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Journal

Clinical and Experimental Pharmacology and Physiology, 42(1)

ISSN

0305-1870

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Publication Date

2015

DOI

10.1111/1440-1681.12320

Peer reviewed

HHS Public Access

Author manuscript

Clin Exp Pharmacol Physiol. Author manuscript; available in PMC 2015 April 02.

Published in final edited form as:

Clin Exp Pharmacol Physiol. 2015 January ; 42(1): 1–13. doi:10.1111/1440-1681.12320.

Pharmacology of manipulating lean body mass

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Summary

1. Dysfunction and wasting of skeletal muscle as a consequence of illness decreases the length and quality of life. Currently, there are few, if any, effective treatments available to address these conditions. Hence, the existence of this unmet medical need has fuelled large scientific efforts.
2. Fortunately, these efforts have shown many of the underlying mechanisms adversely affecting skeletal muscle health.
3. With increased understanding have come breakthrough disease-specific and broad spectrum interventions, some progressing through clinical development.
4. The present review focuses its attention on the role of the antagonistic process regulating skeletal muscle mass before branching into prospective promising therapeutic targets and interventions. Special attention is given to therapies in development against cancer cachexia and Duchenne muscular dystrophy before closing remarks on design and conceptualization of future therapies are presented to the reader.

Keywords

atrophy; cachexia; Duchenne muscular dystrophy; exercise; hypertrophy; regeneration; skeletal muscle

Introduction

Skeletal muscle accounts for nearly half of the mass of the human body. It is not surprising then that this tissue plays a fundamental role in human health and well-being. Skeletal muscle is not only required for locomotion, but it plays an important role in many metabolic pathways and directly impacts our overall quality of life.[1,2] In adults, interventions promoting an increase in muscle mass (hypertrophy) and strength are generally associated

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with positive health consequences, such as delaying the onset and reducing the severity of disease.[3] For these reasons, an increase in muscle size and strength is associated with an increase in longevity.[4] By contrast, skeletal muscle loss (atrophy) is often a reflection of the seriousness of a disease, and has a negative impact on quality of life and the ability to respond to and/or recover from disease.[5–8]

Diseases affecting the maintenance of lean body mass most often occur as muscle loss or wasting conditions. The medical severity of this muscle atrophy will often depend on health status, sex and the type of muscle affected.[9,10] Although most forms of muscle wasting share similar gross phenotypic similarities, the molecular events that lead to atrophy can vary, and these differences are important to consider when designing new therapeutic interventions.[11,12] Outlined here are some of the major muscle disorders where pharmacological interventions are common or desired. The objective of the present review was to show the overlapping threads that tie these interventions together, and to illustrate where synergistic pharmacologies might lead to new treatment paradigms or where new interventions might add value.

Maintenance of Skeletal Muscle: Negative Factors Effecting the Balance

Muscle loss occurs when the equilibrium between myofibrillar protein synthesis and degradation is perturbed (Fig. 1). This occurs in conditions where the protein synthesis machinery fails, either by failing to keep up with the rate at which proteins are being degraded or by making unstable/malformed proteins. A negative balance exists, tilting this balance towards degradation, in genetic neuromuscular disorders, sepsis, fasting, disuse, paralysis and ageing.[13–21] Excessive breakdown of proteins can also occur as an unwanted consequence of therapeutic treatments, such as corticosteroid administration in intensive care, in burn victims and after treatment with lipid-lowering drugs.[22–26] The general consensus is that the increase in degradation involves one or more of the proteolytic pathways: the ubiquitin proteasome pathway, autophagy, the lysosomal pathway, calpains and/or the caspase (or apoptotic) protease pathway.[14,27–29] It is currently unclear whether every proteolytic pathway is involved in the breakdown of muscular proteins. However, it is clear that multiple factors contribute to accelerated muscle loss. More in depth discussion of these disorders is presented here.

Genetic disorders

Inherited myopathies are a large group of deleterious conditions leading to progressive muscle loss, weakness and a shorter lifespan.[30] The most common myopathy, Duchenne muscular dystrophy (DMD), occurs as a result of aberrant expression (or lack) of dystrophin.[31] In the absence of dystrophin, the muscle becomes more prone to damage, and as a result shows a large increase in the rate of muscle protein degradation that cannot be compensated for by simply increasing de novo protein synthesis. Potential therapeutic developments against DMD have focused on rectifying the gene mutation, enhancing muscle function and supressing the resulting inflammatory response that leads to necrosis and fibrosis.

Cachexia

Cachexia is the loss of muscle mass occurring from increased circulation of muscle-wasting factors as a result of disease. Cachexia occurs in some types of cancer, uraemia and stroptozotocin-induced diabetes.[32] Cachexia also results from chronic heart failure, COPD or after the administration of corticosteroids or blood pressure-lowering medications.[26,33–37] Although there is some evidence that muscle protein synthesis is depressed in patients with cachexia, increases in the ubiquitinatin proteasome pathway and autophagy resulting in an increase in the rate of protein degradation also play an important role in this form of muscle loss.[38,39] A hallmark of cachexia is that the loss of lean body mass cannot be prevented or reversed simply by increasing nutritional intake.[40]

Sarcopenia

The decline in muscle mass and decrease in muscle function as a consequence of age in the absence of disease is known as sarcopenia.[41–43] The rate of protein breakdown in muscles of individuals with sarcopenia is the same as that of young controls, suggesting that a deficit in protein synthesis is the basis for age-associated muscle loss.[44] In support of this hypothesis, sarcopenia is characterized by decreased myogenic (anabolic) markers at rest, and on load, increased production of oxidative species, decreased anti-oxidant levels and reduced capacity to activate satellite cells.[43, 45,46] This loss in skeletal muscle plasticity occurs concomitantly with an impaired ability to increase protein synthesis in response to exercise or nutrition.[47–49]

Non-degenerative conditions of muscle wasting

There are several non-degenerative inducers of muscle wasting. These include, malnutrition, inactivity, injury or trauma to nerves (denervation) and/or tendons.[50] Loss of innervation leading to paralysis can occur as a result of damage or death of the motor unit, sectioning of the nerve, or disruption between acetylcholine and its receptor (such as a result of post-synaptic block or neuromuscular blockade while in intensive care unit).[18,51] Experimentally, loss of innervation is often used to model the progression of neuromuscular diseases such as amyotrophic lateral sclerosis.[52–54] In denervated muscles, early atrophy results from an increase in degradation in the absence of changes in protein synthesis.[55] However, after prolonged denervation, there is an increase in protein synthesis that partially preserves muscle mass, suggesting that in some cases compensatory mechanisms exist to maintain muscle in the absence of proper signals (see below).[55,56] In contrast to denervation, tendon trauma or forced inactivity (bedrest) leads to the mechanical unloading of a muscle while still retaining the intact nerve that supplies both electrical activity and trophic factors.[50] As a result, the muscle atrophy is less severe in humans, and is largely the result of decreases in the rate of muscle protein synthesis.[57]

Signalling Underlying the Negative Control of Muscle Mass

Growth arrest and DNA damage-inducible 45 signalling

As aforementioned, both fasting and denervation lead to rapid muscle atrophy. Even though these are very different physiological insults, they share common molecular responses. Both denervation and fasting induce growth arrest and DNA damage-inducible 45 (Gadd45), a

small nuclear protein that alters the transcriptional profile of skeletal muscle towards protein degradation and away from protein synthesis.[11,58,19] Gadd45 increases and is required for normal muscle atrophy after fasting, immobilization and denervation, suggesting that Gadd45 is central to acute *in vivo* skeletal muscle atrophy.[59] In the case of fasting, the increase in Gadd45 is driven by activating transcription factor (ATF) 4.[58] Mice that lack a functional ATF4 do not increase Gadd45, and suffer much less atrophy than control mice when fasted.[58] However, ATF4 knockout mice still activate Gadd45 and atrophy normally after denervation.[11] In denervation, the increase in Gadd45 is therefore not the result of an increase in ATF4, but rather results from the upregulation of the class II histone deacetylases, specifically HDAC4.[11] At present, how immobilization induces Gadd45 and whether Gadd45 is involved in sarcopenia or cachexia has yet to be shown. However, the importance of Gadd45 in skeletal muscle atrophy suggests that Gadd45 is a potential pharmaceutical target for preventing muscle loss.

Tumour necrosis factor superfamily 12 signalling

Cytokines, and in particular tumour necrosis factor (TNF) superfamily 12 (TWEAK), have been shown to play a key role in accelerating the breakdown of skeletal muscle proteins during inflammatory conditions, such as cancer cachexia and chronic heart disease.[60,61] TWEAK signals through nuclear factor kappa B (NF-κB) – a transcription factor involved in immune, inflammatory and cell survival responses that is heavily associated with protein degradation.[62–65] Absence of TWEAK is linked to a slight decrease in muscle cross-sectional area and a decrease in proteasome activity, improved skeletal muscle regeneration, and protection against denervation-mediated muscle wasting in mice.[66,67] Accordingly, deletion of NF-κB has shown to increase muscle mass, force (in fast oxidative muscle fibres), protect against atrophy and enhance muscle regeneration.[68] It is possible that the benefits observed in muscle through inhibition of this pathway are a result of activation of mammalian target of rapamycin (mTOR) through the Akt/growth factor pathway and/or decreased levels of ubiquitin ligases targeting muscle proteins.[69,70] The recent discovery that NF-κB controls the transcription of the muscle-specific E3 ligase, MuRF1, suggests that TWEAK likely drives atrophy through the activation of degradation downstream of NF-κB.[71]

Maintenance of Skeletal Muscle: Positive Factors Affecting the Balance

Muscle mass gains occur during developmental growth, in response to growth factors, diet and exercise.[72–74] As with muscle atrophy, muscle hypertrophy is the result of a change in the net balance between protein synthesis (anabolism) and degradation (catabolism).[63,75–81] However, it has become clear over the past few years that muscle hypertrophy and atrophy are not identical processes in reverse. Even so, improved understanding of the biology of growth has led to diverse approaches for the positive regulation of muscle mass.[82,83] These interventions have been designed to mimic, amplify or block a subset of signalling pathways implicated in muscle growth/wasting, and could in turn impact on hundreds, if not thousands, of muscle remodelling genes/gene regulators.[18,32,40,63,84–90]

Signalling underlying the positive control of muscle mass

Over the last twenty years we have begun to understand the molecular mechanisms underlying the control of skeletal muscle mass development. Some of these are generalized pathways, molecular events that are required for any cell to grow, whereas others appear to be specific for controlling the size of skeletal muscle, independent of other tissues in the body. Therapeutically, it is the muscle-specific events that are the most attractive as a way to decrease side effects of any treatment. However, if more generalized growth pathways can be targeted to muscle this could make them valuable tools in treating muscle diseases. Below, we will briefly describe some of the known pathways that control muscle size in the adult.

Mammalian target of rapamycin pathway

Activation of mTOR is one of the key events involved in muscle growth. mTOR can be activated by: (i) growth factors, through the PI-3kinase/Akt pathway;[91] (ii) mechanical loading, through the removal of the inhibitor TSC2 from the mTOR/Rheb complex;[92] and (iii) feeding, through the GATOR/Rag/Ragulator pathway.[93,94] In this way, mTOR can directly control muscle growth by integrating hormonal, nutritional and loading cues. mTOR activation after exercise correlates with muscle hypertrophy in both rodents and humans.[94,96] Furthermore, when mTOR is specifically blocked by the bacterial macrolide, rapamycin, there is no acute rise in muscle protein synthesis after exercise or feeding, and muscle hypertrophy is prevented after overload.[97–99] Taken together, these data suggest that mTOR is required for the acute response of muscle to feeding and exercise, and is important in the regulation of muscle protein synthesis.

The activation of mTOR directly increases the rate of protein synthesis, and stimulates muscle hypertrophy through phosphorylation of protein 70 S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) also known as PHAS-1.[63,78] Additionally, phosphorylated 4E-BP1 can exert a positive feedback loop by binding to the regulatory associated protein of mTOR (raptor) and further activate mTOR.[100] Phosphorylation of both p70S6K and 4E-BP1 are common readouts of anabolic processes.[101] Activation of this pathway as a means to enhance muscle function has been the focus of intense efforts. For example, chemical activation of Akt has shown to protect against sepsis in mice.[102] Also, exogenous delivery of recombinant human insulin-like growth factor-1 (IGF-1; an upstream activator of Akt) improves muscle function in dystrophic rodents and enhances the benefits of gene therapy.[103,104] IGF-1 treatment seems safe (without side effects humans), and has the potential to improve body composition and metabolism in dystrophic patients.[105–107] Unfortunately, there are reasons to believe that stimulation of Akt through IGF-1 will not produce substantial functional improvements.[73]

Transforming growth factor-β signalling pathway

The transforming growth factor-β (TGF-β) signalling pathway is pivotal in controlling developmental muscle growth. The TGF-β family of ligands that activate this pathway, such as myostatin, TGF-β, bone morphogenic proteins (BMP) or Activin, act through a series of receptors and effector small mother of decapentaplegic (Smad) proteins to regulate hundreds of gene targets, many of which are involved in muscle growth and wasting.[108–110] The TGF-

β family ligands bind to type II and type I transmembrane serine/threonine kinase receptors in the membrane of the muscle cell to exert biological action, predominantly through the Smad2 and Smad3 (Smad2/3) or Smad1, Smad5 or Smad8 (Smad1/5/8) axis (Fig. 2). [111]

The most cited example of Smad2/3 modulation of muscle mass is myostatin, or its absence, which was first found as being responsible of the hypermuscular phenotype of the Belgium Blue cattle.[112] Myostatin is an important factor in cellular differentiation and cell growth inhibition during wasting conditions.[113–115] The inhibition of the TGF-β pathway and positive regulation of muscle growth can occur extracellularly by limiting ligand activity/availability through antibodies, ligand antagonists such as Gasp-1, Myostatin pro-peptides, antisense nucleotides, dominant negative receptor competitors, soluble receptor inhibitors, the BMP Activin membrane-bound inhibitor (Bambi) and Follistatin (Fst).[116–126] Inhibition of the TGF-β pathway can also occur intracellularly by limiting ligand production through molecular interruption of protein production, inhibition of Smad signalling by SKI, release of Notch from the plasma membrane or competitive activation of Smad1/5/8.[121,127–131] In accordance, the absence or direct or indirect inhibition of myostatin produces muscle growth, and improves (in rodents) muscle size and strength in diseases such as Duchenne Muscular Dystrophy, amyotrophic lateral sclerosis and cachexia.[132–138] It is also believed that inhibition of myostatin could increase the success of myoblast transplantation.[134,139]

PGC-1α signalling

Recently, a potential role for the mitochondrial transcriptional cofactor the peroxisome proliferating activated receptor co-activator (PGC-1α) has been suggested.[140] In these experiments, high load caused PGC-1α to be expressed from a different promoter resulting in the production of a novel mRNA that has been termed PGC-1α4. However, almost as quickly as the discovery of PGC-1α4 and its potential role in muscle growth came a report that showed that muscle growth occurred completely normally in the PGC-1α knockout mice who are unable to express PGC-1α4.[141] It is still possible that PGC-1α4 plays a role in the regulation of developmental, but not load-induced, muscle growth through the regulation of either myostatin signaling or a G-protein coupled receptor. However, this remains to be conclusively shown.

G-protein signalling

The hypertrophic potential of β2 agonists has been known for decades.[142] However, the potential of multiple different G-protein activators to produce skeletal muscle hypertrophy suggests that this is a more generalized phenomenon. G-protein activation by Wnt, ghrelin, lysophosphatidic acid, as well as clenbuterol, fenoterol and formoterol, can increase muscle mass.[142–146] Interestingly, this effect is dependent on mTOR, as inactivation of mTOR by rapamycin prevents G-protein coupled muscle hypertrophy, suggesting that there are other therapeutically relevant ways to activate mTOR within muscle.[143,147] One of the clinical drawbacks to this family of therapeutics has been that the current β2 agonists produce cardiac hypertrophy as well as skeletal muscle hypertrophy.[148] However, the discovery of a skeletal muscle specific G-protein coupled receptor (possibly the receptor of Wnt7a) has the potential to revolutionize muscle therapeutics.

Potential Interventions Against Muscle Wasting Conditions

There is a large unmet medical need for an intervention that maintains or re-establishes skeletal muscle mass and function. Current treatments to reduce the loss of muscle mass, such as nutritional supplementation (providing increased calorie intake and special amino acid compositions), hormonal treatment (growth hormone, testosterone, IGF-1 and insulin) and anti-inflammatories, have been largely ineffective or are associated with severe side-effects.[5] Although it would be more cost-effective to develop a 'one for all' treatment than targeting each disease independently, it is unlikely that a single intervention will have the desired therapeutic effect across the variety of conditions associated with muscle atrophy. For these reasons, the following section highlights a selection of targets that in the opinion of the authors are likely to advance through clinical development.

Tgf-β Signalling Pathway

There has been an immense amount of attention devoted to developing strategies to inhibit the TGF-β family ligands, as it largely targets skeletal muscle and holds immense promise as a therapeutic target. As mentioned earlier, these interventions vary from a compound approach, to other approaches, such as the delivery of antibodies against myostatin in cancer patients (phase II trial by Eli Lilly, clinicaltrials.gov identifier: NCT01505530), delivery of pro-peptides that can bind to and block myostatin in solution or by transient DNA expression that elicits muscle growth and improves muscle function.[116,149–151] Encouraging results have also been produced from delivering short interfering hairpin RNA against myostatin, showing increased muscle mass in rodents. Other alternatives to manipulate this pathway are the inhibition of Activin receptor type IIB (ActRIIB) through rAAV delivery of a dominant negative receptor or antibody-targeted receptor inhibitors.[139,152,153] It is well known that Fst and Fst-related proteins can promote hypertrophy in the skeletal muscle of rodents and non-human primates, so it is not surprising that a clinical trial is currently underway to test the potential of Fst in patients with Becker muscular dystrophy (BMD) and sporadic inclusive myositis (NCT01519349).[134,154–157] In addition, Acceleron Pharma/Shire developed ACE-031, a ActRIIB receptor chimera with strong capacity to decrease body fat, increase muscle mass and improve insulin sensitivity.[136,158,159] However, ACE-031 has been discontinued given that a phase II clinical trial raised safety concerns (NCT01099761). Although the safety concerns about ACE-031 have not been published, it is possible that this molecule had off-target effects, given its lack of target tissue specificity.

Another strategy for TGF-β signalling pathway inhibition is the treatment with halofuginone hydrobromide, which in animals and humans, acts as a potent inhibitor of the synthesis of collagen type I at the transcriptional level by inhibiting Smad3 phosphorylation, resulting in ubiquituous reduction of fibrosis and collagen synthesis.[160,160] Importantly, when administered at intended doses, halofuginone hydrobromide has a minimal effect on collagen content in non-fibrotic tissues.[162–165] Halofuginone hydrobromide also produces anti-inflammatory and antifibrotic effects by inhibiting activated peripheral blood T-cell function and pro-inflammatory cytokine production, including interferon-γ and TNF-α, through inhibition of NF-κB activation and p38 mitogen-activated protein kinase (MAPK) in a dose-dependent manner.[160] Finally, halofuginone hydrobromide has recently been

shown to enhance myotube fusion by stimulating serine/threonine protein kinase Akt, MAPK/extracellular- signal-related kinase and MAPK phosphorylation, and inhibiting Smad3 phosphorylation in myotubes, all of which are crucial for myotube formation.[166]

Recently, the protagonist role of the Smad2/3 axis has been challenged by two reports showing that the Smad1/5/8 axis can modulate Smad2/3 activity, and might even have a dominant role over Smad2/3. Almost simultaneously, Sartori *et al.* and Winbanks *et al.* reported that the Smad1/5/8 axis of the TGF-β pathway can promote muscle growth and override Smad2/3 signalling in mice (Fig. 2).[56,167] Using transgenic mice models, Sartori *et al.* showed that phosphorylated Smad2/3 forms a complex with Smad4 to accelerate muscle mass loss, whereas the opposite occurred on its release from the Smad2/3 complex and association with Smad1/5/8 (Fig. 2). Consequently, inhibition of Smad1/5/8 increased denervation-mediated muscle wasting, and overactivation of Smad1/5/8 inhibited myostatin-mediated muscle growth independent of IGF-1 signalling. Winbanks *et al.* used adeno-associated viral vectors (AAV) to activate this pathway, and promote growth in adult mice. AAV-mediated overexpression of the ligand, BMP7, or introduction of a constitutively active ALK3 (a subunit of the BMP receptor) was sufficient to promote muscle growth. Additionally, AAV-mediated upregulation of the naturally occurring Smad1/5/8 inhibitor, Smad6, blocked Fst-mediated muscle growth. Together, these findings highlight the importance of testing the potential of Smad1/5/8-activating interventions for reversal of atrophy and frailty.

Selective Androgen Receptor Modulators

Selective androgen receptor modulators (SARM) are a class of pharmaceuticals intended for oral delivery that offer to stimulate the normal androgen response, but do so with tissue selectivity; that is, to stimulate a normal testosterone-like response in those tissues in need of the targeted effect, but avoiding those tissues where the testosterone effect would be considered adverse. The recent examples in this field are targeting skeletal muscle (i.e. anabolic effects), but avoiding prostate gland (i.e. the androgenic effects). The ratios of activity in these organs that are used in the development SARM therapies are 3:1 to >10:1, indicating the drug has a three- or 10-fold greater effect on skeletal muscle than the prostate.[168,169]

There are many mechanisms by which testosterone and other androgens are believed to provide their effects, but increasingly for muscle mass increases the SARM are believed to act through ‘anticatabolic’ pathways (Fig. 1).[170,171] That is they operate not so much as stimulators of new protein synthesis, but rather through inhibiting the normal degradation rate of muscle protein.[168,169]

Personalized Therapies

The increased understanding on the underlying causes of genetic musculopathies have led to the development of targeted therapies that allow correction or replacement of the deficient gene. A good example is the dystrophin gene, which is mutated in DMD. The dystrophin gene comprises 79 exons, which are interspersed with non-coding introns. Deletions of one or more exons disrupts the synthesis of the dystrophin protein, which plays an essential role

in muscle fibre function. Dystrophinopathies result in progressive muscle fibre degeneration through normal degenerative and inflammatory processes leading to necrosis and fibrosis. The extent and nature of the mutations vary across different subpopulations of DMD patients, but the key factor of the disease is a disruption in the coding of mRNA for dystrophin. If the defective mRNA is 'out of frame', this results in a premature termination of the protein, leading to a truncated and non-functional dystrophin protein within their muscles.

Patients with BMD show intermediate to milder phenotypes with mostly longer to normal life expectancies when compared with DMD patients. Here, the mutations in the dystrophin gene maintain the open reading frame and result in an internally truncated, but semi-functional, protein. Therefore, the specific skipping of one or more exons flanking the deletion in a DMD patient might allow restoration of the mutated open reading frame, introduction of novel, BMD-like dystrophin and conversion of a severe DMD into a typically milder BMD phenotype. As such, the aim of exon-skipping therapies is restoration of coding reading frame so that a shorter, but still functional, dystrophin protein can be produced.

Although exon skipping is a mutation-specific treatment, representing personalized medicine, an important intrinsic advantage compared with conventional gene therapy is that it simultaneously corrects all dystrophin isoforms. It also maintains the original tissue-specific gene regulation. Furthermore, the antisense compounds that induce exon skipping are small, can be engineered synthetically, and are highly sequence-specific.[172,173]

Although the exon-skipping approach has shown promise and several groups have published positive results, the need for many different gene-skipping therapies to cover a wide array of patient mutations is very challenging and indeed some mutations will never be addressed by exon skipping. Analogously, any viral vector or DMD gene therapy will require adequate levels of long-term expression across multiple muscle types in order to be effective. The applicability of AAV has improved the development of this technology, and with more recent CRISPR technology this type of therapy is on the cusp of clinical trials to prove its utility.[174,175] For a recent review on this technology, see Jarmin *et al.* who made a comparison of the two approaches to gene therapy.[173]

Examples of New Therapy Development

Given the complexities of muscle homeostasis shown in Fig. 1, it would seem not only reasonable, but in fact required, that the optimal treatment of muscle loss conditions will involve multiple simultaneous therapeutic interventions. This is especially true for those conditions that arise from diseases originating from loss of protein as a result of genetic defects. As such, new drug developers should consider treatment paradigms that would include simultaneous interventions across multiple pathways as the ultimate end-points. As examples, two disease areas are summarized below.

DMD

Perhaps the best case in point is the treatment of DMD by prednisone. As aforementioned, DMD results from a genetic defect in the dystrophin gene, and is a serious condition leading to patient deaths in the late teens to 20s. The only known effective treatment of this disease is prednisone, a steroidal anti-inflammatory that has been show to act through multiple pathways including:

1. Inhibition of transcription of COX-2, cytokines, cell adhesion molecules and inducible nitric oxide synthase.
2. Blockage of vitamin D_3-mediated induction of osteocalcin gene in osteoblasts.
3. Modification of collagenase gene transcription.
4. Increased synthesis of annexin-1, an important protein for negative feedback to the hypothalamus and anterior pituitary gland.

Interestingly, more focused anti-inflammatories, such as NSAIDs, have not been very helpful in treating the disease and it is clear the multipharmacology nature of prednisone is responsible for its effectiveness in DMD. As such, although exon skipping, or other gene replacement therapies, might be part of the answer, it is clear that combinatorial therapies will also be required, even if it is only because of the fact that not all genetic mutations in DMD are treatable by exon skipping. Other promising treatments include the restoration of dystrophin through exogenous expression of the protein or parts of it, or upregulation or delivery of utrophin, an alternative gene that shares functions with dystrophin.[104,176–178] Complementary approaches include development of new anti-fibrotics, as well as a number of new, multifunction anti-inflammatories.[164] For an overview of the major programs in therapy development for DMD, see the website: http://tinyurl.com/ltkf2do. What should be clear from this overview is the wide diversity of therapies in development.

Cachexia

In addition to calorie supplementation, current therapeutic strategies for cachexia have been based on either blocking cytokine synthesis or action. Thalidomide has been shown to suppress TNF-α production in monocytes *in vitro* and to normalize elevated TNF-alpha levels *in vivo*.[179] A recent randomized, placebo-controlled trial in patients with cancer cachexia showed the drug was well tolerated and effective at attenuating loss of weight and lean body mass in patients with advanced pancreatic cancer.[180] An improvement in lean body mass and improved quality of life were also observed in a randomized, double-blind trial using a protein and energy-dense, omega-3 fatty acid-enriched oral supplement, provided that the concentration of eicosapentaenoic acid was equal to or greater than 2.2 g/day.[181] This was also thought to work through decreasing TNF-α production. However, recent data arising from a large, multicenter, double-blind, placebo-controlled trial showed eicosapentaenoic acid administration alone is not successful in the treatment of weight loss in patients with advanced gastrointestinal or lung cancer.[182] Peripheral muscle proteolysis, as it occurs in cancer cachexia, serves to mobilize amino acids required for the synthesis of liver and tumour proteins. Therefore, the administration of exogenous amino acids might theoretically serve as a muscle-sparing intervention by providing substrates for both muscle

metabolism and gluconeogenesis. Recent studies have shown dietary supplementation with a specific combination of high protein, leucine and fish oil improves muscle function, daily activity and the immune response in cachectic tumour-bearing mice.[183] In addition, when β-hydroxy-β-methylbutyrate, a metabolite derived from leucine catabolism, was used as a supplement in tumour-bearing rats, cachexia was prevented in part by modifying NF-κB expression.[184] Also, a recent phase II study involving the administration of anti-oxidants, pharmaconutritional support, progestogen (megestrol acetate and medroxyprogesterone acetate) and anticyclooxygenase-2 drugs, showed efficacy and safety in the treatment of patients suffering from cachexia as a result of advanced cancer. These data reinforce the use of the multitargeted therapies (nutritional supplementation, appetite stimulants and physical activity regimen) in the treatment of cancer cachexia.[184]

Not surprisingly, myostatin inhibition has also been shown to protect against cachexia-mediated atrophy and increased survival.[137,138,185] Leading this space is Novartis's Activin receptor A/B antibody in phase III clinical trials. In the same therapeutic space, another phase III clinical trial using the SARM category and the Enobosarm from GTX are most advanced.[186]

Opinion on Design and Conceptualization of Future Muscle Therapies

Strategic considerations

The ramifications of the polypharmaceutical nature of treating muscle wasting diseases have the following implications for drug developers:

1. Whenever two or more drugs are taken simultaneously, the potential exists for drug–drug interactions. As such, new drug development in this area should be especially focused on those compounds with reduced or absent ability to negatively interact with other drugs.
2. Combining multiple therapies into one pill has repeatedly proven to enhance patient compliance with a regimen and therefore improved efficacy measures. Unfortunately, for this to be effective, the medicines within a single tablet or capsule need to have similar pharmacokinetic properties that support concomitant administration (e.g. once a day dosing).
3. Drug discovery and development should explore combination therapies very early on in the program, and even co-develop therapies in order to hasten the development and increase the odds of success. Currently, the process, both from a pharmacological and a regulatory perspective, is focused on the preclinical and clinical development of one therapy at a time as single agents. This needs to change if we are going to expeditiously develop polypharmaceutical approaches to complex diseases.

Pharmacological aspect

From a pharmaceutical development perspective, drugs to treat diseases that alter muscle balance can be divided into three categories:

1. Therapies that treat the primary cause of the disease; that is, a cure. For some diseases, such as DMD, this means replacing or fixing the defective gene or gene product; for other diseases, such as cachexia, it means curing the primary cause, which is most often cancer or heart failure.
2. Therapies that do not cure the primary cause of the disease, but work to maintain or restore a normal balance of muscle protein synthesis and degradation, and as such maintain normal muscle function for longer periods of time.
3. Therapies that treat the symptoms of the disease, such as antifibrotics and anti-inflammatories, that reduce the progression of the muscle damage caused by the primary disease processes and thereby indirectly maintain normal muscle function for longer periods of time.

Because muscle cells are constantly renewing, full cures of muscle diseases are difficult to obtain (e.g. so far gene therapy approaches have shown improvements, but not really full reversal of the disease). Therefore, a reasonable strategy for developing therapies in the muscle disease therapeutic area would be to span all three categories.

Conclusions

Future therapies for non-genetic muscle wasting conditions should first identify how muscle balance is being disturbed: decreased synthesis or increased degradation (Fig. 1). Next, the intrinsic (loss of signalling within muscle) or extrinsic (increased inflammatory cytokines) cause of the imbalance needs to be identified. With this information, any muscle wasting disease can be attacked at multiple points: blocking the primary cause and promoting an anabolic response. For example, muscle wasting as a result of generalized inflammation (for example, after burn) would be treated with an anti-inflammatory together with an agent to decrease protein degradation within muscle, such as a SARM. Simultaneous treatment with agents to stimulate anabolic processes, from at simplest a leucine-rich protein diet to more complex products designed to target specific growth pathways, would maximize the recovery or maintenance of muscle mass and function.

As we move forward in the development of drugs that promote muscle anabolism, it is clear from the G-protein, mTOR and TGF-β sections in the present article, that these growth pathways have multiple points of intersection. It is therefore conceivable that novel drugs can be developed that specifically target these intersection points within skeletal muscle and activate multiple growth pathways simultaneously. The development of drugs that have this capacity is the ultimate goal in the battle to increase muscle protein synthesis. However, as should be clear from the discussion here, such a drug alone would have limited use in a disease whose primary feature is an increase in degradation.

In conclusion, there are many opportunities and targets for treating conditions that disrupt the normal balance of muscle mass. As described here, each disease is the result of its ability to alter both synthesis and degradation. Therefore, we need to develop combination or monotherapies that attack each condition with its own unique amalgam of anabolic and anticatabolic agents. Tailoring treatments in this manner will offer enhanced responses and greater efficacy.

Acknowledgments

KB was supported by a project grant from the National Institute on Ageing of the National Institutes of Health under award number R01AG045375. PS is a shareholder in Myostin Therapeutics. KB is a stakeholder in Advanced Muscle Technologies. EB is CSO and shareholder in Akashi Therapeutics.

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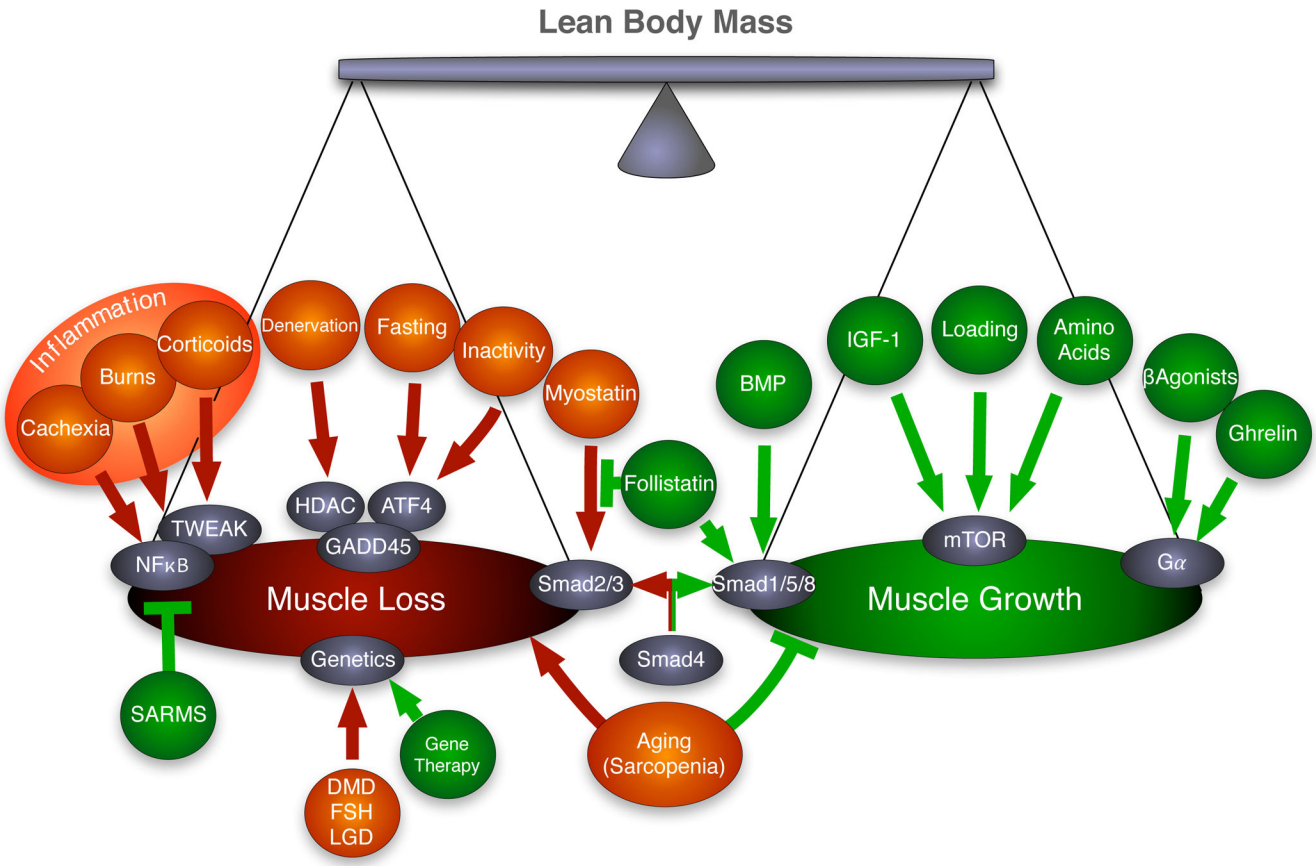

Fig. 1.

The balance between muscle growth and wasting in a variety of conditions. Muscle growth conditions largely signal through mammalian target of rapamycin (mTOR), whereas wasting has multiple triggers and molecular targets including nuclear factor kappa B (NF-κB), growth arrest and DNA damage-inducible 45 (Gadd45) and small mother of decapentaplegic (Smad)2/3. ATF4, activating transcription factor 4; DMD, Duchenne muscular dystrophy; Facioscapulohumeral Muscular Dystrophy (FSH); histone deacetylase (HDAC); Limb-girdle muscular dystrophy (LGD); SARM, selective androgen receptor modulators; TWEAK, tumour necrosis factor superfamily 12.

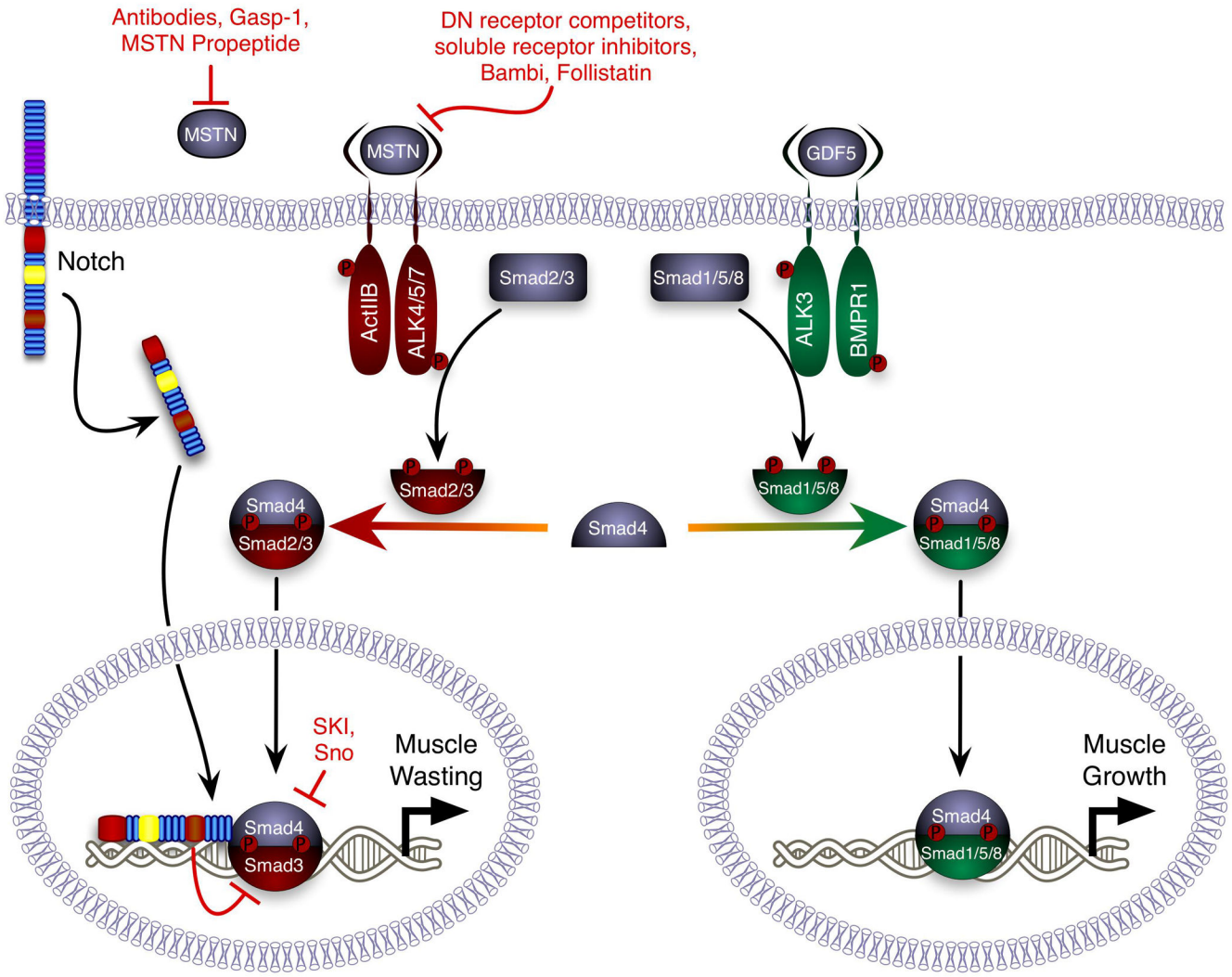

Fig. 2.

The interplay between multiple factors in the transforming growth factor-β (TGF-β) signalling pathway and the control of muscle mass. Myostatin and similar members of the TGF-β superfamily can activate small mother of decapentaplegic (Smad)2/3 and drive transcriptional events that result in the transcription of genes that limit muscle size. This can be prevented at a variety of drug/protein inhibitors (indicated in the red text and red bars). Smad2/3 signalling can also be blocked by growth and differentiation factor 5 (GDF5) and other members of the TGF-β superfamily that activate Smad1/5/8, and this competes for Smad4 binding and shifts transcription. Resistance exercise can also limit Smad2/3 signalling by activating Notch and blocking Smad2/3 transcription. bone morphogenetic protein receptor 1 (BMPR1); dominant negative (DN); and myostatin (MSTN).