Muscle wasting in disease: molecular mechanisms and promising therapies

Shenhav Cohen[1], James A. Nathan[2] and Alfred L. Goldberg[3]

Atrophy occurs in specific muscles with inactivity (for example, during plaster cast immobilization) or denervation (for example, in patients with spinal cord injuries). Muscle wasting occurs systemically in older people (a condition known as sarcopenia); as a physiological response to fasting or malnutrition; and in many diseases, including chronic obstructive pulmonary disorder, cancer-associated cachexia, diabetes, renal failure, cardiac failure, Cushing syndrome, sepsis, burns and trauma. The rapid loss of muscle mass and strength primarily results from excessive protein breakdown, which is often accompanied by reduced protein synthesis. This loss of muscle function can lead to reduced quality of life, increased morbidity and mortality. Exercise is the only accepted approach to prevent or slow atrophy. However, several promising therapeutic agents are in development, and major advances in our understanding of the cellular mechanisms that regulate the protein balance in muscle include the identification of several cytokines, particularly myostatin, and a common transcriptional programme that promotes muscle wasting. Here, we discuss these new insights and the rationally designed therapies that are emerging to combat muscle wasting.

Cachexia
Severe loss of body weight (especially muscle mass), with or without the loss of fat. Cachexia is associated with serious disease, in particular cancer.

[1]Faculty of Biology, Technion Institute of Technology, Haifa, 32000, Israel.
[2]Cambridge Institute for Medical Research, Department of Medicine, University of Cambridge, Cambridge, CB2 OXY, UK.
[3]Department of Cell Biology, 240 Longwood Ave., Harvard Medical School, Boston, Massachusetts 02115, USA.
Correspondence to A.L.G. or S.C.
e-mails: alfred_goldberg@hms.harvard.edu; shenhavc@tx.technion.ac.il

Muscle atrophy occurs in specific muscles with denervation or inactivity, but is also a systemic response to fasting and various diseases. These diseases include sepsis, AIDS, renal and cardiac failure, excessive glucocorticoids (as in Cushing syndrome) and trauma, and muscle atrophy also occurs in 80% of patients with cancer (a condition known as cancer-associated cachexia)[1,2]. During atrophy, the degradation of both myofibrillar and soluble proteins is accelerated. In systemic wasting conditions, protein synthesis also decreases, leading to the rapid loss of muscle mass and body weight, weakness and increased disability. This condition can reduce quality of life and contribute to mortality. Clinical studies and recent findings in tumour-bearing mice suggest that cachexia is likely to be the major immediate cause of death in many patients with cancer, and preservation of muscle mass may therefore increase survival[3]. Thus, the development of countermeasures to block or attenuate this debilitating process is likely to have major therapeutic benefits for a diverse set of clinical conditions.

Previous studies[4,5], primarily in animal models, have demonstrated that muscles atrophying in response to diverse catabolic stimuli all show similar activation of protein degradation by both the ubiquitin–proteasome system (UPS)[6–9] and autophagy[10,11]. Most muscle proteins, particularly myofibrillar components, are degraded by the UPS, and the loss of contractile machinery during atrophy accounts for the reduction in muscle strength. Mitochondria (and other organelles) are also degraded by autophagy, and their loss accounts for the decreased endurance capacity of atrophied muscles. The coordinated induction of numerous components of the UPS in different types of muscle atrophy led us to propose that a set of common transcriptional adaptations activate protein breakdown and reduce protein synthesis, ultimately leading to muscle wasting[6–9]. Microarray studies subsequently identified a set of 120 atrophy-related genes (termed atrogenes) that are induced or repressed in various wasting conditions[6–9]. Among these genes are those encoding ubiquitin, proteasome subunits and key ubiquitin ligases[2,4], as well as many genes encoding proteins that mediate autophagy[10]. Two muscle-specific ubiquitin ligases, muscle-specific RING-finger 1 (MURF1; also known as TRIM63)[12] and atrogin 1 (also known as MAFBX)[8], are markedly induced in almost all types of atrophy. Consequently, MURF1 and atrogin 1 are now widely used as markers of accelerated proteolysis and the atrophy process, although their induction is transient and is seen primarily during the period of rapid weight loss[9]. Neither atrogin 1 nor MURF1 is necessary for normal muscle growth, but loss of either reduces the rates of atrophy upon denervation, glucocorticoid treatment

or fasting[12,13]. During atrophy, atrogin 1 catalyses the degradation of proteins that promote protein synthesis, whereas other ligases, in particular MURF1 and ubiquitin tripartate motif-containing protein 32 (TRIM32), catalyse the ubiquitylation and degradation of the myofibrillar apparatus and the cytoskeleton[13–16].

Whether a muscle grows or atrophies primarily depends on the activity of the phosphoinositide 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) signalling pathway[17,18]. In most cells, this pathway controls cell division, but in non-dividing muscle cells, it stimulates overall protein synthesis and inhibits protein degradation[19]. In muscle, activation of PI3K–AKT signalling promotes net protein accumulation by suppressing forkhead box protein O (FOXO) transcription factors[20], which control the expression of the atrogene programme[21]. However, during fasting and disease, PI3K–AKT–mTOR signalling decreases, and consequently protein synthesis falls; simultaneously, proteolysis increases largely through the FOXO-mediated expression of the atrogene programme[17] (FIG. 1). In fact, activation of FOXO3 alone is sufficient to trigger proteolysis via the UPS[21] and autophagy[10,11], and to cause substantial atrophy[21]. In addition, several other transcription factors are important in causing muscle wasting, including SMAD2 and SMAD3 (REF. 22), glucocorticoid receptors[21,23] and nuclear factor-κB (NF-κB)[24,25], and their inhibition can reduce or block different types of atrophy. SMAD2 and SMAD3 mediate protein loss and atrophy downstream of myostatin, activin A and other transforming growth factor (TGF) family members, which are highly catabolic in muscle[22]. Although the precise roles of these transcription factors in altering the expression of specific genes are unclear, they seem to cooperate with or act by FOXO-mediated transcription[26], as discussed below.

In this article, we discuss the molecular mechanisms and pathophysiology of the muscle atrophy that accompanies various systemic diseases and describe new potential drug targets and emerging therapies that could combat this debilitating loss of muscle. In patients with genetic muscular dystrophies, there are inherent defects in the muscles that create particular therapeutic challenges, and important unanswered questions include to what extent the atrophy process contributes to disease progression and whether treatments that only slow protein loss would be beneficial. Therefore, in this article, we focus our discussion on conditions that cause wasting of normal muscle. As mechanisms that promote protein breakdown contribute to atrophy, inhibition of key regulators of this process or agents that promote muscle-protein synthesis are of major therapeutic promise for the treatment of numerous wasting conditions, ranging from prolonged bed rest to cancer-associated cachexia.

Molecular mechanisms controlling muscle mass

Reduced PI3K–AKT signalling and FOXO activation lead to enhanced proteolysis. The insulin-like growth factor 1 (IGF1)–PI3K–AKT pathway induces muscle growth primarily by stimulating protein synthesis through mTOR kinase and inhibiting degradation induced by FOXO transcription factors[27], although mTOR can also rapidly suppress autophagy[28] and the UPS[17,29]. Overproduction of IGF1 or AKT in mice, either through transgenic expression or by electroporation into muscles, was sufficient to reduce muscle weight loss, induce systemic hypertrophy and increase strength[30,31]. Conversely, reduced PI3K–AKT signalling, as occurs in fasting and catabolic diseases, results in decreased protein synthesis, increased FOXO-mediated proteolysis and fibre atrophy. Such inhibition of PI3K–AKT signalling occurs not only with the decrease in insulin and/or IGF1 levels, as in individuals who are fasting or have type 1 diabetes, but also with increased levels of myostatin, an autocrine inhibitor of normal muscle growth, or its homologue activin A. Indeed, increased levels of both myostatin and activin A occur in pathological states and can cause a rapid loss of muscle mass[22,32,33] (FIG. 1; TABLE 1) (see below).

Many new anticancer treatments currently in use or in clinical trials inhibit PI3K–AKT–mTOR signalling and therefore are likely to also inhibit muscle growth and promote atrophy. Conversely, methods to activate these kinases could be of value to combat muscle wasting, especially during recovery and rehabilitation. Such treatments could be dangerous — for example, in treating cancer-associated cachexia in older people. Stable IGF1 derivatives or antagonists of myostatin–activin A signalling are attractive approaches to reduce FOXO activity in muscle, but they also have the potential to promote tumour growth. Recent findings indicate the use of several other potential drug targets in this pathway to block atrophy. For example, overexpression of the transcription factor JUNB reduces muscle protein breakdown and atrophy by blocking the binding of FOXO3 to atrogene promoters[34]. JUNB is a transcription factor that promotes cell division[35], but in heart and skeletal muscle it is also essential for myofibril integrity and maintenance of normal muscle size[34]. Another intriguing potential target is the exercise-induced transcription coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α), which suppresses FOXO- and NF-κB-dependent transcription during atrophy induced by fasting or denervation[36,37]. Of particular interest is the recently discovered exercise-induced isoform PGC1α4, which can repress myostatin, and induce IGF1 and hypertrophy[38]. In addition to being important for the maintenance of muscle mass and functional capacity with contractile activity, PGC1α is crucial in promoting mitochondrial biogenesis[39] and oxidative metabolism[40]. As the transcription and protein levels of PGC1α[41] and JUNB[34] decrease in various forms of atrophy, and maintaining high levels of these factors can block various types of muscle wasting, agents that inhibit the downregulation of PGC1α and JUNB during atrophy could be of considerable therapeutic benefit (FIG. 1) (see below).

Among the three FOXO family members, FOXO1 and FOXO3 have received the most attention as they are probably activated in all types of atrophy[21], and they both induce components of the UPS[21,42,43] and autophagy[10,11,41]. The activity of these transcription factors is tightly controlled by several post-translational modifications. Phosphorylation by mammalian sterile 20-like protein

Myofibrillar
Consisting of myofibrils. Myofibrils are the organizational units in skeletal muscle composed of aligned filaments that enable contraction. Myofibrils contain mainly myosin in the thick filaments and actin in the thin filaments plus many less abundant regulatory proteins.

Atrogenes
Atrophy-related genes that are similarly induced or suppressed in all types of atrophy in skeletal muscles.

Catabolic diseases
Diseases associated with marked weight loss, particularly loss of muscle mass and strength owing to the accelerated destruction of muscle proteins.
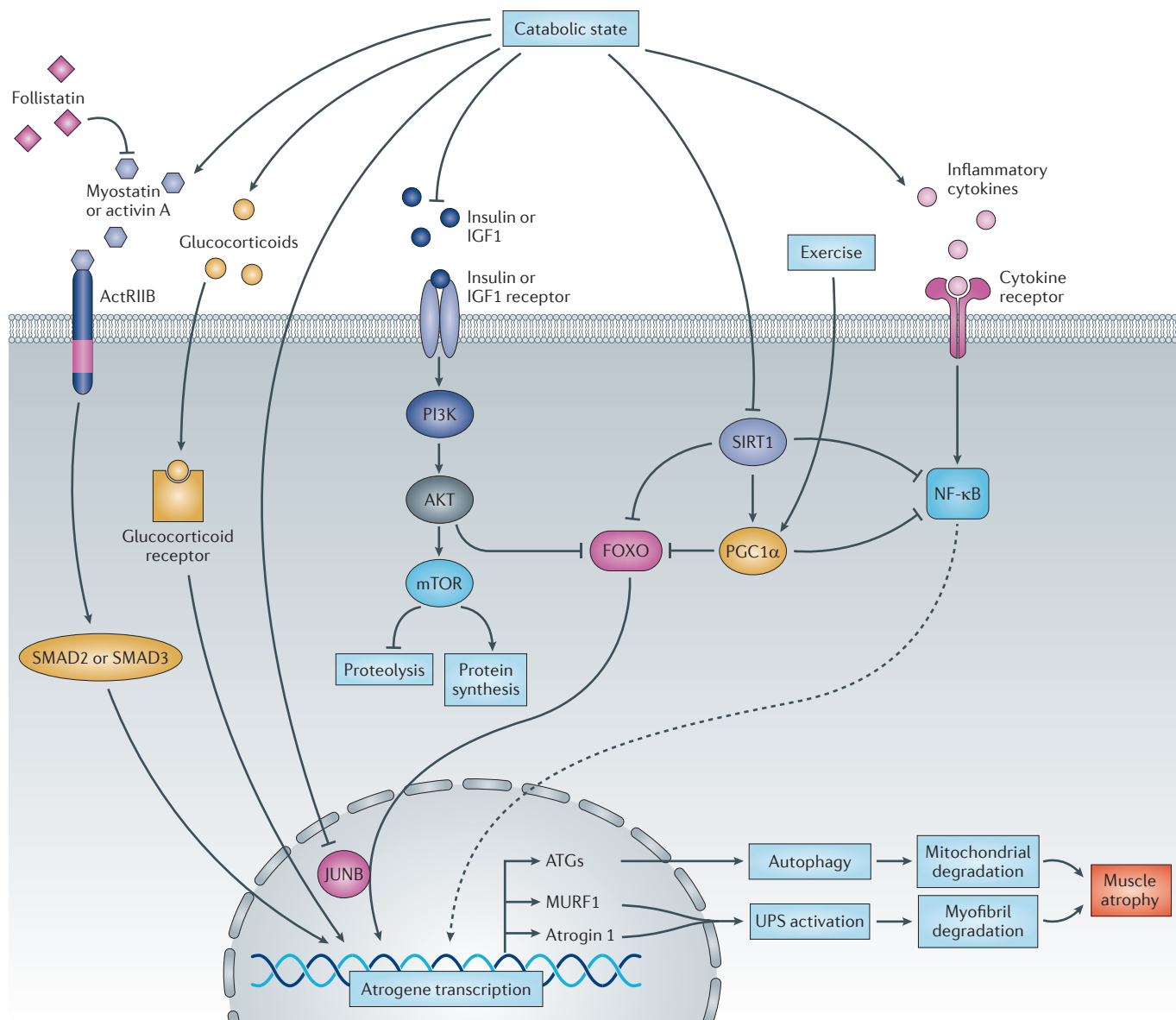
Figure 1 | **Signalling pathways leading to muscle atrophy.** In diverse catabolic states, multiple intracellular signalling pathways stimulate the expression of atrogenes and thus protein degradation via the proteasome and autophagy. These catabolic effects in muscle are mediated by specific transcription factors, including forkhead box protein O (FOXO) proteins, nuclear factor-κB (NF-κB) and SMAD2 or SMAD3. The activation of these transcription factors results from extracellular stimuli or from a decrease in phosphoinositide 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) signalling. This reduction in PI3K–AKT–mTOR activity or increased glucocorticoids results in inhibition of mTOR and protein synthesis, and together with the accelerated proteolysis leads to muscle atrophy. The dashed line indicates that the evidence for NF-κB-mediated induction of atrogene expression is tentative. ActRIIB, activin A receptor, type IIB; ATGs, autophagy-related genes; IGF1, insulin-like growth factor 1; JUNB, transcription factor JunB; MURF1, muscle-specific RING-finger 1; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; SIRT1, NAD-dependent protein deacetylase sirtuin 1; UPS, ubiquitin–proteasome system.

kinase 1 (MST1; also known as STK4)[44] or 5′-AMP-activated protein kinase (AMPK; also known as PRKA)[45,46] stimulates FOXO3. By contrast, phosphorylation by AKT[17,27], deacetylation by the sirtuin family NAD-dependent protein deacetylase sirtuin 1 (SIRT1)[47], ubiquitylation[48], or binding to JUNB[34] or PGC1α[37] inhibits FOXO3 transcriptional activity. In animal models, manipulation of any of these FOXO-modifying mechanisms suppressed FOXO, inhibited overall proteolysis and prevented muscle wasting. Although few of these potential intracellular targets for small-molecule drug development have been pursued, several antagonists of the myostatin–activin A pathway, which act extracellularly to promote AKT signalling and suppress FOXO and SMAD activities[32,33] (see below), are promising agents that are currently in clinical trials.

Table 1 | **Pathological conditions that cause systemic muscle wasting**

Catabolic state	Myostatin activation	Increased glucocorticoids	Insulin resistance
Cancer-associated cachexia	Yes	Yes	Yes
Sepsis	Unclear	Yes	Yes
Critical illness myopathy	Unclear	Yes	Yes
Diabetes	Yes	Yes	Yes
Chronic renal disease	Yes	Yes	Yes
Chronic obstructive pulmonary disease	Yes	Administered medically	Unclear
Heart failure	Yes	Variable, depending on cause	Yes
Trauma	Unclear	Yes	Yes
Burns	Unclear	Yes	Yes
Sarcopenia	Yes	Unclear	Yes

NF-κB is an important transcription factor in several types of atrophy. In stimulating the expression of atrophy genes, FOXO transcription factors do not function alone. The transcription factor NF-κB, which is the key regulator of inflammatory responses and apoptosis, is also important for many types of muscle atrophy[24,25,49,50,51]. In mouse muscles, expression of the IκB super-repressor to inhibit NF-κB reduced atrophy upon denervation in tumour-bearing mice[24], even during fasting (D. L. Lee and A.L.G., unpublished observations). In mice lacking IκB kinase-β (also known as IKK2), atrophy upon denervation was also attenuated[25]. The specific mechanisms by which NF-κB promotes atrophy, and whether it catalyses atrogene expression, remain uncertain, although recent studies have demonstrated that NF-κB is a potent inducer of myostatin[52]. Surprisingly, the various upstream signals that regulate NF-κB function in muscles during different types of wasting are for the most part unknown, although activation of the ubiquitin ligase TRAF6 is likely to be important[53]. Several pro-inflammatory cytokines (tumour necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-1 and interferon-γ (IFNγ))[54] are elevated in sepsis, cancer and other catabolic conditions, and may together trigger muscle wasting, probably (at least in part) by increasing the expression of NF-κB (FIG. 1) or by causing the release of other cytokines[55,252].

Thus far, attempts to prevent or slow the process of muscle wasting by blocking a specific cytokine have been unsuccessful. One recently described inflammatory cytokine strongly implicated in muscle wasting is the TNF-like weak inducer of apoptosis (TWEAK), which binds to the surface receptor FN14 (also known as TNFRSF12A) and causes NF-κB activation[56]. Mice lacking TWEAK showed reduced atrophy upon denervation, a process that normally induces TWEAK[57]. Despite appreciable efforts, NF-κB inhibitors have not emerged. Although small-molecule inhibitors of IKK2 (REF. 58) and suppressors of NF-κB activation[59–61] have been described, their specificity and potency remain questionable.

SMAD2 and SMAD3 mediate myostatin- and activin A-induced wasting. Myostatin, the major autocrine inhibitor of muscle growth, binds to the activin A receptor, type IIB (ActRIIB) in skeletal and cardiac muscle, inducing fibre atrophy through activation of the transcription factors SMAD2 and SMAD3 (REF. 22) (FIG. 1), and through inhibition of the proliferation and differentiation of muscle stem cells, termed satellite cells[62,63]. Myostatin accumulation is associated with insulin resistance and protein loss in the muscle fibre[64,65]. The physiological and pathophysiological mechanisms that regulate myostatin secretion in different conditions are still mostly unknown, although glucocorticoids[66,67] (see below), FOXO1, NF-κB[52], and SMAD2, SMAD3 and SMAD4 can all enhance myostatin expression, and the released myostatin acts in both an autocrine and a circulatory manner, contributing to their catabolic effects (TABLE 1).

In addition to myostatin, several related TGF family members, including activin A and GDF11, bind to ActRIIB and stimulate SMAD2 and SMAD3 production[253]. Similarly to myostatin, these molecules seem to limit normal muscle growth and can induce muscle wasting in patients with disease. Although myostatin is the primary factor limiting muscle growth in mice, in humans, activin A and GDF11 also seem to be of increasing importance with ageing or disease[68]. Furthermore, transgenic mice expressing high levels of follistatin, a natural circulating glycoprotein inhibitor of myostatin and these other TGFβ members, exhibited a marked increase in muscle mass. Thus, follistatin administration is another possible approach to impede atrophy[69]. This increase in muscle mass following TGFβ inhibition seems to be partly due to the activation of the PI3K–AKT pathway and mTOR signalling[22,70], bone morphogenetic protein (BMP) signalling[71], and AMPK, PGC1α[72] and PGC1α4 (REF. 38). Together, these various studies and recent preclinical findings all strongly suggest that inhibiting the myostatin–activin A pathway is a potentially useful approach to block various types of atrophy (TABLE 1). Several promising inhibitors of the myostatin–activin A pathway, including antibodies against myostatin or its receptor ActRIIB, have been generated for the treatment of cancer-associated cachexia, certain myopathies, heart failure, renal failure, obesity and diabetes[32]. Small-molecule inhibitors of TGF signalling are

currently in clinical trials for cancer treatment, and they may be useful for the treatment of cachexia (TABLE 2). However, small molecules that can selectively antagonize myostatin–activin A–SMAD2/SMAD3 signalling in muscle are yet to be identified.

The fundamental cause of muscle wasting is an ordered breakdown of myofibrils. The primary loss of muscle strength during atrophy results from the accelerated destruction of the contractile machinery, the myofibrils, which constitute more than 70% of muscle proteins. Their loss results in a reduction in force generation, fatigue and, if prolonged, physical disability. The mechanisms for myofibril protein breakdown have recently become clearer, and specific ubiquitin ligases have a prominent role. Proteins that form the sarcomere are lost in a specific order during atrophy induced by denervation[13]. Initially, certain small regulatory proteins that stabilize the myosin (thick) filament are ubiquitylated by the FOXO-induced ubiquitin ligase MURF1, and degraded by the 26S proteasome (FIG. 2). Subsequently, the myosin heavy chain undergoes the same fate[13,15,73]. However, the degradation of actin and other thin filament components (for example, tropomyosin) is linked to the breakdown of Z-bands and the cytoskeleton[14] and involves another ubiquitin ligase, TRIM32 (REFS 14,74). Programmes to develop selective inhibitors of MURF1 have been pursued in industry, but this approach will probably not prevent atrophy because the components of the thin filaments (for example, actin and tropomyosin), Z-bands and the cytoskeletal network will still be targeted for degradation by TRIM32, and mitochondria and many soluble proteins will be degraded by autophagy.

MURF1 is specific to atrophying muscle, whereas the ubiquitin ligase TRIM32 is found in many (perhaps all) cell types. Mutations in *TRIM32* cause limb girdle muscular dystrophy 2H[75], and loss of TRIM32 in mice results in considerable neurological defects and myopathy[76,77]. In contrast to atrogin 1 or MURF1, TRIM32 is not induced by fasting[14], and ubiquitylation by TRIM32 and the resulting loss of the desmin cytoskeleton are activated by desmin phosphorylation[14]. Therefore, inhibition of the critical unidentified kinase responsible for the phosphorylation of desmin could be an attractive target to combat atrophy by blocking the initial disassembly of the desmin cytoskeleton and the myofibrils.

In addition to this role in destruction of the cytoskeleton and myofibrils, TRIM32 also reduces PI3K–AKT–FOXO signalling during fasting[78], which results in decreased protein synthesis and increased proteolysis[79]. Therefore, inhibiting TRIM32 function in muscle can actually promote muscle growth[78], and TRIM32 could be an attractive therapeutic target. However, thus far, no clear success has been achieved in developing specific inhibitors of the RING-finger family of ubiquitin ligases, including TRIM32.

The solubilization of the ubiquitylated myofibrillar components also involves a complex containing valosin-containing protein (VCP), also known as the p97 ATPase complex[80], which extracts ubiquitylated proteins

from larger structures (for example, the endoplasmic reticulum membrane or chromatin) before proteasomal degradation; inhibition of VCP prevents atrophy and proteolysis through both the proteasome and autophagy[81,82]. Although the VCP complex is becoming a target for cancer treatment[83], mutations in this protein can cause inclusion body myopathy[84], often with Paget disease and dementia[84], as well as amyotrophic lateral sclerosis[85]. Thus, even though inhibition of VCP can delay the loss of muscle protein[81], its inhibition is only likely to be useful for killing rapidly growing cancer cells, and such treatments are potentially toxic and likely to interfere with protein homeostasis. Similarly, proteasome inhibitors (for example, bortezomib) have proven to be highly successful in the treatment of certain haematological cancers[86]. Although such inhibitors were originally developed to decrease muscle atrophy[86], which they can do[87–89], their use to combat atrophy would be ill-advised because it is likely that they will also alter cell composition and interfere with protein quality control.

Muscle atrophy during systemic disease

Cancer-associated cachexia. Cachexia is a complex metabolic syndrome that accompanies many malignancies. It is characterized by severe loss of muscle mass with or without loss of fat[90] and is associated with increased morbidity and mortality. Studies in tumour-bearing animal models demonstrated that accelerated proteolysis, primarily by the UPS, causes most of the muscle wasting[8,91], and a similar decrease in muscle mass was observed in several clinical studies of cachexia[92,93]. Pro-inflammatory cytokines, such as TNFα, IL-1 and IL-6, have long been considered as mediators of cancer-associated cachexia, although their roles in muscle wasting remain controversial. TNFα was initially believed to have a crucial role in the weight loss observed in tumour-bearing mice[94], and exogenous TNFα can induce the expression of genes involved in proteolysis by the UPS[95–97]. However, this cytokine can have many systemic effects, including anaemia and shock, and several studies have failed to observe a direct effect on protein breakdown in isolated muscles or animals treated with TNFα[98,99]. Such studies are complicated because high levels of one cytokine (such as TNFα) can trigger the release of many other factors, including IL-1 and IL-6 (REF. 252), from macrophages or endothelial cells. With the advent of anti-TNFα therapy, it has been possible to directly assess the role of TNFα blockade in early phase clinical trials (TABLE 2). The TNFα receptor–blocker etanercept[100], and the TNFα-specific monoclonal antibody infliximab[101], did not prevent muscle atrophy in two randomized controlled trials of patients with advanced cancer-associated cachexia. Infliximab also adversely affected patients' quality of life. It seems possible that TNFα contributes to cachexia in certain conditions, but other pro-inflammatory cytokines are also likely to be involved.

IL-1 and IL-6 are upregulated in animal models of cancer-associated cachexia[102], and IL-6 levels correlate with weight loss in certain human cancers[103–106]. Moreover, cachexia can be ameliorated in mice treated with IL-6-targeted antibodies[107]. Based on these observations,

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Sarcomere
The repeated structural and contractile unit along the length of a myofibril delimited by the Z-bands.

Z-bands
The boundaries of sarcomeres where desmin filaments are aligned and thin (actin) filaments are anchored.

Table 2 | **Summary of clinical trials evaluating treatments for muscle wasting**

Drug/target	Disease process	Trial details	Outcomes	Ref or ClinicalTrials.gov identifier
TNFα				
Etanercept (TNFα ligand bound to Fc-IgG1)	Cancer	• IV administration • RCT	No inhibition of muscle wasting	100
Infliximab (TNFα-specific mAb)	Non-small-cell lung cancer	• IV administration • RCT	Trial stopped early because of decreased quality of life in infliximab-treated group	101
IL-6				
ALD518 (BMS-945429; IL-6-specific mAb)	Lung cancer	• IV administration • Phase I/II	No inhibition of muscle wasting	108
Myostatin/activin				
BYM338 (bimagrumab; ActRIIB-specific mAb)	Sarcopenia	• IV administration • RCT	In progress	NCT01669174
	COPD	• IV administration • RCT	In progress	NCT01601600
	Cancer	• IV administration • RCT	In progress	NCT01868685
	Mechanical ventilation	• IV administration • RCT	In progress	NCT01433263
	Sporadic inclusion body myositis	• IV administration • RCT	In progress	NCT01925209 (RESILIENT trial)
LY2495655 (myostatin-specific mAb)	Pancreatic cancer	• IV administration • Phase II	In progress	NCT01505530
Ghrelin receptor				
Ghrelin	COPD	• IV administration • RCT	Improvement in quality-of-life score but not physical activity	246
Anamorelin (growth hormone secretagogue receptor agonist)	Cancer	• Oral administration • RCT	Improvement in symptoms score	247
	Non-small-cell lung cancer	• Oral administration • RCT	In progress	NCT01387282
SUN11031 (ghrelin agonist)	COPD	• SC administration • Phase II	Increased muscle mass but not function	NCT00698828
Androgen receptor				
Enobosarm	Ageing	• Oral administration • Phase II	Increased muscle mass and function	248
	Cancer	• Oral administration • RCT	Increase muscle mass but not function	NCT01355484 and NCT01355497 (POWER trials); NCT00467844 (REF. 249)
MT-102 (SARM)	Non-small-cell lung cancer	• Oral administration • RCT	In progress	ACT-ONE trial[250]
GSK2849466 (SARM)	Healthy volunteers	• Oral administration • Phase I	No serious adverse events	NCT01696604
LGD-4033 (SARM)	Healthy volunteers	• Oral administration • Phase I	No serious adverse events	251

ActRIIB, activin A receptor, type IIB ; COPD, chronic obstructive pulmonary disease; IgG1, immunoglobulin G1; IV, intravenous; IL-6, interleukin-6; mAb, monoclonal antibody; RCT, randomized controlled trial; SARM, selective androgen receptor modulator; SC, subcutaneous; TNFα, tumour necrosis factor-α.

several clinical trials with IL-6-specific monoclonal antibodies are underway (TABLE 2). In a Phase I/II trial, the IL-6 inhibitor ALD518 was safe in patients with advanced non-small-cell lung cancer (NSCLC) and had beneficial effects on anaemia and anorexia; however, there was no clear effect on lean body mass[108]. It is possible that IL-1 and IL-6 inhibitors will only show limited benefits in clinical trials, similar to the limitations observed with anti-TNFα therapy, because there are multiple inflammatory cytokines originating in the tumour or stromal cells that may be involved in activating muscle proteolysis in these complex diseases.

Potentially the most exciting recent discoveries regarding the aetiology and treatment of cancer-associated cachexia concern the role of the myostatin–activin A–SMAD signalling pathway. Myostatin and activin A are both upregulated in patients with various types of malignancies (for a review see REF. 32) and, as previously discussed, both the loss of myostatin and its increased expression have dramatic effects on muscle mass. It is therefore not surprising that inhibition of myostatin–activin A signalling is an attractive therapeutic approach for many types of muscle wasting (TABLES 2,3). The most dramatic example of the potential benefits of inhibiting myostatin–activin A signalling is in the treatment of tumour-bearing mice with an ActRIIB decoy receptor[3,109–111]. Treatment with the ActRIIB decoy receptor prevented cachexia development in several cancer models[3,109–111], increased muscle function[109–111] and even prolonged survival[3]. Moreover, when this treatment was initiated after cachexia had developed, it completely reversed not only skeletal muscle loss, but also the decrease in cardiac mass (a previously unappreciated aspect of the cachexia syndrome), even though circulating levels of the pro-inflammatory cytokines TNFα, IL-6 and IL-1 remained high[3]. An even more striking result was that the survival of the tumour-bearing mice increased even though the rate of tumour growth was not slowed[3]. Therefore, myostatin–activin signalling seems to be the dominant mechanism that regulates muscle mass in certain, perhaps many, cancers, which makes targeting this pathway a tractable and promising option for reducing cachexia and its associated morbidity. Moreover, even if this pathway is not the primary driver of muscle wasting, inhibition of SMAD2 and SMAD3 signalling will promote muscle build-up and reduce cachexia.

Rapid muscle atrophy in acute critical illness (burns, sepsis and trauma). Acute severe injury (for example, burns) or systemic inflammation (for example, in sepsis) causes a particularly rapid loss of skeletal muscle, which, in its most severe form, can lead to debilitating critical illness myopathy (CIM), also known as acute quadriplegic myopathy, acute care myopathy or critical care myopathy. This profound loss of muscle, which includes the loss of respiratory skeletal muscles, can lead to an inability to wean patients from mechanical ventilation, and often ultimately to respiratory failure and death. Despite the clear evidence that CIM is a life-threatening illness[112,113], our understanding of its aetiology is incomplete. This is probably due to the complex pathophysiology of CIM, in that there are multiple factors that may contribute to the rapid atrophy, including sepsis, inflammation, corticosteroids, inactivity, neuromuscular blocking agents, reduced caloric intake and insulin resistance (TABLE 1). Moreover, histological analyses of muscle biopsies demonstrate three different patterns of atrophy in CIM: myopathy with abnormal variation in fibre size, fibre atrophy and single-fibre necrosis; thick filament myopathy with selective loss of myosin (known as myosinopathy)[114], which may be associated with high-dose corticosteroid treatment and neuromuscular blocking agents; and necrotizing myopathy with phagocytosis

of muscle fibres[115]. Despite these complex pathological findings, accelerated protein degradation by activation of FOXO and the UPS is the principal mechanism for the loss of muscle mass in CIM[116,117].

The release of glucocorticoids in sepsis and burns activates several transcription factors required for the induction of muscle wasting, including FOXO1, FOXO3, NF-κB, PPARβ/δ[21,43,118,119] and myostatin[66]. In cultured myotubes, glucocorticoids induce PPARβ/δ, which causes FOXO1 expression. Interestingly, the PPARβ/δ inhibitor GSK0660 can decrease muscle wasting in rats treated with dexamethasone, or during sepsis induced by caecal ligation and puncture[118]. Therefore, treatment of acute muscle wasting with PPARβ/δ inhibitors merits further study.

The role of myostatin–activin A signalling in CIM is unclear. Myostatin mRNA expression in muscle is rapidly increased following burn injury in rats, but not following sepsis or endotoxin treatment[66]. Treating rats with the glucocorticoid receptor antagonist RU486 prevented this increase in myostatin expression[66]. A similar increase in myostatin mRNA levels was also observed after dexamethasone treatment in cultured myotubes[120,121] and in the muscles of mice[122]. Although glucocorticoid receptor antagonists or adrenalectomy can prevent muscle wasting in models of fasting and cancer, other studies found no beneficial effect of these drugs on muscle wasting following burn injury[123] or uraemia[124], and the loss of adrenal steroids and their many protective functions was deleterious to the stressed individual. Furthermore, other groups have failed to observe an increase in myostatin expression with sepsis or dexamethasone treatment. In fact, one group reported a decrease in myostatin mRNA expression, although the protein levels were unchanged[125]. It therefore remains to be determined whether myostatin signalling is altered in humans with sepsis or burns, and whether myostatin receptor blockade reduce muscles wasting in these conditions.

Muscle wasting in chronic diseases. Muscle wasting is also a substantial component of many chronic diseases. As these conditions are often incurable, any therapies to prevent muscle loss and thus improve the patients' quality of life would be highly beneficial. Patients with heart failure[126], chronic kidney disease (CKD)[127] and chronic obstructive pulmonary disease (COPD)[128–130] have profound muscle wasting that can deleteriously affect their prognosis. Despite the diverse nature of these diseases, they all seem to increase muscle proteolysis, primarily through the UPS[21,127,131–133] and the coordinated induction of atrogenes by FOXO transcription factors.

CKD can induce a catabolic state characterized by hypoalbuminaemia and loss of muscle mass (reviewed in REF. 134). The metabolic acidosis that complicates renal injury stimulates muscle proteolysis through activation of the UPS. In addition, this increased proteolysis and rapid muscle loss requires glucocorticoids[135]. Adrenalectomized rats with metabolic acidosis failed to develop muscle wasting, and muscle proteolysis increased only when acidosis was accompanied by low insulin and physiological levels of glucocorticoids[135]. More recently, it was shown that high levels of

glucocorticoids can reduce PI3K signalling and therefore activate FOXO1 (REF. 136). However, patients with CKD also have high levels of multiple cytokines that seem to contribute to muscle loss, including TNFα, IL-6 and myostatin[252], which signal through the signal transducer and activator of transcription (STAT) pathway.

In addition to the UPS, caspase 3 activation has been proposed to contribute to the accelerated proteolysis observed in CKD. In cultured myotubes, caspase 3 can cleave actomyosin to form a 14 kDa actin fragment, which is then degraded by the UPS[137]; this actin fragment has also been detected in mouse models as well as in
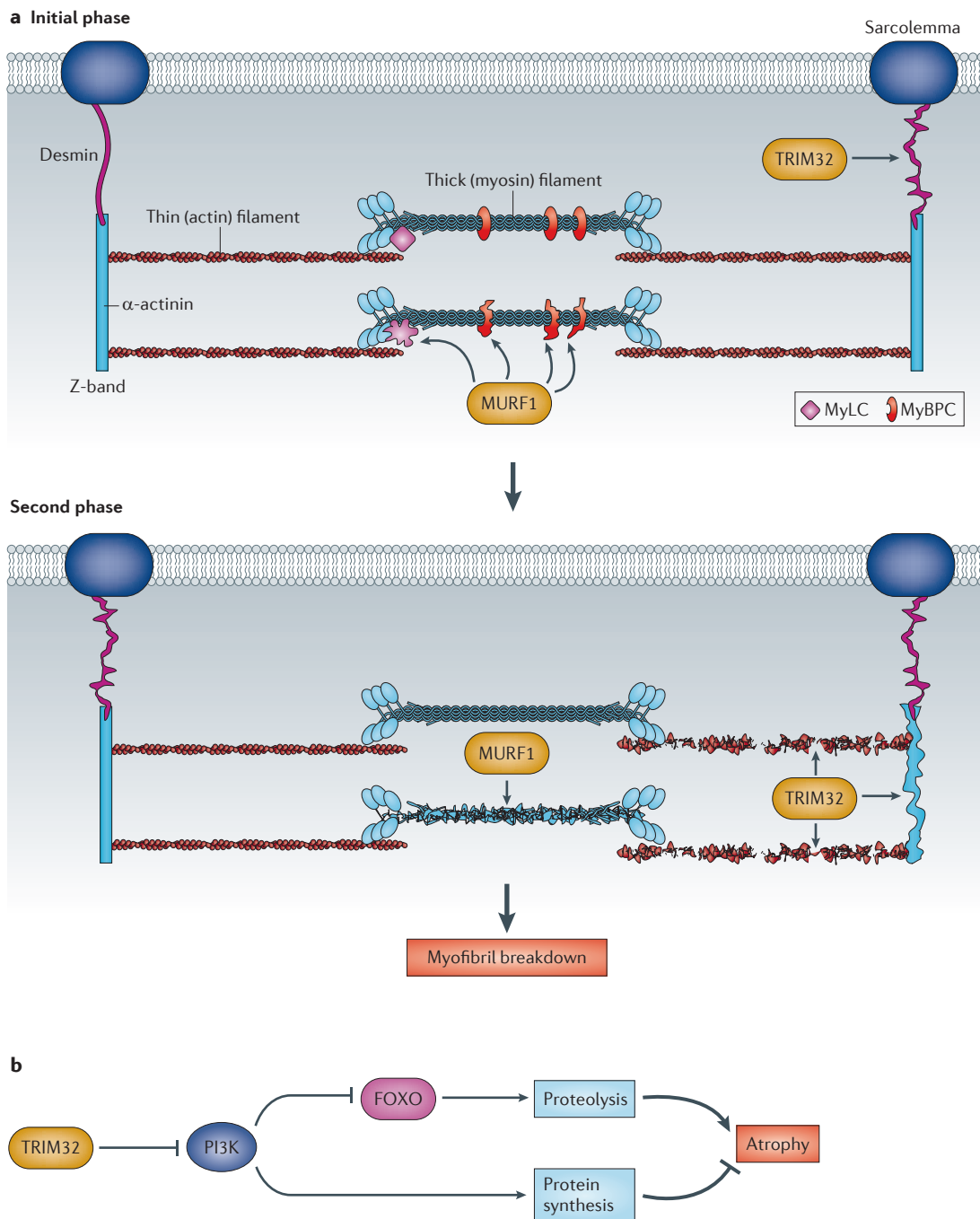
Figure 2 | **Mechanisms of myofibril breakdown and atrophy.** **a** | Loss of myofibrils during atrophy is primarily mediated by two crucial ubiquitin ligases: muscle-specific RING-finger 1 (MURF1) and ubiquitin tripartate motif-containing protein 32 (TRIM32). MURF1 catalyses the loss of thick filament-stabilizing proteins and then of myosin itself. Loss of the thin filament requires TRIM32, which also catalyses the destruction of the Z-band and the desmin cytoskeleton. **b** | In addition to its role in degradation of myofibrils and the cytoskeleton, TRIM32 also reduces phosphoinositide 3-kinase (PI3K)–AKT signalling, which results in decreased protein synthesis and increased proteolysis. FOXO, forkhead box protein O; MyBPC, myosin binding protein C; MyHC, myosin heavy chain; MyLC, myosin light chain.

the muscles of patients with CKD on haemodialysis[138]. It has also been shown that inhibition of caspase 3 reduced proteolysis in the muscles of rats[137]. Initially, such changes were proposed to generate protein fragments degraded by the N-end rule ubiquitylation system, which is activated in atrophying muscles[139,140]; however, recently, caspase 3 was also reported to activate proteasomes in myotubes by cleaving two proteasomal ATPase subunits[141]. Similar modifications of the 19S subunits were observed in a mouse model of CKD[141]. However, another study failed to demonstrate any decrease in actomyosin degradation or proteasome activity during denervation-induced atrophy in caspase-3-deficient mice[142], even though apoptosis was suppressed. Thus, the exact role of the caspases in atrophy — either in the activation of proteolysis by the UPS or in causing some apoptosis in this highly catabolic state — remains uncertain. Notably, the overexpression of the principal intracellular apoptosis inhibitor X-chromosome-linked inhibitor of apoptosis protein (XIAP), which inactivates several caspases, ameliorated muscle loss in a transgenic CKD mouse model[143]. It may be that apoptosis and caspase activation partially contribute to the muscle loss in CKD and other chronic conditions in which continued loss of cell proteins by atrogene induction may eventually lead to the activation of caspases. Fibre apoptosis has been observed during insect morphogenesis and in the muscles of aged sarcopenic animals, in which marked atrophy by the UPS precedes muscle apoptosis[144].

COPD is currently incurable and a major cause of morbidity and mortality worldwide[145], and skeletal muscle wasting is commonly observed in these patients[130]. Similar muscle wasting may also complicate other lung conditions and muscle atrophy can be substantial in patients with pulmonary hypertension[146–148]. Although muscle atrophy in lung disease can be caused by several mechanisms discussed above (that is, sepsis, inflammation and reduced physical activity) (TABLE 1), other factors, especially hypoxia, may also contribute. Rats exposed to hypoxia showed decreased exercise capacity and muscle mass[149] and increased proteasome activity[150]. In humans, chronic exposure to high altitude is associated with decreased muscle mass[151]. The mechanism of hypoxia-induced muscle wasting is unknown, but some clinical studies have suggested that oxygen supplementation may improve muscle function[152].

Patients with congestive cardiac failure often exhibit substantial skeletal muscle wasting, often termed cardiac cachexia (reviewed in REF. 153). As in the other disease states discussed above, proteolysis by the UPS is activated, and myostatin–activin A signalling is increased. Myostatin is of particular interest in heart failure as, similar to skeletal muscle, cardiomyocytes express ActRIIB, the myostatin–activin A receptor[154]. Therefore, activation of myostatin–activin A signalling may reduce cardiac hypertrophy but trigger skeletal muscle wasting in patients with congestive heart failure. Interestingly, although mice with a cardiac-specific deletion of the myostatin gene can develop heart failure, they do not develop skeletal muscle wasting[155], unlike normal mice. Conversely, when myostatin was overexpressed in

cardiomyocytes, circulating levels of myostatin increased, and skeletal muscle wasting was evident. It is not generally appreciated that the circulating TGF family members myostatin, activin A and GDF11 have similar catabolic effects in cardiac and skeletal muscle. Indeed, in cancer-associated cachexia, there is a profound loss of cardiac muscle, and administration of a ActRIIB decoy receptor antagonist to tumour-bearing mice not only preserved muscle mass but also prevented cardiac atrophy[3]. However, this reversal of cardiac atrophy may not be exclusively due to inhibition of myostatin and activin A, as one study recently showed that GDF11, another circulating ligand of ActRIIB, reverses the cardiac hypertrophy and left ventricular failure that frequently occurs in aged mice and humans[156]. Thus, these three activators of SMAD signalling have important (but potentially distinct) regulatory effects on both types of striated muscle, and blocking their actions may have applications in treating cardiac disease, as well as the wasting of skeletal muscle.

Sarcopenia. Probably the most frequent but least understood type of systemic muscle loss is sarcopenia, which is seen in older patients without overt disease[157]. This phenomenon differs from other types of wasting, as the muscle loss develops slowly and occurs over a number of years. Sarcopenia may affect as much as 15% of the population aged over 65 years and approximately 50% of individuals aged over 80 years. This loss of muscle substantially reduces the quality of life and physical activity of older people[158], and the increased frailty leads to falls, fractures and hospital admissions. Examining the mechanisms and progression of sarcopenia in patients is difficult. Older patients have numerous co-morbidities that may directly contribute to the muscle wasting, therefore the sarcopenia may be due to a combination of factors (TABLE 1), including immobility, loss of androgens, decreased levels of growth hormone and insulin resistance; the relative importance of these factors varies between individuals.

Determining the underlying mechanisms of sarcopenia is hampered by the slow nature of its development. Sarcopenia is also observed in rats, and early reports concluded that the proteasome activity in muscle decreased with ageing[159]. However, we showed that in atrophying muscles from aged rats there was a twofold to threefold increase in 26S proteasome content, as well as a general increase in protein ubiquitylation[160]. These muscles, however, did not show activation of the same atrogene programme that is characteristic of rapid atrophy. Although these findings do indicate increased proteolysis in sarcopenia, clear evidence from animal studies shows that loss of mitochondria, mitochondrial dysfunction and apoptosis are also increased in age-related muscle wasting (reviewed in REF. 161). Aged rodents have increased apoptosis in skeletal muscles, which also occurs in humans with sarcopenia (for example, in percutaneous muscle biopsies of 70-year-old men compared with those from 20-year-old men)[162]. To further identify the mechanisms underlying the development of sarcopenia, one recent study determined global gene expression

N-end rule ubiquitylation system
A pathway for ubiquitylation that targets degradation proteins with unusual amino-terminal residues, which may be generated by proteolytic cleavage of normal cell proteins.

Sarcopenia
The gradual loss of skeletal muscle mass seen in aged humans and animals.

Table 3 | **Summary of studies of myostatin–activin pathway inhibitors in mice**

Drug/compound	Disease process	Delivery route	Outcomes	Refs
ActRIIB decoy	Bowel and lung cancer	Subcutaneous injection	Reversed skeletal and cardiac muscle wasting, and prolonged survival	3
Myostatin-specific antibody	Lung cancer	Subcutaneous injection	Inhibited muscle wasting and improved muscle function	109,111
Myostatin-specific peptibody	Renal failure	Subcutaneous injection	Inhibited muscle wasting	207
JA-16 (myostatin-specific antibody)	Heart failure	Intraperitoneal injection	Inhibited muscle wasting	155
Myostatin-specific antibody	Disuse (for example, hindlimb immobilization in plaster cast)	Subcutaneous injection	Inhibited muscle wasting and improved muscle function	245
Myostatin-specific antibody	Sarcopenia	Subcutaneous injection	Inhibited muscle wasting	176,245
STAT3 small-molecule inhibitor (C188-9)	Renal failure	Subcutaneous injection	Inhibited muscle wasting	252

ActRIIB, activin A receptor, type IIB; STAT3, signal transducer and activator of transcription 3.

profiles in rats aged between 6 and 27 months[163]. The genes with the greatest changes in expression in sarcopenia (for example, *PPARGC1A* (which encodes PGC1α)) were associated with the pathways controlling mitochondrial content and oxidative metabolism. Therefore, preventing mitochondrial decline with therapies that increase the expression of *PPARGC1A* may be of therapeutic benefit.

The sequential mRNA profiling of muscles as sarcopenia developed demonstrated not only a progressive decrease in mitochondrial biogenesis, but also many changes characteristic of denervation atrophy (including the induction of acetylcholine receptors)[163]. Morphological studies have also documented that increased fibre denervation is an important contributor to sarcopenia[164,165], presumably owing to the increase in age-related motor neuron death. Unfortunately, none of the therapies being developed to combat excessive proteolysis in the muscle can restore innervation to these denervated fibres.

As ageing progresses, protein synthesis in muscle declines, partly due to a reduction in anabolic hormones, especially testosterone and growth hormone. Testosterone levels may fall by as much as 60% in men between 25 and 85 years of age, and by 30% in women[166,167]. Growth hormone also declines progressively after 30 years of age[168,169], and consequently circulating levels of IGF1 also decline with ageing. Hormone replacement has been examined as a possible therapy for sarcopenia[170]. However, treatment with growth hormone reduced muscle wasting but failed to increase exercise capacity, and testosterone replacement in hypogonadal men had only modest effects on muscle mass and function[171]. The myostatin–activin A pathway is also activated in patients with sarcopenia (reviewed in REF. 32). Aged myostatin-null mice lost less muscle mass than aged

control mice[172], and a myostatin antagonist attenuated sarcopenia in aged mice[173]. Levels of myostatin and activin A are also increased in elderly men and women[174,175]. Studies examining whether blocking myostatin may be a therapeutic target to combat sarcopenia demonstrated that administrating a myostatin-specific antibody to aged mice increased their exercise capacity[176] and reduced the decline in muscle mass[177]. Inhibiting myostatin–activin A signalling is therefore a highly promising therapeutic strategy to combat muscle loss in older people.

Treatment strategies and promising agents
As common proteolytic pathways are activated during diverse types of atrophy, targeting certain key components of these common mechanisms is likely to be beneficial in many diseases. Currently, the only validated treatment is exercise, which reduces various types of atrophy[178–180] and forms the mainstay of clinical management. However, exercise is not a practical option for bed-ridden, frail, sarcopenic or older individuals, or those with acute illnesses. Thus, there is an urgent and yet unmet medical need to develop drug therapies that will increase muscle mass and strength to improve patient quality of life and survival.

Potential drug targets to block wasting (PGC1α, JUNB and SIRT1). During exercise, several key factors that maintain skeletal muscle mass and promote hypertrophy are induced, including PGC1α[36,37], PGC1α4 (REF. 38), JUNB[181] and SIRT1 (REF. 182). The decrease in the levels of these proteins in various types of atrophy seems to contribute to the debilitating loss of muscle mass. Thus, agents that increase the levels of PGC1α, JUNB or SIRT1 could be of therapeutic benefit to slow muscle wasting in various catabolic states.

In normal muscle, especially type I (REF. 183) and IIA or IIX fibres[184], PGC1α and PGC1β are important for the production of mitochondria and oxidative metabolism. They promote these processes by co-activating genes together with several transcription factors, including the oestrogen-related receptor-α (ERRα)[185], striated muscle activator of RHO signalling (STARS)[186], myocyte enhancer factor 2 (MEF2)[187], PPARα and nuclear respiratory factor 1 (NRF1)[36,37] (for a review see REF. 188). In catabolic states, the loss of PGC1α helps to stimulate the atrophy process by activating FOXO transcription factors and NF-κB, thus promoting protein degradation[36]. Overexpression of PGC1α or PGC1β in mouse skeletal muscle increased mitochondrial content[189] and prevented atrophy upon denervation or fasting[37]. Furthermore, transgenic mice overexpressing PGC1α in muscle had an extended lifespan and did not show sarcopenia[190]. Indeed, overexpression of PGC1α4 promotes muscle hypertrophy and can protect against the development of cachexia[38]. Interestingly, overexpression of the PGC1α homologue in transgenic flies also reduced age-related wasting and extended lifespan[191]. Pharmacological activation of PGC1α signalling has been examined using the AMPK agonist 5-aminoimidazole-4-carboxamide riboside (AICAR). Prolonged treatment of mice with AICAR increased the content of PGC1α[192] in muscle, increased aerobic metabolism and improved the resistance to fatigue upon running[193]. However, AICAR treatment did not delay the atrophy induced by denervation[37] and, surprisingly, in cultured myotubes AICAR increased MURF1 and atrogin 1 expression and protein degradation[194,195], which was the opposite of what was observed in adult muscle *in vivo*. Thus, AICAR administration does not seem to be a promising approach to reduce atrophy.

JUNB is a transcription factor that is best known for its role in promoting cell division[35], but it is also important for the maintenance of postnatal muscle size, even though muscle is a postmitotic tissue[34]. Downregulation of JUNB in adult muscle fibres causes atrophy, and its overexpression can slow atrophy by inhibiting FOXO3-induced proteolysis[34]. JUNB overexpression can also induce muscle growth independently of changes in the AKT–mTOR pathway and without causing satellite-cell proliferation[34]. This growth effect may result from inhibition of the TGFβ–SMAD pathway. Recent evidence has also implicated JUNB in the maintenance of sarcomere architecture and function in striated muscle[196]. Thus, preventing the loss of JUNB in atrophying muscle may delay wasting by increasing myofibril stability, reducing proteolysis and increasing protein synthesis.

SIRT1 is a member of the sirtuin family of class III NAD+-dependent protein deacetylases. Its overexpression or activation is protective in animal models of various metabolic, neurodegenerative, inflammatory and neoplastic diseases, and it augments lifespan in *Caenorhabditis elegans* (for reviews see REFS 197–199). SIRT1 overexpression in adult muscle induced rapid fibre hypertrophy[47], and blocked atrophy by inducing PGC1α[200] and reducing proteolysis, FOXO transcription factors[47] and NF-κB activity (D. L. Lee and A.L.G.,

unpublished observations); these effects on muscle size required the deacetylation activity of SIRT1 (REF. 47). By increasing the proliferation of muscle precursor cells, SIRT1 may also promote muscle growth, maintenance and repair[201]. Thus, SIRT1 seems to be a master regulator of muscle growth and atrophy, energy homeostasis and metabolism. Activating SIRT1 or inhibiting its loss during atrophy may therefore be an attractive approach to prevent muscle wasting. An alternative therapeutic approach would be to identify the protein acetylase that is responsible for the activation of FOXO transcription factors and NF-κB, and to inhibit its activity. Recently, it was demonstrated that the protein deacetylase GCN5 has such a role and that downregulation of GCN5 can prevent muscle wasting (D. L. Lee and A.L.G., unpublished observations). Thus, GCN5 represents an attractive drug target; moreover, finding enzyme inhibitors is much easier than finding enzyme activators (for example, an activator of SIRT1).

Myostatin and activin A antagonists. The classical work of Lee and co-workers[202] first showed that myostatin is an autocrine factor that normally limits muscle size. Because of the growing evidence indicating that increased production of myostatin and its analogue, activin A, contribute to several forms of atrophy, inhibition of myostatin–activin A–GDF11 signalling is a promising therapy for multiple types of systemic wasting. Several agents have now been developed to antagonize myostatin–activin A–SMAD signalling, including follistatin[69,203], soluble (decoy) forms of ActRIIB[3], antibodies that bind myostatin or block its receptor[68,204] and a recombinant myostatin propeptide[205] (TABLES 2,3). Small-molecule inhibition of STAT3 can also decrease myostatin levels[252]. Myostatin is synthesized from a propeptide that is cleaved to generate an amino-terminal secretory domain and a carboxy-terminal mature receptor-binding peptide[69,206]. Administration of this propeptide prevented the mature myostatin from binding to the receptor, increased body and muscle mass, and enhanced muscle repair and regeneration after severe muscle injury in normal mice[205].

Notably, the administration of an ActRIIB decoy in cancer-associated cachexia models, including in colon 26 (C26) tumour-bearing mice, fully reversed skeletal muscle loss and cardiac atrophy, and dramatically prolonged the survival of the tumour-bearing animals[3]. These effects were attributed to the blocking of the activation of muscle protein breakdown by FOXO-induced expression of ubiquitin ligases[3]. This treatment also stimulated muscle stem cell growth, but the physiological consequences of this action on the satellite cells remain unclear. Furthermore, inhibition of myostatin–activin A signalling has proven beneficial for muscle wasting and insulin resistance in several diseases, including renal failure[207], heart failure[155], obesity[208] and diabetes[209]. However, additional trials are essential to determine whether the build-up of muscle mass by ActRIIB antagonism improves muscle function, quality of life or prolongs lifespan in diverse catabolic conditions. It is noteworthy that inhibition of myostatin in

mouse muscle results in myofibre hypertrophy rather than hyperplasia, although it is possible that the proliferation of satellite cells, which accompanies fibre hypertrophy, is also required for this response[210,211].

Although it remains uncertain whether these approaches will be beneficial to patients with inherited muscle diseases, these therapies can increase the regenerative potential of satellite cells, which is an important compensatory response that slows the progression of muscular dystrophy. Inhibiting myostatin–activin A signalling seems to be the most promising approach for preventing the wasting of normal muscles rather than muscular dystrophies in which the muscles are inherently defective, or amyotrophic lateral sclerosis, in which they are denervated. In fact, initial clinical trials of the decoy receptors, which were undertaken to treat patients with dystrophy, were unsuccessful and were terminated owing to bleeding (ClinicalTrials.gov identifier: NCT01099761). This unwanted side effect was probably caused by the binding of other TGF family members to these circulating receptors and, presumably, may be avoided by more selective antibody-based approaches that only target ActRIIB[63], myostatin or activin A. The development of therapies that block myostatin, activin A or GDF11 should be advanced by clinical trials to treat inclusion body myositis, a highly debilitating, adult-onset condition in which SMAD signalling is activated. Novartis has begun clinical trials with an antibody (BYM338) that binds to activin type II receptors (ActRIIA and ActRIIB) and therefore specifically prevents myostatin, activin A and GDF11 from binding to these receptors. These trials will be considered by the Food and Drug Administration for accelerated approval[68].

IGF1 analogues and ghrelin. IGF1 is a 7.5 kDa polypeptide that is structurally related to insulin. It is a circulating hormone secreted by the liver in response to pituitary growth hormone, but it is also an autocrine factor that is released by muscle fibres. IGF1 stimulates protein synthesis, myoblast differentiation and muscle hypertrophy, and it inhibits protein degradation and many systemic forms of wasting. However, because of its rapid clearance, IGF1 itself is not a suitable therapeutic agent. 'Long arginine' IGF1 is a modified form of IGF1 that has a long circulation time, binds to more tissue targets and is more potent than endogenous IGF1 (REF. 212). Its ability to induce nerve growth and promote myoblast proliferation offers greater therapeutic potential. However, several more potent variants have been developed that have prolonged circulation times and have reduced association with inhibitory IGF1-binding proteins[213].

The hormone ghrelin increases the levels of growth hormone, and therefore IGF1, and increases body mass in healthy subjects[214]. Ghrelin can also reduce atrophy induced by dexamethasone, fasting or denervation[215]. Several clinical trials have been initiated to evaluate its safety and therapeutic benefits (TABLE 2). Although ghrelin is well tolerated and may improve certain symptoms associated with cachexia, it is unclear whether it preserves muscle mass and function (TABLE 2). Ghrelin agonists,

such as anamorelin, have the advantage of being orally active, and clinical trials are in progress to evaluate their efficacy in treating cachexia (TABLE 2). However, despite these promising effects of ghrelin and related agents, any therapy that increases IGF1 levels may increase the risk of neoplasia or growth of pre-existent cancers, and increasing levels of growth hormone may also induce peripheral insulin resistance and diabetes[216].

β₂-adrenoreceptor agonists and phosphodiesterase inhibitors. Muscle growth can also be stimulated independently of IGF1 through activation of the G protein-coupled β₂-adrenoreceptor, which causes cAMP accumulation and protein kinase A activation[217], as well as stimulating PI3K–AKT–mTOR signalling[79]. Thus, β₂-adrenoreceptor agonists, in addition to stimulating the breakdown of glycogen and lipids, enhance protein synthesis, inhibit protein degradation and can reduce atrophy upon denervation[218], immobilization[219], cancer[220] or ageing[221]. Through the stimulation of protein kinase A[222] and thereby PI3K–AKT–mTOR[219] signalling, the β₂-adrenoreceptor agonist clenbuterol decreased atrogene induction and proteolysis through both proteasomal degradation and autophagy[223]. In animal husbandry, there has been appreciable interest in the use of clenbuterol and related agonists to promote muscle growth. Presumably, the anabolic effects of adrenaline evolved as a physiological mechanism to maintain muscle mass in exercising or stressed individuals. Although potentially attractive as a treatment for muscle wasting, there has been little interest in the clinical applications of clenbuterol because of concerns regarding potential cardiovascular side effects[217,224], such as cardiac arrhythmias. However, the actual extent of these potential adverse effects has not been reported, and under certain conditions, increasing cardiac output may be beneficial. Interestingly, the use of clenbuterol or related agents to promote growth of farm animals has been banned, as β₂-adrenoreceptor agonists remain present in the meat from treated animals.

Another class of drugs that increase cAMP levels are the selective inhibitors of phosphodiesterase 4 (PDE4), which have been used for the treatment of COPD[225,226] because of their anti-inflammatory properties[227] and their capacity to reduce airflow obstruction[227,228]. Interestingly, PDE inhibitors can reduce muscle atrophy in rat models of sepsis[229,230], cancer[229], diabetes[231], denervation and immobilization[232], COPD[233,234] and fasting[235]. Similar to the β₂-adrenoreceptor agonists, PDE4 inhibitors can decrease atrogene induction and protein degradation by the proteasome[236,237] during atrophy.

Androgens and selective androgen receptor modulators. The androgenic steroid testosterone binds to nuclear receptors in muscle and increases protein synthesis and muscle mass[238]. Surprisingly, despite its widespread misuse in athletics, the effects of testosterone on muscle have not been extensively studied experimentally. This is probably because it has relatively minor effects on muscle mass in rodents[239], although testosterone is highly anabolic in humans. Even though testosterone and its analogues can induce muscle growth[238] and increase the number

of satellite cells[240], its clinical use is substantially limited by severe side effects, in particular the increased risk of developing prostate hypertrophy, cancer[241], masculinization and behavioural abnormalities. Therefore, non-steroidal selective androgen receptor modulators (SARMs) are being developed to overcome these issues. SARMs bind to the androgen receptor and display tissue-selective activation of androgenic signalling. SARMs cannot be metabolized into dihydrotestosterone or oestrogens, thus reducing the risk of developing prostatic hyperplasia. They seem to have promising therapeutic potential in various conditions, including cancer-associated cachexia, sarcopenia and osteoporosis, and in castrated men after prostate surgery. Several SARMs have been investigated in clinical trials[242,243] (TABLE 2). For example, the SARM enobosarm demonstrated encouraging results in Phase I and II clinical trials in patients with cancer-associated cachexia, in which it increased lean body mass and seemed to improve functional performance[244]. However, Phase III trials of this drug failed to meet their co-primary endpoints of preserving total muscle mass and physical function (ClinicalTrials.gov identifiers: NCT01355497 and NCT1355484, see Further Information). Whether SARMs are useful in combination with other drugs to treat muscle wasting remains to be tested.

Concluding remarks

Substantial progress has been made recently in our understanding of the molecular mechanisms that mediate the loss of muscle mass in disease. Several novel mechanisms have been discovered that are attractive drug targets, and several new rationally designed therapies for muscle wasting have entered, or are entering, clinical trials. At present, myostatin and activin A antagonists (and perhaps SARMs) are the most promising drugs to combat systemic atrophy. However, the potential future use of these agents as therapies requires more than simply the demonstration of increased muscle mass in humans. Therapeutic trials in this area involve major challenges for the pharmaceutical and biotechnology industries. The usual challenge in drug development — to demonstrate efficacy *in vivo* while ensuring safety — is particularly difficult in this area. Rigorously evaluating strength, nitrogen balance or endurance is challenging, especially in older individuals or those who are ill. Additionally, improvements in the quality of life are difficult to quantify. These are also major challenges for regulatory agencies, as drugs that only improve quality of life have never been approved by the FDA, despite their potential value. Perhaps the most dramatic therapeutic result and the simplest to license would be if these treatments prolonged survival as they do in mouse models of cancer[3]. A final challenge with therapies that build muscle will be to prevent the misuse of such agents to enhance athletic performance, especially as anabolic steroids and growth hormones have already been widely misused. New methods will have to be developed to monitor the multiple types of agents that can antagonize myostatin–activin A signalling. Although these challenges are considerable, the potential medical benefits of such therapies are likely to be substantial.

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Enobosarm (Ostarine; GTx-024): http://www.gtxinc.com/Pipeline/OstarineMK2866.aspx?Sid=4

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