhibitors

In addition to the more ‘classical’ pathways involved in muscle and bone development discussed already, recent studies have suggested that the activin signaling pathway—well known for the suppressive effects of myostatin on muscle mass—may represent another shared pathway between muscle and bone. Myostatin is a member of the TGF-β superfamily and a muscle-derived hormone that was first discovered in 1997 [108]. Myostatin deficiency results in increased muscle mass in several species, including humans [108, 109]. Conversely, increases in myostatin may partially explain the muscle wasting observed in patients with chronic diseases such as renal failure [110], HIV [111], and chronic obstructive pulmonary disease [112]. Myostatin exerts these effects on muscle by binding to a transmembrane receptor, activin receptor IIB (ActRIIB), ultimately activating Smad family proteins and downstream signals that lead to muscle protein breakdown via the ubiquitin-proteasome system. There is also a closely related ActRIIA that binds additional activin ligands (but can weakly bind myostatin) and shares some functional overlap with ActRIIB in muscle [113, 114]. More recently, the activin signaling pathway has also been shown to affect bone development and remodeling. Polymorphisms in the myostatin gene are associated with peak bone mineral density [115]. Myostatin knockout mice display increased BMD and bone mineral content (BMC) [24, 116] and greater callus size following osteotomy [117]. These anabolic effects on bone were predominantly believed to be related to increased mechanical loading, secondary to increased skeletal muscle mass. However, direct effects of activin/myostatin on bone are also possible, as bone marrow stromal cells and osteoblasts express activin receptors, and modulating the pathway in vitro appears to affect bone cell differentiation [118•, 119•].

The activin signaling pathway is an attractive therapeutic target for musculoskeletal disease, given the evidence to suggest that it might function to negatively regulate both bone and muscle mass. Indeed, several inhibitors of this pathway have already been developed, including myostatin-neutralizing antibodies/propeptide, recombinant follistatin (an

endogenous inhibitor that binds and sequesters ligands), follistatin derivatives, and soluble activin receptors [114]. Mice treated with such agents demonstrated substantial increases in muscle mass and strength [26, 118•]. Positive effects on muscle mass were also reported in mouse models of androgen deficiency [120], muscular dystrophy [121], and cancer cachexia [122]. In addition to the expected effects on skeletal muscle, ActRIIB-Fc also increased bone formation rates and bone mineral density in mice and demonstrated direct effects on osteoblast activity [118•, 123•]. Similar bone anabolic responses were also seen in primates administered soluble ActRIIA [124]. Interestingly, a myostatin propeptide had no effect on bone parameters in mice, despite increasing muscle mass [125•]. This difference in tissue response highlights the possibility that specific components in the pathway could be exploited therapeutically to achieve different benefits (eg, bone and muscle anabolic, only muscle anabolic, etc.), depending on the disease context.

In addition to these preclinical studies, inhibitors of the activin signaling pathway have also been tested in human phase 1 and 2 trials. A recombinant human myostatin antibody (MYO-029, Stamulumab) was found to be generally safe in healthy individuals (NCT00563810) and adults with muscular dystrophy [126]. Although MYO-029 resulted in improved contraction in single muscle fibers [127], increases in muscle mass on DXA were not statistically significant. Moreover, no improvement in muscle strength was observed in 116 patients with muscular dystrophy [126], although this study was not adequately powered to detect changes in muscle function. In a double-blind, placebo-controlled study of 48 postmenopausal women, a single dose of ACE-031 (soluble ActRIIB decoy receptor) resulted in significant increases in lean mass (3.3 %) and thigh muscle volume (5.1 %) on DEXA and MRI after 1 month [128•]. Although grip strength was measured at baseline, changes following treatment were not reported. ACE-031 also resulted in a significant increase in bone-specific ALP and decrease in C-telopeptide, indicating increased bone remodeling. The company reported a significant 3.4 % increase in bone mineral density (BMD) at 113 days in a phase 1b trial of 60 postmenopausal women on ACE-031 (www.acceleron.pharma.com/), although this trial has not been published or subject to peer review.

Targeting this pathway is not without its issues however. First noted in trials of children with DMD (NCT01099761, clinicaltrials.gov) and postmenopausal women receiving higher doses of ACE-031 [128•], side effects include nose-bleeds and skin telangiectasia. Although not serious in itself, this phenomenon does raise concerns about unrecognized, systemic effects of ActRIIB inhibition. Other off-target effects include significant reduction in serum FSH levels (43 %) after a single dose of ACE-031 (3 mg/kg), most likely related to suppression of activin/GnRH signaling [128•], and alteration of fat mass and metabolism [105]. In this regard, antibodies directed against activin receptors could potentially offer a means to avoid off target effects seen with soluble receptor administration by allowing more specific targeting of IIA vs IIB receptor and varied blockade kinetics. A recent study showed that a novel anti-ActRIIA antibody (BYM338) was twice as effective as a myostatin-specific inhibitor (D76A) in increasing muscle mass in mice [129•]. The effect of BYM338 was partly myostatin-independent as confirmed by its effects in myostatin mutant mice. Interestingly, BYM338 also resulted in increases in muscle IGF-1 and prevented

glucocorticoid-induced muscle wasting by reducing levels of E3 ubiquitin ligases, MAFbx, and MuRF1.

Clinical studies of various activin pathway inhibitors are still in their infancy, but they have yielded promising results for the therapeutic potential of modulating this pathway to treat musculoskeletal disease. Larger, prospective studies are, of course, necessary to establish the long-term safety and efficacy of these agents.

Myokines and Future Directions

In addition to myostatin, a number of recent studies have demonstrated other muscle-secreted factors—termed myokines—that can serve as paracrine/endocrine factors to influence other organ systems, including bone. These myokines include myostatin, LIF, IL-6, IL-7, BDNF, IGF-1, FGF-2, FSTL-1, and irisin [25]. Given that we have already discussed therapeutics targeted at 2 of these myokines (myostatin and IGF-1, albeit secondary to GH), it is likely that further study of muscle-bone interactions will reveal other myokines as candidate therapeutic targets to treat musculoskeletal disease.

Importantly, many of these myokines could impact bone and muscle secondarily, through actions on other tissues and organs, as the interconnectedness of bone and muscle extends well beyond just one another. In recent years, endocrine pathways have been elucidated that connect bone metabolism to the pancreas, fat, and brain; all organs also interconnected with muscle. It is not unreasonable to suspect that impinging upon a “middle man” could exert profound effects on muscle and/or bone. One such example can be envisaged for the myokine IL-6, which has been demonstrated to increase the secretion of insulin from the pancreas [130]. Insulin could then feed into the bone–pancreas endocrine loop to exert secondary effects upon bone [27••]. Such systems biology-based approaches to understanding the interaction of muscle and bone—with each other and other organs—could even result in treatments for musculoskeletal disease that target entirely different organ systems to exert their effect on muscle and bone (eg, CNS or fat). This represents a truly exciting future direction for study.

Conclusions

Osteoporosis and sarcopenia are closely related conditions characterized by age-related involution of the bone-muscle unit. Functionally, this progressive muscle and bone loss leads to falls, fractures, deconditioning, and further muscle wasting, all of which can be exacerbated by additional disease pathology. While previous efforts to reduce fracture were heavily geared toward treating bone as a separate organ, our increasing understanding of bone-muscle interactions has highlighted that targeting the bone-muscle unit as a whole may break this ‘vicious cycle’ of musculoskeletal atrophy even more effectively. There has been significant progress in the development of novel anabolic agents for bone and muscle, most notably SARMs and activin pathway inhibitors, and exciting new opportunities for targeting myokines may be on the horizon.

Developing therapeutic treatments to holistically treat musculoskeletal disease is not without significant challenge however, and one of the largest hurdles is related neither to the targets

nor their biology. Rather, it lies within the definition of the condition itself; sarcopenia and its end-points are poorly defined. Moreover, functional outcomes and markers are needed to clarify a positive outcome in the 'musculoskeletal unit' and guide efficacy trials. These topics are under vigorous debate and are of critical importance for advancing musculoskeletal therapeutics. As with any therapeutic development, safety has also been a concern. Telangiectasia, bleeding, and gonadotropin suppression in patients on activin pathway inhibitors highlight our incomplete understanding of systemic activin/myostatin signaling [128•]. Similar issues have been overcome for other pathways, however. To avoid undesirable systemic effects of androgens, SARMs selectively target muscle and bone. Concerns of nonphysiological GH levels, and possible related side effects, with GH administration have been addressed with GH secretagogues, which preserve IGF-1-mediated negative feedback of GH [60]. Finally, systems biology-based research may prompt us to consider other tissues that participate in bone-muscle interactions, such as fat and nerves, as future potential therapeutic targets [8••]. Collaborative efforts by basic scientists, clinicians, and industry are needed to address these complex issues and energize the clinical development of novel bone-muscle therapies.

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References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Australian Bureau of Statistics. [Accessed Dec 2013] 2005. Available at: http://www.abs.gov.au/ausstats/abs@.nsf/cat/4823.0.55.001

2. Connelly, LB.; Woolf, A.; Brooks, P. Cost-effectiveness of interventions for musculoskeletal conditions. In: Jamison, DT.; Breman, JG.; Measham, AR.; Alleyne, G.; Claeson, M.; Evans, DB., et al., editors. Disease control priorities in developing countries. 2nd edn.. Oxford University Press and World Bank; Washington, DC: 2006. p. 963-8.

3. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006; 17(12):1726–33. doi:10.1007/s00198-006-0172-4. [PubMed: 16983459]

4••. Bonewald LF, Kiel DP, Clemens TL, Esser K, Orwoll ES, O'Keefe RJ, et al. Forum on bone and skeletal muscle interactions: summary of the proceedings of an ASBMR workshop. J Bone Miner Res. 2013; 28(9):1857–65. doi:10.1002/jbmr.1980. [PubMed: 23671010] This perspective article summarizes an ASBMR workshop on bone-muscle interactions, outlines current concepts in the field, and research questions to stmulate potential therapeutic strategies for musculoskeletal disorders.

5. Cooper C, Dere W, Evans W, Kanis JA, Rizzoli R, Sayer AA, et al. Frailty and sarcopenia: definitions and outcome parameters. Osteoporos Int. 2012; 23(7):1839–48. doi:10.1007/s00198-012-1913-1. [PubMed: 22290243]

6. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998; 147(8): 755–63. [PubMed: 9554417]

7. Scott D, Hayes A, Sanders KM, Aitken D, Ebeling PR, Jones G. Operational definitions of sarcopenia and their associations with 5-year changes in falls risk in community-dwelling middle-aged and older adults. Osteoporos Int. 2014; 25(1):187–93. doi:10.1007/s00198-013-2431-5. [PubMed: 23800748]

8••. DiGirolamo DJ, Kiel DP, Esser KA. Bone and skeletal muscle: neighbors with close ties. J Bone Miner Res. 2013; 28(7):1509–18. doi:10.1002/jbmr.1969. [PubMed: 23630111] This perspective article reviews bone-muscle interactions throughout development and aging and highlights the predominant nature of muscle in this relationship.

9. Schoenwolf, GC.; Bleyl, SB.; Brauer, PR.; Francis-West, PH. Chapter 8: Development of the musculoskeletal system. Larsen's human embryology. 4th edition. Churchill Livingstone/Elsevier; Philadelphia: 2009.

10•. Karasik D, Kiel DP. Evidence for pleiotropic factors in genetics of the musculoskeletal system. Bone. 2010; 46(5):1226–37. doi:10.1016/j.bone.2010.01.382. [PubMed: 20149904] This review covers genetic aspects of bone-muscle interactions, providing valuable insight in the common genetic etiology of osteoporosis and sarcopenia.

11. Kahn J, Shwartz Y, Blitz E, Krief S, Sharir A, Breitel DA, et al. Muscle contraction is necessary to maintain joint progenitor cell fate. Dev Cell. 2009; 16(5):734–43. doi:10.1016/j.devcel.2009.04.013. [PubMed: 19460349]

12. Nowlan NC, Bourdon C, Dumas G, Tajbakhsh S, Prendergast PJ, Murphy P. Developing bones are differentially affected by compromised skeletal muscle formation. Bone. 2010; 46(5):1275–85. doi:10.1016/j.bone.2009.11.026. [PubMed: 19948261]

13. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop. 2000; 20(1):71–4. [PubMed: 10641693]

14. Shaw NJ, White CP, Fraser WD, Rosenbloom L. Osteopenia in cerebral palsy. Arch Dis Child. 1994; 71(3):235–8. [PubMed: 7979497]

15. Sharir A, Stern T, Rot C, Shahar R, Zelzer E. Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. Development. 2011; 138(15):3247–59. doi:10.1242/dev.063768. [PubMed: 21750035]

16. Slizewski A, Schonau E, Shaw C, Harvati K. Muscle area estimation from cortical bone. Anat Rec. 2013 doi:10.1002/ar.22788.

17. Szulc P, Beck TJ, Marchand F, Delmas PD. Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men—the MINOS study. J Bone Miner Res. 2005; 20(5):721–9. doi:10.1359/JBMR.041230. [PubMed: 15824844]

18. Rikkonen T, Sirola J, Salovaara K, Tuppurainen M, Jurvelin JS, Honkanen R, et al. Muscle strength and body composition are clinical indicators of osteoporosis. Calcif Tissue Int. 2012; 91(2):131–8. doi:10.1007/s00223-012-9618-1. [PubMed: 22733383]

19. Keyak JH, Koyama AK, LeBlanc A, Lu Y, Lang TF. Reduction in proximal femoral strength due to long-duration spaceflight. Bone. 2009; 44(3):449–53. doi:10.1016/j.bone.2008.11.014. [PubMed: 19100348]

20••. Lebrasseur NK, Achenbach SJ, Melton LJ III, Amin S, Khosla S. Skeletal muscle mass is associated with bone geometry and microstructure and serum insulin-like growth factor binding protein-2 levels in adult women and men. J Bone Miner Res. 2012; 27(10):2159–69. doi:10.1002/jbmr.1666. [PubMed: 22623219] This observational study of ~ 590 patients reported an association between appendicular muscle mass, bone cortical thickness at remote sites, and serum insulin-like growth factor (IGF) binding protein-2 (IGFBP-2) levels. This further supports the integrated nature of bone-muscle cross-talk.

21. Harry LE, Sandison A, Paleolog EM, Hansen U, Pearse MF, Nanchahal J. Comparison of the healing of open tibial fractures covered with either muscle or fasciocutaneous tissue in a murine model. J Orthop Res. 2008; 26(9):1238–44. doi:10.1002/jor.20649. [PubMed: 18404722]

22. Gopal S, Majumder S, Batchelor AG, Knight SL, De Boer P, Smith RM. Fix and flap: the radical orthopaedic and plastic treatment of severe open fractures of the tibia. J Bone Joint Surg (Br). 2000; 82(7):959–66. [PubMed: 11041582]

23. Reverte MM, Dimitriou R, Kanakaris NK, Giannoudis PV. What is the effect of compartment syndrome and fasciotomies on fracture healing in tibial fractures? Injury. 2011; 42(12):1402–7. doi:10.1016/j.injury.2011.09.007. [PubMed: 21993369]

24. Elkasrawy MN, Hamrick MW. Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. J Musculoskelet Neuronal Interact. 2010; 10(1):56–63. [PubMed: 20190380]

25. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012; 8(8):457–65. doi:10.1038/nrendo.2012.49. [PubMed: 22473333]

26. Cianferotti L, Brandi ML. Muscle-bone interactions: basic and clinical aspects. Endocrine. 2013 doi:10.1007/s12020-013-0026-8.

27••. DiGirolamo DJ, Clemens TL, Kousteni S. The skeleton as an endocrine organ. Nat Rev Rheumatol. 2012; 8(11):674–83. doi:10.1038/nrrheum.2012.157. [PubMed: 23045255] This review presents bone as an endocrine organ based on the discovery of unique ‘osteokines’ with effects in glucose and energy homeostasis.

28. Christoforidis A, Maniadaki I, Stanhope R. Growth hormone / insulin-like growth factor-1 axis during puberty. Pediatr Endocrinol Rev. 2005; 3(1):5–10. [PubMed: 16369208]

29. Perrini S, Carreira MC, Conserva A, Laviola L, Giorgino F. Metabolic implications of growth hormone therapy. J Endocrinol Invest. 2008; 31(9 Suppl):79–84. [PubMed: 19020393]

30. Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A, Giorgino F. The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. J Endocrinol. 2010; 205(3):201–10. doi:10.1677/JOE-09-0431. [PubMed: 20197302]

31••. Mavalli MD, DiGirolamo DJ, Fan Y, Riddle RC, Campbell KS, van Groen T, et al. Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. J Clin Invest. 2010; 120(11):4007–20. doi:10.1172/JCI42447. [PubMed: 20921627] This study delineates specific effects of GH signaling in muscle via the generation of 2 different mouse models. Effects of GH in muscle development depend on IGF-1, whilst effects on insulin sensitivity are IGF-1-independent.

32. Rojas Vega S, Knicker A, Hollmann W, Bloch W, Struder HK. Effect of resistance exercise on serum levels of growth factors in humans. Hormone and metabolic research = Hormonund Stoffwechselforschung = Hormones et metabolisme. 2010; 42(13):982–6. doi:10.1055/s-0030-1267950.

33. Jennische E, Hansson HA. Regenerating skeletal muscle cells express insulin-like growth factor I. Acta Physiol Scand. 1987; 130(2):327–32. doi:10.1111/j.1748-1716.1987.tb08144.x. [PubMed: 3300170]

34. Miller WL, Eberhardt NL. Structure and evolution of the growth hormone gene family. Endocr Rev. 1983; 4(2):97–130. doi:10.1210/edrv-4-2-97. [PubMed: 6345149]

35. Carter-Su C, Schwartz J, Smit LS. Molecular mechanism of growth hormone action. Annu Rev Physiol. 1996; 58:187–207. doi:10.1146/annurev.ph.58.030196.001155. [PubMed: 8815791]

36. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev. 2008; 29(5):535–59. doi:10.1210/er.2007-0036. [PubMed: 18436706]

37. Hill PA, Tumber A, Meikle MC. Multiple extracellular signals promote osteoblast survival and apoptosis. Endocrinology. 1997; 138(9):3849–58. doi:10.1210/endo.138.9.5370. [PubMed: 9275074]

38. Hock JM, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology. 1988; 122(1):254–60. doi:10.1210/endo-122-1-254. [PubMed: 3335207]

39. DiGirolamo DJ, Mukherjee A, Fulzele K, Gan Y, Cao X, Frank SJ, et al. Mode of growth hormone action in osteoblasts. J Biol Chem. 2007; 282(43):31666–74. doi:10.1074/jbc.M705219200. [PubMed: 17698843]

40. Laron Z. Do deficiencies in growth hormone and insulin-like growth factor-1 (IGF-1) shorten or prolong longevity? Mech Ageing Dev. 2005; 126(2):305–7. doi:10.1016/j.mad.2004.08.022. [PubMed: 15621211]
41. Zhao HY, Liu JM, Ning G, Zhao YJ, Chen Y, Sun LH, et al. Relationships between insulin-like growth factor-I (IGF-I) and OPG, RANKL, bone mineral density in healthy Chinese women. Osteoporos Int. 2008; 19(2):221–6. doi:10.1007/s00198-007-0440-y. [PubMed: 17703270]
42. Leger B, Derave W, De Bock K, Hespel P, Russell AP. Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation. Rejuvenation Res. 2008; 11(1):163–75B. doi:10.1089/rej.2007.0588. [PubMed: 18240972]
43. Ghiron LJ, Thompson JL, Holloway L, Hintz RL, Butterfield GE, Hoffman AR, et al. Effects of recombinant insulin-like growth factor-I and growth hormone on bone turnover in elderly women. J Bone Miner Res. 1995; 10(12):1844–52. doi:10.1002/jbmr.5650101203. [PubMed: 8619364]
44. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, et al. Effects of human growth hormone in men over 60 years old. N Engl J Med. 1990; 323(1):1–6. doi:10.1056/NEJM199007053230101. [PubMed: 2355952]
45. Reed ML, Merriam GR, Kargi AY. Adult growth hormone deficiency - benefits, side effects, and risks of growth hormone replacement. Front Endocrinol. 2013; 4:64. doi:10.3389/fendo.2013.00064.
46. Liu H, Bravata DM, Olkin I, Nayak S, Roberts B, Garber AM, et al. Systematic review: the safety and efficacy of growth hormone in the healthy elderly. Ann Intern Med. 2007; 146(2):104–15. [PubMed: 17227934]
47. Papadakis MA, Grady D, Black D, Tierney MJ, Gooding GA, Schambelan M, et al. Growth hormone replacement in healthy older men improves body composition but not functional ability. Ann Intern Med. 1996; 124(8):708–16. [PubMed: 8633830]
48. Blackman MR, Sorkin JD, Munzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, et al. Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA. 2002; 288(18):2282–92. [PubMed: 12425705]
49. Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. Growth Horm IGF Res. 2008; 18(6):455–71. doi:10.1016/j.ghir.2008.05.005. [PubMed: 18710818]
50. Samaras, T., editor. Human Body Size and the Laws of Scaling: Physiological, Performance, Growth, Longevity and Ecological Ramifications. Nova Science Publishers; New York: 2007.
51. Melmed GY, Devlin SM, Vlotides G, Dhall D, Ross S, Yu R, et al. Anti-aging therapy with human growth hormone associated with metastatic colon cancer in a patient with Crohn's colitis. Clin Gastroenterol Hepatol. 2008; 6(3):360–3. doi:10.1016/j.cgh.2007.12.017. [PubMed: 18255351]
52. Holt LJ, Turner N, Mokbel N, Trefely S, Kanzleiter T, Kaplan W, et al. Grb10 regulates the development of fiber number in skeletal muscle. FASEB. 2012; 26(9):3658–69. doi:10.1096/fj.11-199349.
53. Solomon AM, Bouloux PM. Modifying muscle mass—the endocrine perspective. J Endocrinol. 2006; 191(2):349–60. doi:10.1677/joe.1.06837. [PubMed: 17088404]
54. Veldhuis JD, Patrie JM, Frick K, Weltman JY, Weltman AL. Administration of recombinant human GHRH-1,44-amide for 3 months reduces abdominal visceral fat mass and increases physical performance measures in postmenopausal women. Eur J Endocrinol. 2005; 153(5):669–77. doi:10.1530/eje.1.02019. [PubMed: 16260425]
55. Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell FE Jr, Clasey JL, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. Ann Intern Med. 2008; 149(9):601–11. [PubMed: 18981485]
56. White HK, Petrie CD, Landschulz W, MacLean D, Taylor A, Lyles K, et al. Effects of an oral growth hormone secretagogue in older adults. J Clin Endocrinol Metab. 2009; 94(4):1198–206. doi:10.1210/jc.2008-0632. [PubMed: 19174493]
57. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev. 2004; 25(3):389–425. doi:10.1210/er.2003-0003. [PubMed: 15180950]
58. Chen Y, Zajac JD, MacLean HE. Androgen regulation of satellite cell function. J Endocrinol. 2005; 186(1):21–31. doi:10.1677/joe.1.05976. [PubMed: 16002532]

59••. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010; 95(6):2536–59. doi:10.1210/jc.2009-2354. [PubMed: 20525905] These guidelines published by the Endocrine Society outline reccomendations for testing and treating androgen deficiency.

60. Bhasin S, Calof OM, Storer TW, Lee ML, Mazer NA, Jasuja R, et al. Drug insight: testosterone and selective androgen receptor modulators as anabolic therapies for chronic illness and aging. Nat Clin Pract Endocrinol Metab. 2006; 2(3):146–59. doi:10.1038/ncpendmet0120. [PubMed: 16932274]

61. Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA, et al. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. J Clin Endocrinol Metab. 2006; 91(10):3908–15. doi:10.1210/jc.2006-0173. [PubMed: 16849417]

62. Tracz MJ, Sideras K, Bolona ER, Haddad RM, Kennedy CC, Uraga MV, et al. Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. J Clin Endocrinol Metab. 2006; 91(6):2011–6. doi:10.1210/jc.2006-0036. [PubMed: 16720668]

63. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol. 2005; 63(3):280–93. doi:10.1111/j.1365-2265.2005.02339.x.

64. Srinivas-Shankar U, Roberts SA, Connolly MJ, O'Connell MD, Adams JE, Oldham JA, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 2010; 95(2):639–50. doi:10.1210/jc.2009-1251. [PubMed: 20061435]

65•. Spitzer M, Huang G, Basaria S, Travison TG, Bhasin S. Risks and benefits of testosterone therapy in older men. Nat Rev Endocrinol. 2013; 9(7):414–24. doi:10.1038/nrendo.2013.73. [PubMed: 23591366] This review summarizes benefits of testosterone therapy, its indications and risks in older men, particularly relating to cardiovascular disease, prostate conditions, and erythrocytosis.

66. Institute of Medicine TNAP. Testosterone and aging: clinical research directions. Liverman, CT.; Blazer, DG., editors. National, Academies Press; Washington, D.C.: 2004.

67•. Zhang X, Sui Z. Deciphering the selective androgen receptor modulators paradigm. Expert Opin Drug Discov. 2013; 8(2):191–218. doi:10.1517/17460441.2013.741582. [PubMed: 23231475] This review covers pharmacologic aspects in the development of SARMs and summarizes preclinical and clinical data.

68. Mohler ML, Bohl CE, Jones A, Coss CC, Narayanan R, He Y, et al. Nonsteroidal selective androgen receptor modulators (SARMs): dissociating the anabolic and androgenic activities of the androgen receptor for therapeutic benefit. J Med Chem. 2009; 52(12):3597–617. doi:10.1021/jm900280m. [PubMed: 19432422]

69. Dalton JT, Barnette KG, Bohl CE, Hancock ML, Rodriguez D, Dodson ST, et al. The selective androgen receptor modulator GTx-024 (enobosarm) improves lean body mass and physical function in healthy elderly men and postmenopausal women: results of a double-blind, placebo-controlled phase II trial. J Cachex Sarcopenia Muscle. 2011; 2(3):153–61. doi:10.1007/s13539-011-0034-6.

70•. Dobs AS, Boccia RV, Croot CC, Gabrail NY, Dalton JT, Hancock ML, et al. Effects of enobosarm on muscle wasting and physical function in patients with cancer: a double-blind, randomised controlled phase 2 trial. Lancet Oncol. 2013; 14(4):335–45. doi:10.1016/S1470-2045(13)70055-X. [PubMed: 23499390] This clinical trial of enobosarm, a nonsteroidal SARM, showed significant increases in lean body mass but no effect in bone mineral density in patients with cancer cachexia. A phase 3 trial is currently underway.

71. Basaria S, Collins L, Dillon EL, Orwoll K, Storer TW, Miciek R, et al. The safety, pharmacokinetics, and effects of LGD-4033, a novel nonsteroidal oral, selective androgen receptor modulator, in healthy young men. J Gerontol A Biol Sci Med Sci. 2013; 68(1):87–95. doi:10.1093/gerona/gls078. [PubMed: 22459616]

72•. Papanicolaou DA, Ather SN, Zhu H, Zhou Y, Lutkiewicz J, Scott BB, et al. A phase IIA randomized, placebo-controlled clinical trial to study the efficacy and safety of the selective androgen receptor modulator (SARM), MK-0773 in female participants with sarcopenia. J Nutr

Health Aging. 2013; 17(6):533–43. doi:10.1007/s12603-013-0335-x. [PubMed: 23732550] The steroidal SARM, MK-0773, resulted in significant increases in lean body mass in frail elderly women but this did not translate to improvements in muscle strength.

73•. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of Vitamin D in skeletal muscle: form, function, and metabolism. Endocr Rev. 2013; 34:33–83. doi:10.1210/er.2012-1012. [PubMed: 23169676] This review summarizes effects of vitamin D in skeletal muscle from animal, cell and human studies.

74. Amling M, Priemel M, Holzmann T, Chapin K, Rueger JM, Baron R, et al. Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. Endocrinology. 1999; 140(11):4982–7. [PubMed: 10537122]

75. Schubert L, DeLuca HF. Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch Biochem Biophys. 2010; 500(2):157–61. doi:10.1016/j.abb.2010.05.029. [PubMed: 20515645]

76•. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? Arch Biochem Biophys. 2012; 523(1):123–33. doi:10.1016/j.abb.2012.04.001. [PubMed: 22503810] This article examines the tissue distribution of VDR, including organs where its presence is controversial such as liver and skeletal muscle.

77. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. J Clin Invest. 2012; 122(5):1803–15. doi:10.1172/JCI45890. [PubMed: 22523068]

78. Yamamoto Y, Yoshizawa T, Fukuda T, Shirode-Fukuda Y, Yu T, Sekine K, et al. Vitamin D receptor in osteoblasts is a negative regulator of bone mass control. Endocrinology. 2013; 154(3):1008–20. doi:10.1210/en.2012-1542. [PubMed: 23389957]

79. Lam NN, Triliana R, Sawyer RK, Atkins GJ, Morris HA, O'Loughlin PD, et al. Vitamin D receptor overexpression in osteoblasts and osteocytes prevents bone loss during vitamin D-deficiency. J Steroid Biochem Mol Biol. 2014 doi:10.1016/j.jsbmb.2014.01.002.

80. Endo I, Inoue D, Mitsui T, Umaki Y, Akaike M, Yoshizawa T, et al. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology. 2003; 144(12):5138–44. doi:10.1210/en.2003-0502. [PubMed: 12959989]

81. Girgis CM, Clifton-Bligh RJ, Mokbel N, Cheng K, Gunton JE. Vitamin D signaling regulates proliferation, differentiation and myotube size in C2C12 skeletal muscle cells. Endocrinology. 2014; 155(2):347–57. doi:10.1210/en.2013-1205. [PubMed: 24280059]

82. Lee SG, Lee YH, Kim KJ, Lee W, Kwon OH, Kim JH. Additive association of vitamin D insufficiency and sarcopenia with low femoral bone mineral density in noninstitutionalized elderly population: the Korea National Health and Nutrition Examination Surveys 2009-2010. Osteoporos Int. 2013; 24(11):2789–99. doi:10.1007/s00198-013-2378-6. [PubMed: 23652463]

83. Snijder MB, van Schoor NM, Pluijm SM, van Dam RM, Visser M, Lips P. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. J Clin Endocrinol Metab. 2006; 91(8):2980–5. doi:10.1210/jc.2006-0510. [PubMed: 16684818]

84. Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res. 2004; 19(2):265–9. doi:10.1359/jbmr.2004.19.2.265. [PubMed: 14969396]

85. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA. 2005; 293(18):2257–64. doi:10.1001/jama.293.18.2257. [PubMed: 15886381]

86. Broe KE, Chen TC, Weinberg J, Bischoff-Ferrari HA, Holick MF, Kiel DP. A higher dose of vitamin d reduces the risk of falls in nursing home residents: a randomized, multiple-dose study. J Am Geriatr Soc. 2007; 55(2):234–9. doi:10.1111/j.1532-5415.2007.01048.x. [PubMed: 17302660]

87. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, et al. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med. 2006; 354(7):669–83. doi:10.1056/NEJMoa055218. [PubMed: 16481635]

88. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. 2013 doi:10.1016/S0140-6736(13)61647-5.

89•. Ceglia L, Niramitmahapanya S, Morais MD, Rivas DA, Harris SS, Bischoff-Ferrari H, et al. A randomized study on the effect of vitamin D3 supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. J Clin Endocrinol Metab. 2013 doi: 10.1210/jc.2013-2820. This RCT of 21 older women showed an increase in myonuclear VDR and muscle fiber size amongst those receiving vitamin D supplementation (4000 IU d for 4 months). However, functional parameters were unchanged, possibly due to the small sample size.

90•. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010; 303(18):1815–22. doi:10.1001/jama.2010.594. [PubMed: 20460620] This well-known RCT of 2256 community-dwelling older women reported an increased risk of falls and fractures in the 3 months following vitamin D mega-supplementation (500,000 IU oral).

91•. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011; 96(1):53–8. doi:10.1210/jc.2010-2704. [PubMed: 21118827] This article summarizes the IOM recommendations for 25OHD target levels (50 nmol/L) and daily vitamin D doses (800 IU in adults >70years). Extra-skeletal benefits of vitamin D were reportedly “not yet compelling”.

92•. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011; 96(7):1911–30. doi:10.1210/jc.2011-0385. [PubMed: 21646368] In contrast to the IOM recommendations, the Endocrine society advocated a higher 25OHD target level of 75 nmol/L and daily doses of at least 1500-2000 IU in the older age-group.

93. Korpelainen R, Keinanen-Kiukaanniemi S, Heikkinen J, Vaananen K, Korpelainen J. Effect of impact exercise on bone mineral density in elderly women with low BMD: a population-based randomized controlled 30-month intervention. Osteoporos Int. 2006; 17(1):109–18. doi:10.1007/s00198-005-1924-2. [PubMed: 15889312]

94. Martyn-St James M, Carroll S. Meta-analysis of walking for preservation of bone mineral density in postmenopausal women. Bone. 2008; 43(3):521–31. doi:10.1016/j.bone.2008.05.012. [PubMed: 18602880]

95. Winters-Stone KM, Dobek J, Nail L, Bennett JA, Leo MC, Naik A, et al. Strength training stops bone loss and builds muscle in postmenopausal breast cancer survivors: a randomized, controlled trial. Breast Cancer Res Treat. 2011; 127(2):447–56. doi:10.1007/s10549-011-1444-z. [PubMed: 21424279]

96. Winters KM, Snow CM. Detraining reverses positive effects of exercise on the musculoskeletal system in premenopausal women. J Bone Miner Res. 2000; 15(12):2495–503. doi:10.1359/jbmr.2000.15.12.2495. [PubMed: 11127215]

97. Gilsanz V, Wren TA, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculo-skeletal development of young women with low BMD. J Bone Miner Res. 2006; 21(9):1464–74. doi:10.1359/jbmr.060612. [PubMed: 16939405]

98. Dudley-Javoroski S, Shields RK. Muscle and bone plasticity after spinal cord injury: review of adaptations to disuse and to electrical muscle stimulation. J Rehabil Res Dev. 2008; 45(2):283–96. [PubMed: 18566946]

99. Serrano-Urrea R, Garcia-Meseguer MJ. Malnutrition in an elderly population without cognitive impairment living in nursing homes in Spain: study of prevalence using the Mini Nutritional Assessment test. Gerontology. 2013; 59(6):490–8. doi:10.1159/000351763. [PubMed: 23949114]

100. Berner LA, Becker G, Wise M, Doi J. Characterization of dietary protein among older adults in the United States: amount, animal sources, and meal patterns. J Acad Nutr Diet. 2013; 113(6):809–15. doi:10.1016/j.jand.2013.01.014. [PubMed: 23491327]

101. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008; 87(1):150–5. [PubMed: 18175749]

102. Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. Curr Opin Clin Nutr Metab Care. 2009; 12(1):86–90. doi:10.1097/MCO.0b013e32831cef8b. [PubMed: 19057193]

103. Milne AC, Potter J, Vivanti A, Avenell A. Protein and energy supplementation in elderly people at risk from malnutrition. Cochrane Database Syst Rev. 2009; (2):CD003288. doi:10.1002/14651858.CD003288.pub3. [PubMed: 19370584]

104. Miller CT, Fraser SF, Levinger I, Straznicky NE, Dixon JB, Reynolds J, et al. The effects of exercise training in addition to energy restriction on functional capacities and body composition in obese adults during weight loss: a systematic review. PLoS One. 2013; 8(11):e81692. doi:10.1371/journal.pone.0081692. [PubMed: 24409219]

105. Buehring B, Binkley N. Myostatin - the holy grail for muscle, bone, and fat? Curr Osteoporos Rep. 2013; 11(4):407–14. doi:10.1007/s11914-013-0160-5. [PubMed: 24072591]

106. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet. 2007; 370(9588):657–66. doi:10.1016/S0140-6736(07)61342-7. [PubMed: 17720017]

107. Radford LT, Bolland MJ, Mason B, Horne A, Gamble GD, Grey A, et al. The Auckland calcium study: 5-year post-trial follow-up. Osteoporos Int. 2013 doi:10.1007/s00198-013-2526-z.

108. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature. 1997; 387(6628):83–90. doi:10.1038/387083a0. [PubMed: 9139826]

109. Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, et al. Myostatin mutation associated with gross muscle hypertrophy in a child. N Engl J Med. 2004; 350(26):2682–8. doi:10.1056/NEJMoa040933. [PubMed: 15215484]

110. Han DS, Chen YM, Lin SY, Chang HH, Huang TM, Chi YC, et al. Serum myostatin levels and grip strength in normal subjects and patients on maintenance haemodialysis. Clin Endocrinol. 2011; 75(6):857–63. doi:10.1111/j.1365-2265.2011.04120.x.

111. Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S, et al. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. Proc Natl Acad Sci U S A. 1998; 95(25):14938–43. [PubMed: 9843994]

112. Ju CR, Chen RC. Serum myostatin levels and skeletal muscle wasting in chronic obstructive pulmonary disease. Respir Med. 2012; 106(1):102–8. doi:10.1016/j.rmed.2011.07.016. [PubMed: 21840694]

113. Elliott B, Renshaw D, Getting S, Mackenzie R. The central role of myostatin in skeletal muscle and whole body homeostasis. Acta Physiol. 2012; 205(3):324–40. doi:10.1111/j.1748-1716.2012.02423.x.

114. Tsuchida K, Nakatani M, Hitachi K, Uezumi A, Sunada Y, Ageta H, et al. Activin signaling as an emerging target for therapeutic interventions. Cell Commun Signal. 2009; 7:15. doi:10.1186/1478-811X-7-15. [PubMed: 19538713]

115. Zhang ZL, He JW, Qin YJ, Hu YQ, Li M, Zhang H, et al. Association between myostatin gene polymorphisms and peak BMD variation in Chinese nuclear families. Osteoporos Int. 2008; 19(1):39–47. doi:10.1007/s00198-007-0435-8. [PubMed: 17703271]

116. Hamrick MW. Increased bone mineral density in the femora of GDF8 knockout mice. Anat Rec A Discov Mol Cell Evol Biol. 2003; 272(1):388–91. doi:10.1002/ar.a.10044. [PubMed: 12704695]

117. Kellum E, Starr H, Arounleut P, Immel D, Fulzele S, Wenger K, et al. Myostatin (GDF-8) deficiency increases fracture callus size, Sox-5 expression, and callus bone volume. Bone. 2009; 44(1):17–23. doi:10.1016/j.bone.2008.08.126. [PubMed: 18852073]

118•. Digirolamo DSV, Clemens T, Lee S-J. Systemic administration of soluble 483 activin receptors produces differential anabolic effects in muscle and bone in mice. J Bone Miner Res. 2011; 1167(Suppl.) This abstract presented at ASBMR meeting 2011 reported direct effects of activin receptor signaling in osteoblasts. Mice treated with ActRII-Fc fusion proteins showed both increases in muscle (15%–40%) and trabecular (~13%) bone over 4 weeks.

119•. Bowser M, Herberg S, Arounleut P, Shi X, Fulzele S, Hill WD, et al. Effects of the activin A-myostatin-follistatin system on aging bone and muscle progenitor cells. Exp Gerontol. 2013; 48(2):290–7. doi:10.1016/j.exger.2012.11.004. [PubMed: 23178301] This study examined age-related differences in the expression and activity of myostatin, activin A, and follistatin in mice. Myostatin was particularly important in the impaired proliferative capacity of aged muscle and bone cells, making it an appropriate therapeutic target in treating sarcopenia-osteoporosis.

120. Koncarevic A, Cornwall-Brady M, Pullen A, Davies M, Sako D, Liu J, et al. A soluble activin receptor type IIb prevents the effects of androgen deprivation on body composition and bone health. Endocrinology. 2010; 151(9):4289–300. doi:10.1210/en.2010-0134. [PubMed: 20573726]

121. Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, et al. Functional improvement of dystrophic muscle by myostatin blockade. Nature. 2002; 420(6914):418–21. doi: 10.1038/nature01154. [PubMed: 12459784]

122. Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, et al. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. Cell. 2010; 142(4):531–43. doi: 10.1016/j.cell.2010.07.011. [PubMed: 20723755]

123•. Chiu CS, Peekhaus N, Weber H, Adamski S, Murray EM, Zhang HZ, et al. Increased muscle force production and bone mineral density in ActRIIB-Fc-treated mature rodents. J Gerontol A Biol Sci Med Sci. 2013; 68(10):1181–92. doi:10.1093/gerona/glt030. [PubMed: 23525481] Aged mice treated with ActRIIB-Fc fusion protein showed enhanced muscle size and function after 3 days. Similar features were observed in hypogonadal male mice (ie, orchidectomized) and improvements in bone mineral density were also reported.

124. Lotinun S, Pearsall RS, Davies MV, Marvell TH, Monnell TE, Ucran J, et al. A soluble activin receptor Type IIA fusion protein (ACE-011) increases bone mass via a dual anabolic-antiresorptive effect in Cynomolgus monkeys. Bone. 2010; 46(4):1082–8. doi:10.1016/j.bone. 2010.01.370. [PubMed: 20080223]

125•. Arounleut P, Bialek P, Liang LF, Upadhyay S, Fulzele S, Johnson M, et al. A myostatin inhibitor (propeptide-Fc) increases muscle mass and muscle fiber size in aged mice but does not increase bone density or bone strength. Exp Gerontol. 2013; 48(9):898–904. doi:10.1016/j.exger. 2013.06.004. [PubMed: 23832079] Aged mice injected with a myostatin inhibitor (propeptide-Fc) showed increases in muscle mass but not bone volume. This raised questions about the precise role of myostatin signaling in bone density in aged animals.

126. Wagner KR, Fleckenstein JL, Amato AA, Barohn RJ, Bushby K, Escolar DM, et al. A phase I/IItrial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol. 2008; 63(5):561–71. doi:10.1002/ana.21338. [PubMed: 18335515]

127. Krivickas LS, Walsh R, Amato AA. Single muscle fiber contractile properties in adults with muscular dystrophy treated with MYO-029. Muscle Nerve. 2009; 39(1):3–9. doi:10.1002/mus. 21200. [PubMed: 19086063]

128•. Attie KM, Borgstein NG, Yang Y, Condon CH, Wilson DM, Pearsall AE, et al. A single ascending-dose study of muscle regulator ACE-031 in healthy volunteers. Muscle Nerve. 2013; 47(3):416–23. doi:10.1002/mus.23539. [PubMed: 23169607] This RCT reported increases in muscle mass and serum markers of bone formation in healthy postmenopausal women receiving a single dose of ACE-031, a soluble ActRIIB decoy receptor. Although side-effects were minor —nose bleeds, skin telangiectasia and transient drop in FSH levels—they highlight an incomplete understanding of systemic effects of activin signaling.

129•. Lach-Trifilieff E, Minetti GC, Sheppard K, Ibebunjo C, Feige JN, Hartmann S, et al. An antibody blocking Activin Type II Receptor induces strong skeletal muscle hypertrophy and protects from atrophy. Mol Cell Biol. 2014; 34(4):606–18. doi:10.1128/MCB.01307-13. [PubMed: 24298022] Mice injected with a novel anti ActRII antibody (BYM338) had greater increases in muscle mass than mice receiving a myostatin inhibitor alone. This agent also protected muscles from glucocorticoid-induced atrophy and has potential therapeutic implications.

130. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med. 2011; 17(11):1481–9. doi:10.1038/nm.2513. [PubMed: 22037645]