Therapeutic approaches for muscle wasting disorders

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Abstract

Muscle wasting and weakness are common in many disease states and conditions including aging, cancer cachexia, sepsis, denervation, disuse, inactivity, burns, HIV-acquired immunodeficiency syndrome (AIDS), chronic kidney or heart failure, unloading/microgravity, and muscular dystrophies. Although the maintenance of muscle mass is generally regarded as a simple balance between protein synthesis and protein degradation, these mechanisms are not strictly independent, but in fact they are coordinated by a number of different and sometimes complementary signaling pathways. Clearer details are now emerging about these different molecular pathways and the extent to which these pathways contribute to the etiology of various muscle wasting disorders.

Therapeutic strategies for attenuating muscle wasting and improving muscle function vary in efficacy. Exercise and nutritional interventions have merit for slowing the rate of muscle atrophy in some muscle wasting conditions, but in most cases they cannot halt or reverse the wasting process. Hormonal and/or other drug strategies that can target key steps in the molecular pathways that regulate protein synthesis and protein degradation are needed. This review describes the signaling pathways that maintain muscle mass and provides an overview of some of the major conditions where muscle wasting and weakness are indicated. The review provides details on some therapeutic strategies that could potentially attenuate muscle atrophy, promote muscle growth, and ultimately improve muscle function. The emphasis is on therapies that can increase muscle mass and improve functional outcomes that will ultimately lead to improvement in the quality of life for affected patients.

Keywords: Aging; Cancer cachexia; HIV-AIDS; Muscle atrophy; Muscle diseases; Muscle wasting; Sarcopenia; Signaling pathways

Abbreviations: 1/2RT, one-half relaxation time; 4E-BP1, eukaryotic translation initiation factor4E binding protein 1; AC, adenylate cyclase; ActRII, activin type II receptor; ALK, activin receptor-like kinase; AP-1, activator protein 1; atrogin/MAFbx, muscle atrophy F-box; BMP-1/TLD, bone morphogenetic protein 1/tolloid; C/EBP, CCAAT/enhancer binding protein; Ca 2+ , calcium; caspase, cysteine proteases which cleave after aspartic acid; CdK2, cyclin-dependent kinase 2; COPD, chronic obstructive pulmonary disorder; CRE, cAMP response element; CREB, cAMP response element binding protein; CSA, cross-sectional area; DHPR, dihydropyridine receptor; DMD, Duchenne muscular dystrophy; EC, excitation–contraction; EDIRL, extensor digitorum longus; eEF2, eukaryotic elongation factor 2; eEF2K, eukaryotic elongation factor 2 kinase; eIF4E, eukaryotic translation initiation factor4E; ER, endoplasmic reticulum; ERK1, extracellular signal-regulated kinase 1; FDB, flexor digitorum brevis; FGFR1, fibroblast growth factor receptor 1; FLRG, follistatin related gene; FOXO, forkhead box O; G, inhibitory G proteins; Gβγ, stimulatory G protein; GASP-1, growth and differentiating-associated factor associated serum protein 1; GDF-8, growth and differentiating factor 8; GDP, guanosine diphosphate; GH, growth hormone; GPCR, G protein coupled receptor; Gl, inhibitory G protein coupled receptor; Gβγ, growth factor receptor-bound protein 2; GSK3β, glycogen synthase kinase 3β; GTP, guanosine triphosphate; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor 1; IGF-II, insulin-like growth factor II; IGFBP, insulin-like growth factor binding proteins; IKK, IκB kinases; IL-1β, interleukin-1β; IL-15, interleukin-15; IRS, insulin-receptor substrate; JAK, Janus-activated kinase; JNK, c-Jun amino-terminal kinases; LIF, leukemia inhibitory factor; MD-CMD, merosin-deficient congenital muscular dystrophy; MEK, MAPK kinase; MRF, myogenic regulatory factors; mTOR, mammalian target of rapamycin; MuRF, muscle ring finger protein; MyHC, myosin heavy chain; NF-κB, nuclear factor κB; NFAT, nuclear factor of activated T cells; NOS, nitric oxide synthase; PD-13, 3’-phosphoinositide-dependent protein kinase 1; P13K, phosphoinositide 3 kinase; PIF, proteolysis-inducing factor; PKA, protein kinase A; PKC, protein kinase C; PtdIns (3,4,5)P3, phosphatidylinositol-4,5-triphosphate; PtdIns (4,5)P2, phosphatidylinositol-4,5-bisphosphate; Raf, MAPK kinase kinase; Rb, retinoblastoma protein; ROS, reactive oxygen species; RyR3, ryanodine receptors; SDH, succinate dehydrogenase; SERCA, sarco/endoplasmic reticulum calcium ATPase; SIRS, systemic inflammatory response syndrome; SMM, sphingomyelinase; SOCS, suppressors of cytokine signaling; SR, sarcomplasmic reticulum; STAT3, signal transducers and activators of transcription; TGF-β, transforming growth factor β; TFN-α, tumour necrosis factor α; TPT, time taken to reach peak twitch tension.

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doi:10.1016/j.pharmthera.2006.11.004
1. Introduction

Many different conditions are associated with skeletal wasting and weakness. Muscle wasting can occur as a consequence of diseases such as muscular dystrophies or cancer (cachexia). Similarly, aging is associated with a progressive loss of muscle leading to increasing frailty, weakness, and loss of functional independence. The mechanisms underlying the loss of skeletal muscle differ between the various conditions, thus therapies used to combat wasting and restore muscle function will also differ. For example, the loss of muscle mass may have a neurogenic origin (e.g., denervation), or it could result from cytokine elevation activating protein degradative pathways (e.g., cancer cachexia, HIV-acquired immunodeficiency syndrome [AIDS]). This knowledge is important since a severe loss of functional muscle mass contributes to patient mortality.

Muscles maintain their mass and function because of a balance between protein synthesis and protein degradation associated with equal rates of anabolic and catabolic processes, respectively. Muscles grow (hypertrophy) when protein synthesis exceeds protein degradation. Conversely, muscles shrink (atrophy) when protein degradation dominates. Understanding the pathways that regulate skeletal muscle mass is crucial for the development of successful nutritional or drug interventions that can attenuate wasting and weakness and improve muscle structure and function.

There have been several recent reviews devoted to intracellular signaling during skeletal muscle atrophy and hypertrophy (Jackman & Kandarian, 2004; Rennie et al., 2004; Attaix et al., 2005; Bartoli & Richard, 2005; Cao et al., 2005; Costelli et al., 2005; Glass, 2005; Nader, 2005; Nair, 2005; Bassel-Duby & Olson, 2006; Kandarian & Jackman, 2006) and the purpose of this review is not to simply repeat this information. Rather, this review is designed to provide an overview of some of the major conditions where muscle wasting and weakness are indicated and also to provide information on some therapeutic strategies that could potentially attenuate muscle atrophy, promote muscle growth, and ultimately improve muscle function. This review differs from other reviews in that our discussion is biased toward therapies that do not just modulate muscle structure but instead emphasizes those approaches that could improve functional outcomes that would meaningfully improve patient quality of life.

The major pathways leading to muscle breakdown are the ubiquitin-proteasome pathway (Attaix et al., 2005; Cao et al., 2005; Tisdale, 2005), calpain-calpastatin pathway (Bartoli & Richard, 2005; Costelli et al., 2005; Hasselgren et al., 2005), lysosomal pathway (Farges et al., 2002; Busquets et al., 2006), and apoptosis or programmed cell death (Lee et al., 2004; Leeuwenburgh et al., 2005; Siu & Alway, 2006). The muscle wasting which occurs in the majority of disorders, can be explained by activation of one or more of these pathways. However, not all pathways are activated in every condition. We will first identify a number of different muscle wasting conditions and describe the mechanisms responsible for the loss of muscle mass (and associated weakness) in each case. We will then describe a number of different therapeutic approaches for attenuating muscle wasting and weakness.

2. Muscle wasting conditions

This review does not attempt to cover every condition where muscle wasting is indicated. For example, we have not described the muscle wasting associated with conditions such as sepsis,
cancer cachexia, chronic obstructive pulmonary disease (COPD), chronic heart failure, chronic kidney disease, or HIV-AIDS. Interested readers are directed to appropriate texts where pharmacotherapies for these conditions are discussed in detail, and with a bias towards clinical medicine (e.g., Mitch & Goldberg, 1996; Schols, 2003; Filippatos et al., 2005; Hasselgren et al., 2005; Schulze & Spate, 2005; Strassburg et al., 2005; Wyke et al., 2005; Wyke & Tsidale, 2005; Yarasheski et al., 2005; Mantovani et al., 2006; Mitch, 2006). Instead we have focused attention on many of the muscle wasting conditions that have received far less attention, including aging, disuse atrophy, denervation, and muscular dystrophies.

2.1. Age-related muscle wasting and weakness

Some of the most serious consequences of aging relate to its effects on skeletal muscle. ‘Sarcopenia’ is the term widely used to describe the slow, progressive loss of muscle mass with advancing age. It is derived from the Latin words ‘sarco’ and ‘penia’, with a broad meaning of ‘poverty of flesh’. Sarcopenia is characterized not only by the loss of skeletal muscle mass, but also by the gradual decline in muscle functional properties, including a decrease in force producing capacity and maximum velocity of shortening, and a general slowing of contraction and relaxation. Beyond the age of 85 years the loss of mass and strength can become so severe that some frail elders are unable to complete even the simplest of tasks (Iannuzzi-Sucich et al., 2002). Whilst the effects of aging on skeletal muscle are inevitable, it is unclear whether these intrinsic changes are immutable or reversible.

The age-related decline in muscle function is believed to be related to a decrease in both muscle quantity (mass) and a decrease in muscle quality (a term encompassing many factors including strength per muscle cross-sectional area [CSA], fiber proportions and metabolic characteristics). The loss of muscle quantity occurs through a decrease in contractile protein content via the loss of individual muscle fibers and a decrease in the size of remaining muscle fibers (Fig. 1). This age-related loss of contractile tissue is likely explained by differences in the rates of protein degradation and protein synthesis (Yarasheski, 2003).

Whilst the exact molecular mechanism for this age-related loss of protein has yet to be elucidated fully, it is likely complex and involve multiple cell-signaling pathways. Recent studies examining gene expression in aged skeletal muscle, whilst inconclusive, have suggested that alterations in both protein synthesis and degradation lead to the decline in skeletal muscle mass (Kayo et al., 2001; Welle et al., 2003; Giresi et al., 2005). Interestingly, it has been suggested that muscle protein synthesis may actually increase in a futile attempt to maintain muscle mass and that increased protein degradation is the prime determinant of sarcopenia (Kimball et al., 2004). Other age-related changes in gene expression consistent with impaired oxidative defense, decreased activity of mitochondrial proteins, and differential expression in genes regulating energy metabolism, DNA-damage repair, stress response, immune/inflammatory response, RNA binding and splicing, and proteasome degradation have also been reported and may contribute to sarcopenia (Giresi et al., 2005).

Whilst the mechanism for the age-related loss of muscle mass remains unclear, it is known that aging causes a decline in the size and number of muscle fibers. These changes affect the different muscle fiber types selectively, a process termed ‘age-related motor-unit remodeling’, involving muscle fiber denervation and subsequent reinnervation (see Fig. 1; Larsson et al., 1978; Brown & Hassel, 1996; Kadihresan et al., 1996; Urbanchek et al., 2001; Vandervoort, 2002). In addition to the remodeling of the aged muscle towards a slower phenotype, CSA is reduced significantly in type II fibers, whereas type I fibers remain relatively unaffected (Jakobsson et al., 1990; Dedkov et al., 2003). Similarly, it is mostly the type II fibers that are lost with aging, leaving a higher proportion of type I fibers in the muscle (Tomonaga, 1977; Larsson & Edström, 1986; Lexell, 1997; Luff, 1998; Chan et al., 2001).

The age-related decline in muscle quality is believed to involve a number of different mechanisms, including an increase in the level of intramuscular collagen and fat, a decrease

![Fig. 1](image-url)
in the ability of the remaining myofibers to produce force (a decline in specific force), and alterations in excitation–
contraction (EC) coupling affecting the time course of muscle
contraction (Delbono et al., 1995; Höök et al., 2001; Plant &
Lynch, 2002).

The age-related alteration in the time-course of contraction is
demonstrated in Fig. 2, indicating that both the time taken to
reach peak twitch tension (TPT) and the time taken for the
muscle twitch response to relax (one-half relaxation time, \(\frac{1}{2}RT\))
are increased in old muscle. Whilst neurogenic factors such as
motor unit remodeling are implicated in the slowing of con-
traction, myogenic factors must also be considered. Specifically,
age-related changes in calcium (Ca\({}^{2+}\)) handling during con-
traction and relaxation play an important role in the slowing of
contraction. Studies on single mechanically skinned muscle
fibers from the mouse found a decrease in sarcoplasmic reticulum (SR) Ca\({}^{2+}\) release channel function (Plant & Lynch,
2002), and studies on homogenized rat muscles have shown that
aging is associated with a reduction in the maximum rate of ATP
hydrolysis by the sarco/endooplasmic reticulum Ca\({}^{2+}\) ATPase
(SERCA), which is responsible for lowering intracellular Ca\({}^{2+}\)
levels by actively pumping Ca\({}^{2+}\) back into the SR. This is likely
mediated via an age-related alteration in the environment of the
nucleotide domain and/or an accumulation of nitrotyrosine
residues on the SERCA protein (Viner et al., 1996; Schertzer
et al., 2005). It is also important to note that activity, stability,
and digestibility (defined as the ability of an enzyme to increase
the rate of protein breakdown) of many enzymes in skeletal
muscle may be impaired during aging, which can also affect the
time course of contraction (Mooradian & Wong, 1991).

Finally, the effects of posttranslational modifications on
myosin may also play a role in the age-related changes to skeletal
muscle. Höök et al. (2001) used a single fiber in vitro motility assay
to show that in both human and rat skeletal muscle there is
an age-related slowing (18–25%) in the speed of actin filaments propelled by type I myosin. It was suggested that this was a result
of oxidation, glycosylation, or nitration of myosin.

Although it is generally agreed that the deleterious effects of
aging on muscle are inevitable, debate exists as to whether these
intrinsic changes are immutable or reversible. As the proportion
of older persons in the population increases, sarcopenia will
dramatically impact many lives and place ever-increasing de-
mands on health care. Therefore, there is a profound need for
therapeutic strategies that can slow or ameliorate the effects of
aging on muscle structure and function and so restore muscle
size and strength in the frail elderly so that functional inde-
pendence and quality of life can be maintained in the elderly
(Larsson & Ramamurthy, 2000; Lynch, 2004a,b).

2.2. Muscle disuse, immobilization, and unloading

A prolonged decrease in muscle contractile activity, such as
that in a sedentary lifestyle, during periods of prolonged bed rest,
inactivity as a consequence of congestive heart failure, COPD,
limb casting and muscle unloading (i.e., microgravity), is asso-
ciated with changes in the expression of metabolic, structural,
and contractile proteins in skeletal muscle (Jones et al., 2004;
Giger et al., 2005; Krawiec et al., 2005). These disuse-induced
changes result in a decreased muscle fiber CSA, decreased
muscle mass, reduced force producing capacity, and a general
shift towards a fast muscle phenotype (Stevenson et al., 2003).

The loss of muscle mass associated with disuse is believed to
be mediated via a decrease in muscle protein synthesis and an
increase in protein degradation (Taillandier et al., 1996;
Stevenson et al., 2003; Reid, 2005). Evidence for an inhibition
of protein synthesis pathways includes a decrease in protein
levels of Akt and decreased Akt phosphorylation which has
been linked to a number of signaling pathways controlling
protein synthesis (Bodine et al., 2001a,b). Likely attributed to
the decreased phosphorylation of Akt, disuse is associated with
a decrease in the expression of p70S6k, a positive regulator of
cell growth. In addition, 2 factors involved in translation and
elongation, eukaryotic translation initiation factor 4E (eIF4E)
and eukaryotic elongation factor 2 (eEF2), are inhibited during
periods of disuse (Bey et al., 2003; Stevenson et al., 2003).

Increased protein degradation associated with disuse atrophy
has been attributed to the activation of 3 distinct proteolytic
pathways: the Ca\({}^{2+}\)-dependent calpains (Tidball & Spencer,
2002), lysosomal cathepsins (Taillandier et al., 1996), and the
ubiquitin–proteasome system (for review, see Reid, 2005).
Analysis of gene expression during disuse atrophy in rat skeletal
muscle revealed a rapid increase in mRNA encoding for E3 ubiquitin protein ligases (atrogin/muscle atrophy F-box
[MAFbx], muscle ring finger protein [MuRF], and Nedd4)
during the first few days of disuse, whilst mRNA encoding for
cathepsins C, D, and L was increased maximally after 7–
14 days of disuse (Stevenson et al., 2003). These results suggest
that whilst the ubiquitin–proteasome system may be responsible
for the majority of the proteolysis associated with disuse, the
lysosomal proteases play an important role during prolonged
periods of disuse. Interestingly, only small changes in the
mRNA levels of Ca\({}^{2+}\)-dependent proteases were observed
during disuse atrophy (Stevenson et al., 2003).

Previous studies have been equivocal regarding the potential
role of calpains and calpastatin in disuse atrophy (Taillandier
et al., 1996; Tidball & Spencer, 2002), suggesting that further

![Fig. 2. Representative isometric twitch responses of isolated EDL muscles from adult (16 months old) and aged (28 months old) rats. Note the reduction in maximum force and the slower contraction and relaxation of the twitch response with aging (modified from Schertzer et al., 2005).]({}
work is required to fully elucidate the role of this pathway and muscle proteolysis during disuse.

Results from animal studies have demonstrated that muscle disuse affects fast- and slow-twitch skeletal muscles differently, with greater atrophy in predominantly slow-twitch muscles such as the soleus (Thomason & Booth, 1990) than in fast-twitch skeletal muscles (Bigard et al., 1998). Associated with the disuse-induced morphological alterations in skeletal muscles are changes in metabolism and function.

When muscles are unloaded, normal weight bearing muscles (containing predominantly slow-twitch myosin heavy chain [MyHC] isoform) shift towards a fast-twitch phenotype with a faster velocity of shortening and rate of relaxation (Bigard et al., 1998; Stevenson et al., 2003). Whilst the exact mechanisms responsible for this change in phenotype have yet to be fully elucidated, they are likely to involve both neuronal and myogenic factors. Related to these changes in contractile properties is an apparent increase in the glucogenic dependency of skeletal muscle undergoing disuse-induced atrophy. Interestingly there does not appear to be any change to the total oxidative capacity of the muscle, which may explain the observation that muscle fatigueability is essentially unchanged despite the shift towards a faster phenotype (Bigard et al., 1998; Stein & Wade, 2005).

In addition to the loss of muscle mass and associated changes in muscle phenotype, disuse atrophy is often accompanied by detrimental changes to bone strength as a consequence of the reduced mechanical loading which is normally placed on the bones by contracting muscles (Aguirre et al., 2006). Such changes in bone strength can have severe consequences, as the bones can become brittle and susceptible to fractures that result in casting and bed rest, and continued disuse.

2.3. Muscular dystrophies

Muscular dystrophies are a group of disorders characterized by muscle fiber degeneration and progressive muscle weakness. Mutations in individual genes have been identified for various muscular dystrophies. In some cases, mutations in genes that are expressed throughout the body produce a selective pathology in striated muscles (Brown et al., 2001; Watchko et al., 2002). Conversely, some muscular dystrophies are the result of mutations in genes expressed predominantly or exclusively in striated muscles (Brown et al., 2001; Watchko et al., 2002). In general, gene mutations can cause muscular dystrophies by compromising sarcolemmal integrity or cytoskeletal structure within striated muscle (Watchko et al., 2002). Mutations in the cytoskeletal structural support proteins dystrophin and laminin α-2 are 2 of the most common and severe of the muscular dystrophies.

In general, the absence of an integral protein in muscular dystrophies compromises the structural integrity of striated muscle by disrupting the normal arrangement of cytoskeletal proteins at the sarcolemma and/or basal lamina, including the sarcoglycan or dystroglycan complexes. The dystroglycan complex includes α-dystroglycan, β-dystroglycan, dystrophin, and laminin-2 (Watchko et al., 2002). Muscle laminin-2 is a heterotrimeric protein (frequently referred to as merosin) comprised of α2-, β1-, and γ1-laminin-2 proteins (Watchko et al., 2002). The sarcoglycan complex is a cluster of transmembrane proteins, including α-, β-, γ-, and δ-sarcoglycan, which interact with β-dystroglycan. Dystroglycan and sarcoglycan complexes are hypothesized to provide a functional link between the basal lamina (through the sarcolemma) to the N-terminal end of F-actin, thereby providing cytoskeletal structural support. The functional coupling of this protein network protects muscle integrity during periods of mechanical stress, such as repeated cycles of contraction and relaxation. A number of other proteins that act as important cellular signaling effectors are associated with dystrophin (or other proteins in the complex) including nitric oxide synthase (NOS), calmodulin and growth factor receptor-bound protein 2 (Grb2) (Grozdanovic et al., 1997; Bia et al., 1999; Russo et al., 2000; Wehling et al., 2001; Oak et al., 2003).

Dystrophin is a very large (427 kD) protein associated with the cytoplasmic face of the sarcolemma. Because dystrophin binds to both F-actin and β-sarcoglycan, it is the primary link between the extracellular matrix and the myofiber. In the absence of dystrophin, the expression of dystrophin associated proteins are severely compromised and/or inappropriately localized within the cell (Matsumura & Campbell, 1994; Muller et al., 2001). Dystrophin plays a critical role in stabilizing the dystrophin associated proteins at the muscle membrane, since the dystrophin-glycoprotein complex is restored in extremely rare dystrophin-positive (revertant) muscle fibers in dystrophic muscle (Matsumura et al., 1993).

Mutations in the dystrophin gene located on chromosome Xp21, which lead to a complete absence of the protein or a loss of critical functional domains result in Duchenne muscular dystrophy (DMD) (Hoffman et al., 1987). DMD is an X-linked recessive disorder that has been reported to be the most common lethal inherited childhood disease, affecting approximately 1 in 3500 live male births (Blake et al., 2002; Tidball & Wehling-Henricks, 2004). DMD patients generally have up to 40-fold higher levels of serum creatine kinase (CK), and striated muscles have fibrotic and fat infiltration, highly variable myofiber CSAs with many centrally nucleated nuclei (Hoffman et al., 1988). DMD patients have progressive and severe muscle wasting and weakness, are wheelchair bound before their teenage years, and usually die of respiratory or cardiac failure in their 20s (Hoffman et al., 1988).

Mutations in the gene encoding laminin α2 can result in merosin-deficient congenital muscular dystrophy (MD-CMD), another severe form of muscular dystrophy with a very early onset (Pegoraro et al., 1998). A number of mutations that cause the absence or a truncated form of laminin α2 have been identified in MD-CMD patients (Guicheney et al., 1997; Allamand & Guicheney, 2002). MD-CMD patients have comprehensive myofiber breakdown near the time of birth and severe muscular wasting and weakness ensuing, since the extent of muscle degeneration greatly exceeds the regenerative potential (Pegoraro et al., 1998). Currently, there is no effective treatment for this disease and it often leads to death in early childhood (Allamand & Guicheney, 2002; Emery, 2002). Given that the primary defect
in many of the muscular dystrophies is a mutation in a single gene, genetic manipulation will eventually have tremendous implications for these diseases. Indeed recent viral delivery methodologies show promise in small animals, including mouse models of DMD (Gregorevic et al., 2004a; Wang et al., 2005; Gregorevic et al., 2006; Inagaki et al., 2006). However, significant problems in vector design and delivery must be overcome for these methodologies to reach widespread clinical use in humans (Scott et al., 2002). As such, there is an immediate need for clinically relevant treatment strategies for muscular dystrophies.

2.4. Impaired neural transmission

Skeletal muscle degeneration can occur when neural innervation becomes impaired through injury, surgery, exposure to various medications (intravenous corticosteroids and neuromuscular blocking agents), or that associated with numerous pathologies, including (but not limited to) spinal muscular atrophy, Bell’s palsy, poliomyelitis, and amyotrophic lateral sclerosis (ALS; Krivickas et al., 2002; Gordon et al., 2003; Thomas & Zijdewind, 2006). Regardless of the initiating event, denervation of skeletal muscle leads to rapid muscle wasting and weakness (Finol et al., 1981; Viguie et al., 1997; Huey & Bodine, 1998; Patterson et al., 2006).

Neural innervation is required for the development and survival of skeletal muscle fibers and in addition plays an important role in the maintenance of both muscle mass and fiber type proportions. Whilst the previously described proteolytic pathways, Ca2+-dependent calpains, lysosomal cathepsins, and ubiquitin-proteasome pathway, play an important role in denervation-induced muscle atrophy (Weinstein et al., 1997; Batt et al., 2006), recent evidence has implicated apoptosis (programmed cell death) as a contributing mechanism. Migheli et al. (1997) have shown that apoptosis is associated with skeletal muscle denervation, but not other pathological conditions such as the dystrophinopathies or inflammatory myopathies. However, Tews and Goebel (1997) demonstrated high levels of the proapoptotic marker Bax in denervated muscles from patients with spinal muscular atrophy.

Any imbalance in the numerous pro- and antiapoptotic factors will initiate a chain of events leading to cell death (for review, see Sandri, 2002; Tews, 2005). The BCL-2 family has been proposed as an important regulator of apoptosis and is divided into 3 distinct subgroups: anti-apoptotic, including Bel-2, Bel-X1, Bel-W, A1, and Mcl-1; multidomain proapoptotic, including Bax, Bak, and Bok; and BH3-only proapoptotic, which includes Bad, Bid, Bim, Bik, Dp5/Hrk, Noxa, and Puma (Chao & Korsmeyer, 1998; Danial & Korsmeyer, 2004).

The 2 well-documented members of the BCL-2 family of apoptotic markers are Bel-2 and Bax. Bax has been found to heterodimerize with Bel-2 and homodimerize with itself (Olvai et al., 1993). Importantly, when Bax forms a heterodimer with Bel-2, apoptosis is suppressed; thus the ratio of Bel-2 and Bax in a cell is important (Rosse et al., 1998; Antonsson et al., 2000). When a Bax homodimer is formed it increases permeability of the mitochondrial outer membrane, resulting in the release of cytochrome c (amongst others, Kluck et al., 1997; Kuwana et al., 1998; Antonsson et al., 2000) and the activation of the caspase (cysteine proteases which cleave after aspartic acid) family of proteases (Kluck et al., 1997; Kuwana et al., 1998). Caspsases are believed to be the effectors of cell death and include caspase 9, which activates downstream caspases 2, 3, 6, and 7 (Slee et al., 1999). Caspases target and destroy specific proteins within the cell, resulting in the active cellular self-destruction characteristic of apoptosis.

The most commonly used animal model of muscle denervation is ‘sciatic nerve resection’ in the rat, and it involves the removal of a 5–10 mm section of the sciatic nerve that innervates the entire hindlimb (Dow et al., 2005; Csukly et al., 2006). The loss of neural innervation to skeletal muscle results in numerous biochemical, morphological, and functional changes, resulting in significant muscle fiber atrophy and denervation (Finol et al., 1981; Carlson & Faulkner, 1996; Viguie et al., 1997; Weinstein et al., 1997; Huey & Bodine, 1998; Batt et al., 2006).

Patterson et al. (2006) examined denervation-induced changes to rat fast- and slow-twitch skeletal muscle fibers between 0 and 50 days and found that in addition to the previously well-documented rapid and marked atrophy of skeletal muscles, there was a significant shift in muscle phenotype towards a more ‘intermediate’ phenotype consisting of fast–slow hybrid fibers. Long-term denervation was associated with a decrease in the force producing capacity of extensor digitorum longus (EDL) and soleus muscle fibers which accounted for the decrease in specific (normalized) force producing capacity of denervated muscles (Finol et al., 1981).

Regardless of the initiating event, the loss of neural innervation to skeletal muscle results in a decrease in muscle mass and altered contractile properties through multiple signaling pathways, including apoptosis. Denervation is one of the few muscle wasting conditions where apoptosis plays a significant role in the etiology of muscle wasting and weakness.

3. Interventions for muscle wasting

Developing therapeutic interventions to prevent or reverse muscle wasting and weakness associated with the aforementioned conditions is of increasing importance for 2 reasons. Firstly, patients suffering from severe muscle wasting and weakness often require the use of all their muscle strength to complete even simple tasks, such as rising from a chair, and thus can lose their functional independence rapidly. In the most extreme cases, muscle wasting and weakness is associated with increased morbidity and mortality (Metter et al., 2002). Secondly, the associated socioeconomic impact of muscle wasting and weakness places an ever-increasing burden on healthcare systems and primary caregivers. The following section describes a number of therapeutic interventions for muscle wasting and weakness. Some of these are used clinically, whilst others are in preclinical testing. Importantly, unique strategies to combat skeletal muscles wasting may be required for specific conditions, since the underlying mechanisms responsible for muscle wasting may differ in each disease.
3.1. Exercise

Some muscle wasting disorders are characterized by a slow but progressive loss of muscle mass, such as that concomitant with advancing age, whereas other conditions such as cachexia are usually associated with a much more rapid loss of muscle structure and function. The loss of muscle mass is sometimes complicated and hastened by other medical conditions (e.g., cardiovascular disease) and medications (such as β-blockers) that can potentially prevent or reduce the capacity of some people to perform physical activity (Peel & Mossberg, 1995), which then can feed a vicious circle of progressive impairment and loss of functional independence.

Physical activity can provide beneficial effects for many muscle wasting disorders, most especially sarcopenia where exercise can confer long-term benefits on muscle function, reducing the frequency of falls and promoting independence and quality of living for the elderly (Singh, 2002; Lynch, 2004a, b,c). Resistance (strength) training is the most effective exercise for slowing the rate of loss of muscle mass and maintaining or improving muscle strength, whereas aerobic exercise training has important beneficial effects for cardiovascular fitness. An exercise prescription involving resistance training for muscle strength, aerobic exercise for maintaining cardiovascular fitness, as well as flexibility and balance exercises, has been proposed as the best combination for maintaining independent living (Singh, 2002). Although exercise alone cannot stop or completely reverse the effects of many disease states on muscle structure and function, it is generally agreed that it can slow down these effects and should be considered an important intervention wherever possible (depending on the level of functional impairment). Other factors, such as alterations in circulating levels of muscle anabolic hormones and growth factors, must also be considered when developing strategies to combat muscle wasting. Thus, nutritional and/or drug strategies are also needed to help attenuate the loss of muscle mass and restore muscle size and strength so as to maintain or improve patient quality of life.

3.2. Nutritional

Muscle wasting is associated with reduced protein synthesis, increased protein breakdown (catabolism), and increased oxidative cell damage. One strategy that may have immediate clinical application for muscle wasting is nutritional intervention, including the administration of protein powders and specific amino acids for attenuating muscle protein losses and providing an intracellular milieu favorable for promoting protein synthesis (Dorrens & Rennie, 2003; Volpi et al., 2003; Yanarheski, 2003). Most research in this area has focused on nutritional interventions for the muscle wasting associated with the muscular dystrophies and cancer cachexia, although similar strategies will have application for a broad range of muscle wasting disorders.

For cancer cachexia early intervention with nutritional supplementation may improve outcome in some patients (Argiles, 2005). Nutritional supplements containing anti-inflammatory agents such as polyunsaturated fatty acid (PUFA) and eicosapentanoic acid (EPA) have been shown to be more beneficial to malnourished patients than nutritional supplementation alone. Although not consistent across all studies, EPA supplementation has shown to attenuate wasting, improve immune function, to have some antitumor effects and to improve clinical outcomes (van Bokhorst-de van der Schueren, 2005).

Alteration of glutamine metabolism is correlated with aging and is thought to reflect increased proteolysis in aged as well as in diseased skeletal muscle and in critically ill patients (Gamrin et al., 1996; Parton et al., 2003; Stuurenb erg et al., 2006). In severe muscular dystrophies like DMD, the loss of muscle mass is associated with significant protein wasting, as evident from a negative whole body leucine balance, and a significant decrease in glutamine availability in the postabsorptive state (Hankard et al., 1999). Glutamine has been proposed to be a ‘conditionally essential’ amino acid in DMD (Hankard et al., 1998, 1999).

Acute oral administration of glutamine to DMD patients had a protein-sparing effect, decreasing estimates of whole body protein degradation and de novo glutamine synthesis (Hankard et al., 1999). Oral glutamine or amino acid supplementation over 10 days inhibited whole-body protein degradation equally in DMD (Mok et al., 2006). In most cases, larger trials of nutrition interventions for DMD patients have been unsuccessful, with no improvements in manual and quantitative measurements of muscle strength (Escolar et al., 2005). Similar conclusions were drawn from a review of amino acid feeding studies for patients with ALS where L-leucine, L-valine, and L-isoleucine or, alternatively, L-threonine have been used as experimental interventions with no evidence for an effect of either treatment on muscle strength or disability (Parton et al., 2003). With sarcopenia and perhaps for neuromuscular diseases, one of the major problems with loss of muscle during advancing age is an inability of muscle to respond adequately despite an increased availability of nutrients (Wackerhage & Rennie, 2006).

The potential for creatine monohydrate supplementation to increase lean body mass makes it relevant for application to the treatment of muscle wasting conditions (Persky & Brazeau, 2001; Groeneveld et al., 2003; Payne et al., 2006a,b). After cellular uptake, creatine is phosphorylated to phosphocreatine (PCr) by CK reaction using ATP. At subcellular sites with high-energy requirements (e.g., at the myofibrillar apparatus during muscle contraction), CK catalyses the phosphorylation of PCr to ADP to regenerate ATP. PCr is therefore available as an energy source, serving as an energy buffer and an energy transport vehicle. Additional benefits of creatine supplementation have also been observed for high-intensity long-endurance tasks (e.g., shortening of recovery periods after physical exercise; Terjung et al., 2000), which may help patients in the performance of the tasks of daily living. Creatine supplementation has been advocated for older adults, since it has been shown to enhance muscle strength even after only 5–7 days of administration (Tarnopolsky, 2000; Gotshalk et al., 2002). Creatine supplemented to food (10%) of pregnant mdx dystrophic mice and then to pups, strongly decreased the muscle histopathology in young mdx mice at 24, 28, and 34 days of age (Passaquini et al., 2002).
In contrast, in exercised adult mdx mice no improvement in muscle histology was evident after creatine feeding of mice for 4–8 weeks (De Luca et al., 2003). Tarnopolsky et al. (2004) showed that 4 months of creatine supplementation increased lean body mass and handgrip strength, and a reduced marker of bone breakdown was well tolerated in children with DMD. Creatine supplementation has thus been found efficacious for patients with muscular dystrophy and mitochondrial myopathies, capable of attenuating the age-related loss of muscle mass, and helpful for facilitating rehabilitation after disuse atrophy (Pearlman & Fielding, 2006).

Studies have shown that supplementation with β-hydroxy-β-methylbuturate (HMB), a metabolite of leucine, can have beneficial effects for increasing muscle strength. Giving HMB to older adults involved in resistance training, increased their strength, and decreased fat mass (Panton et al., 2000; Vukovich et al., 2001). HMB has also been proposed for treating patients with muscle wasting disorders. For example, combining the amino acids arginine and glutamine with HMB reversed lean tissue loss in cancer and AIDS patients (Clark et al., 2000; Rathmacher et al., 2004). Studies have shown that HMB can preserve lean body mass and attenuate protein degradation through downregulation of the increased expression of key regulatory components of the ubiquitin-proteasome proteolytic pathway, together with stimulation of protein synthesis (Smith et al., 2004a,b). No studies have yet investigated the effects of HMB administration on patients with muscular dystrophy or other conditions involving dysfunctional muscle repair after injury. Although nutritional strategies have merit in attempting to attenuate muscle wasting, they are most effective when combined with exercise (wherever possible) and a complementary approach for drug therapies for treating severe wasting disorders. The focus of the review thus shifts to manipulating the pathways regulating muscle mass through targeted drug approaches and/or improvement of cell-mediated therapy.

One potential GH secretagogue that has been suggested as a potential therapeutic strategy for muscle wasting conditions is ghrelin (Janssen et al., 2004). Ghrelin has been observed (at high pharmacological doses) to stimulate the GH/IGF-I axis; however, a clear link between increased levels of ghrelin and skeletal muscle growth has yet to be established (Janssen et al., 2004).

Much of the work characterizing the effects of IGFs on myogenesis has been conducted in vitro and it is evident that IGFs (similar to many other growth factors) may either stimulate or inhibit muscle precursor cell differentiation depending on its concentration, the various cell lines utilized, and the constituents of the serum/medium (Florini et al., 1986). Similarly insulin-like growth factor II (IGF-II) has been shown to affect both proliferation and differentiation during myogenesis. IGF-II is important for myoblast differentiation (Fiorini et al., 1991) and act as a critical survival factor for myoblasts during the transition from proliferating to differentiating cells (Stewart & Rotwein, 1996). IGF-II has also been reported to increase myoblast proliferation, an effect dependent on the dose used in vitro (Fiorini et al., 1986; Bach et al., 1995). In fact, the protein fragments of (bovine) IGF-II involved in promoting myogenic precursor cell proliferation have been characterized partially (Konishi et al., 1989). Compared to IGF-I, it has been shown that IGF-II is a more potent differentiation factor (Ewton et al., 1994). This effect may be due to the greater mitogenic effect of IGF-I, which could effectively delay differentiation (Ewton et al., 1994). IGF-II has been shown to reduce levels of fibroblast growth factor receptor 1 (FGFR1) in proliferating myoblasts, whereas IGF-I stimulates FGFR1 expression (Sheehan & Allen, 1999). Since FGFR1 mediated signaling is known to be a
potent stimulator of myoblast proliferation and inhibitor of differentiation (Allen & Boxhorn, 1989), downregulation of FGFR1 by IGF-II may be one of the mechanisms underlying the differential effects of IGF-II and IGF-I on myoblast proliferation or differentiation. In fact, this effect may be important in explaining the synergistic effect of FGF and IGF-I coadministration on increasing myoblast proliferation, whereas IGF-I and IGF-II do not have synergistic effects (Doumit et al., 1993).

Studies conducted in vivo have demonstrated that IGF-II delayed the early stages of myogenesis, whilst enhancing later stages (i.e., during differentiation into myotubes) as evidenced by an increased CSA of myotubes, 7 days after intramuscular injection of the myotoxic agent notexin (Kirk et al., 2003). The IGF-II-mediated delay in early stages of myogenesis was associated with decreased MyoD and myogenin expression 1 and 2 days after myotoxic injury, respectively (Kirk et al., 2003). In addition, histological estimations showed that IGF-II administration was associated with fewer myogenic precursor cells and a larger proportion of phagocytes (Kirk et al., 2003). These results indicate that provision of exogenous IGF-II at a time point when endogenous mechanisms increase its expression (i.e., during differentiation into myotubes) may enhance myogenesis (Kirk et al., 2003).

IGF-I is increased dramatically during the formation of new myofibers in regenerating skeletal muscle (Jennische & Hansson, 1987; Marsh et al., 1997). IGF-I has been shown to increase both the proliferation and differentiation of satellite cells (Barton-Davis et al., 1999) and alter the timing of myogenic regulatory factors (MRF) and other cell cycle related proteins involved in myogenesis (Engert et al., 1996). IGF-I also causes hypertrophy of existing myofibers through IGF-I receptor mediated pathways that are related to increased protein synthesis and reduced protein degradation (Frost et al., 1997; Bark et al., 1998; Rommel et al., 2001; Stitt et al., 2004). Activation of the IGF-I receptor and intracellular signaling through phosphoinositide 3 kinase (PI3K)-Akt/PKB-mammalian target of rapamycin (mTOR) and inhibition of glycogen synthase kinase 3β (GSK3β) is involved in the muscle hypertrophy response (Fig. 3; Rommel et al., 2001). Similarly, IGF-I receptor-mediated activation of PI3K-Akt/PKB and inhibition of forkhead box O (FOXO) transcription factors and muscle specific ubiquitin ligases (i.e., atrogin/MAFbx-1...
and MuRF1) are involved in the anticatabolic responses (Fig. 3; Stitt et al., 2004).

Whilst the exact pathway linking PI3K to p70S6K, a positive regulator of protein translation (Rommel et al., 2001) is not known, it is believed that PI3K phosphorylates the membrane phospholipid phosphatidylinositol-4,5-bisphosphate, creating a lipid-binding site on the cell membrane for a serine/threonine kinase called AKT1 (the predominant skeletal muscle subtype, also referred to as protein kinase B). AKT1 is phosphorylated at the membrane by the kinase 3′-phosphoinositide-dependent protein kinase (PDK) 1. Once activated, AKT1 phosphorylates a number of proteins involved in protein synthesis, gene transcription and cell proliferation and survival (Bodine et al., 2001b; Rommel et al., 2001; Pallafacchina et al., 2002). One such pathway involves AKT1 activation of the mTOR pathway and eventually increases activation of p70S6K, a positive regulator of protein translation, and inhibits PHAS-1 (4E-BP1), a negative regulator of the protein elf4E (Nave et al., 1999; Lai et al., 2004). p70S6K activation mediates the phosphorylation of the 40S ribosomal S6 protein, which leads to an upregulation of the translation of mRNA encoding for ribosomal proteins and elongation factors (Jefferies et al., 1997).

Other signaling pathways activated through PI3K-AKT1 phosphorylation include GSK3 (Bodine et al., 2001b) and FOXO1, a member of the forkhead class of transcription factors (Sandri et al., 2004). GSK3 is inactivated by AKT1 phosphorylation, and since GSK3 normally acts to inhibit the translation initiation factor elf2B, blockade of GSK3 by AKT1 might promote translation initiation and protein synthesis (Bodine et al., 2001b; Rommel et al., 2001). AKT1 signaling is involved not only in “muscle hypertrophic” pathways, but it has been implicated in the inhibition of “muscle atrophy” signaling pathways. AKT1 inactivation of FOXO1 leads to nuclear exclusion and the inhibition of the forkhead transcriptional program, which is necessary for the induction of both MuRF1 and atrogin/MAFbx (Sandri et al., 2004; Stitt et al., 2004). Both muRF1 and atrogin/MAFbx encode ubiquitin ligases, which function to conjugate ubiquitin to protein substrates (Bodine et al., 2001a), and have been shown to be upregulated in numerous models of muscle atrophy (Bodine et al., 2001a; Gomes et al., 2001; Wray et al., 2003). Thus, by inactivating FOXO1, AKT1 blocks the induction of FOXO1-mediated atrophy signaling (Fig. 3).

IGF-I is a critical growth factor during the initial, early, and later stages of myogenesis and has been shown to stimulate muscle precursor cell proliferation, differentiation, and fusion (Semsarian et al., 1999; Jacquemin et al., 2004; Machida & Booth, 2004). As such, the growth promoting aspects of IGF-I have been used to ameliorate the decline in skeletal muscle mass and function in various muscle wasting conditions (Barton-Davis et al., 1998; Barton et al., 2002; Shavlakadze et al., 2004, 2005). The effects of IGF-I administration in vivo appear to be dependent on the age of the host, IGF-I isoform used, and dose and/or route of administration (Barton et al., 2002; Gregorevic et al., 2002a,b, 2004b; Barton, 2006). Models of high-level IGF-I over-expression (e.g., transgenic mice) have indicated clearly that IGF-I causes skeletal muscle hypertrophy and increases force generating capacity in mdx dystrophic mice (Barton et al., 2002) and can attenuate muscle wasting in various conditions such as aging (Barton-Davis et al., 1998), chronic left ventricular dysfunction (Schulze et al., 2005), excessive angiotensin II levels (Song et al., 2005), and denervation (Shavlakadze et al., 2005). Conversely, data from our laboratory have demonstrated that the predominant effect of systemic administration of recombinant IGF-I protein at a relatively low dose (~ 1.5 mg/kg/day) to mdx mice was to induce a shift toward more oxidative, fatigue resistant, slow-twitch muscle phenotype as evidenced from increased muscle fiber succinate dehydrogenase (SDH) activity (Gregorevic et al., 2002a,b, 2004b; Schertzer et al., 2006) and a prolonged time course of the isometric twitch in the EDL and soleus muscles (Gregorevic et al., 2004b). Interestingly, these changes occurred in the absence of changes to myofiber CSA, muscle mass, or maximum force producing capacity (Gregorevic et al., 2002a,b, 2004b; Schertzer et al., 2006). The comparison between transgenic animals and (a potentially viable therapeutic approach such as) recombinant protein delivery highlight the disparities in different models of endocrine factor delivery and warrants further characterization. Specifically, the role of insulin-like growth factor binding proteins (IGFBP), which could limit or potentiate the bioavailability of IGF-I (and IGF-II) must be characterized, since they could play an important role in IGF-related therapeutics.

Stimulation of the IGF-I pathway in skeletal muscle may be a particularly relevant therapeutic approach in specific skeletal muscle wasting conditions, such as sarcopenia. The results to date suggest that IGF-I can simultaneously counteract many of the age-related changes in skeletal muscle including decreased mass, absolute force production, specific force production, and motor unit remodeling (described in Section 2.1). Viral-mediated over-expression of IGF-I reverses the age-related reduction in mass and absolute force production the skeletal muscles of old mice (Barton-Davis et al., 1998). Furthermore, it has been demonstrated that transgenic over-expression of IGF-I reverses the age-related decline in specific force of mouse flexor digitorum brevis (FDB) single fibers and reversed the age-related decline in the magnitude of intracellular Ca2+ transients induced by action potentials (Gonzalez et al., 2003). This effect is likely associated with a restoration in specific EC coupling components, which are compromised in aged skeletal muscle. Aging is associated with a decrease in dihydropyridine receptors (DHPR) and uncoupling of the normal ratio of DHPR to ryanodine receptors (RyRs) (Renganathan et al., 1997a,b). Transgenic mice that over-express IGF-I had a significant (52%) increase in the number of DHPRs in EDL muscles (Renganathan et al., 1997b). IGF-I has been shown to increase the transcription of the DHPRα1 subunit by inducing a calmodulin kinase/calcineurin-mediated signaling cascade that culminates in cAMP response element binding protein (CREB) binding to the promoter region of the DHPR α1 subunit gene in order to increase the rate of transcription (Zheng et al., 2002, 2004). Thus, it appears that IGF-I can restore the specific force of aged muscle fibers by increasing specific components of EC coupling involved in charge movement and intracellular Ca2+ regulation.
IGF-I is also a particularly relevant therapeutic agent for aging skeletal muscle because of its neurogenic effects. Transgenic over-expression of IGF-I in mice has been shown to attenuate some of the characteristic age-related motor unit remodeling (Messi & Delbono, 2003). Furthermore, in an approach that is more relevant to therapeutic application, it has been demonstrated that (spinal cord) motor neuron targeting of IGF-I (by fusing a tetanus toxin fragment-C to recombinant IGF-I protein) reverses the age-related decline in specific force of mouse single fibers (Payne et al., 2006a,b). These findings demonstrate the multiple ways that IGF-I can attenuate age-related deleterious changes to skeletal muscle and provide insight into future therapeutic interventions.

Although stimulation of the IGF-I pathway has significant potential in some muscle wasting conditions, provision of exogenous IGFs (or insulin) with the aim to ameliorate muscle wasting may be futile in certain conditions or disease states associated with increased proinflammatory cytokines. A proinflammatory cytokine-mediated state of acquired “IGF resistance” may negate the anabolic (and anabolic) actions of IGF-I in skeletal muscle. Specifically, tumor necrosis factor α (TNF-α) and IL-1β have also been shown to induce IGF-I resistance and inhibit protein synthesis in proliferating myoblasts (Broussard et al., 2003, 2004; Strle et al., 2004). Even at very low concentrations, TNF-α impairs IGF-I-induced protein synthesis in primary myoblasts and C2C12 cells (Broussard et al., 2003). Similarly, low levels of IL-1β inhibit IGF-I-mediated increases in protein synthesis in myoblasts and C2C12 cells (Broussard et al., 2004). TNF-α and IL-1β also reduce protein levels of myogenin (Broussard et al., 2003, 2004), thereby providing a mechanism of reduced myogenic differentiation potential of muscle cells during proinflammatory cytokine exposure. Suppression of MyoD (Langen et al., 2004) and myogenin (Broussard et al., 2003, 2004) may be the critical effectors of proinflammatory cytokine-mediated inhibition of proliferation and differentiation during myogenesis.

The IGF-I resistance induced by TNF-α and IL-1β is not mediated by the ability of IGF-I to activate the intrinsic tyrosine kinase activity of the IGF-I receptor (Broussard et al., 2003, 2004). TNF-α and IL-1β reduce tyrosine phosphorylation of insulin-receptor substrate (IRS)-1 and IRS-2 proteins, independent of changes to the IGF-I receptor phosphorylation state (Broussard et al., 2003, 2004). The intracellular cascades responsible for proinflammatory cytokine-mediated IGF-I resistance are not fully characterized, but appear to involve ceramide from both de novo- and sphingomyelinase (SMase)-dependent pathways (Strle et al., 2004). In addition, c-Jun amino-terminal kinases (JNK) play a central role in TNF-α-induced IGF-I resistance in myoblasts in a remarkably similar way to insulin resistance in obesity and type II diabetes (Hirosumi et al., 2002; Strle et al., 2006). Because the insulin and IGF-I receptor signaling pathways are highly homologous, including signaling through IRS-1 and IRS-2 docking proteins, much of the recent work delineating the role of inflammation and proinflammatory cytokine-induced insulin resistance in skeletal muscle may be applicable to IGF-I resistance, growth retardation, cachexia, and skeletal muscle wasting (Yamamoto et al., 1992; Baltensperger et al., 1993; Blenis, 1993; Myers et al., 1993; Skolnik et al., 1993; Sun et al., 1993). These pathways may involve extracellular signals such as prolonged high levels of free fatty acids (FFA), glucose and intracellular signals such as the generation of reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, JNKs, IκB kinase (IKK), protein kinase C (PKC)-θ, suppressors of cytokine signaling (SOCS) proteins, and a potential positive feed back cycle involving an activator protein 1 (AP-1) and/or nuclear factor κB (NF-κB)-mediated increase in the transcription of proinflammatory cytokines (Fig. 3; Aggarwal, 2000; Qi & Pekala, 2000; Baud & Karin, 2001; Yu et al., 2002; Bennett et al., 2003; Papa et al., 2004; Ueki et al., 2004; Varfolomeev & Ashkenazi, 2004; Beere, 2005; Kaneto et al., 2005; Wellen & Hotamisligil, 2005). Further investigation of the intracellular pathways involved in proinflammatory cytokine-mediated IGF-I resistance is warranted and may aid in characterization and treatment of specific muscle wasting disorders, particularly in the tailoring of treatments for conditions involving upregulation of proinflammatory cytokines. Given evidence that at least some of the intracellular effectors of GH are independent of IGF-I upregulation (Sotiropoulos et al., 2006), stimulation of the GH-receptor pathway may be an attractive target in certain muscle wasting conditions in order to circumvent the potential for IGF-I resistance.

3.4. Testosterone and selective androgen receptor modulators

Recent well-controlled, double-blinded studies have demonstrated unequivocally that androgens, such as testosterone, regulate muscle mass in humans (Herbst & Bhasin, 2004); however, the effect of androgens on subsequent muscle strength is less clear (Axell et al., 2006). Satellite cells and myonuclei are the predominant sites of AR expression (Altuwaijri et al., 2004; Sinha-Hikim et al., 2004) and androgen administration increases satellite cell numbers in animals and humans in a dose-dependent manner. Androgens also increase androgen receptor levels in satellite cells which may enhance the sensitivity of satellite cells to androgen treatment (Chen et al., 2005). To date, the mechanisms by which androgens might increase satellite cell numbers are not known, but it is thought that they may potentially regulate satellite cell proliferation and differentiation (Chen et al., 2005).

Anecdotal evidence of muscle anabolism and enhanced physical performance in steroid-using athletes suggests that increased muscle bulk is explicitly linked to enhanced muscle function (Wilson, 1988). In humans, there is clear evidence that physiological testosterone administration increases lean body mass in conditions of low circulating androgens. Testosterone replacement therapy has been used effectively to counteract loss of lean body mass in hypogonadal men (Brodsky et al., 1996; Bhasin et al., 1997), in older men with normal or low serum testosterone (Snyder et al., 1999, 2000; Ferrando et al., 2002), and HIV-infected men with low serum testosterone (Bhasin and Javanbakht, 1999). Similarly, muscle growth has been achieved in euonadal states after supraphysiological administration to young, healthy men (Griggs et al., 1989; Bhasin et al., 1996), and HIV-infected men with normal testosterone levels (Fairfield et al., 2005; Wellen & Hotamisligil, 2005). Further investigation of the intracellular pathways involved in proinflammatory cytokine-mediated IGF-I resistance is warranted and may aid in the characterization and treatment of specific muscle wasting disorders, particularly in the tailoring of treatments for conditions involving upregulation of proinflammatory cytokines. Given evidence that at least some of the intracellular effectors of GH are independent of IGF-I upregulation (Sotiropoulos et al., 2006), stimulation of the GH-receptor pathway may be an attractive target in certain muscle wasting conditions in order to circumvent the potential for IGF-I resistance.
et al., 2001). However, although some studies have demonstrated enhanced muscle strength following androgen administration (Schroeder et al., 2003), others have failed to detect a significant functional effect of androgen therapy despite gains in muscle mass (Wang et al., 2004).

Testosterone administration has been proposed as a possible therapy to counteract the muscle atrophy and weakness that occurs with prolonged weightlessness or bed rest; however, its ability to ameliorate the loss of muscle mass and preserve strength (in the latter condition) has not been demonstrated. In patients with myotonic dystrophy, a condition where muscle wasting may be associated with low circulating levels of adrenal androgens, testosterone supplementation increased muscle mass but did not improve muscle strength (Griggs et al., 1989). Similarly, early studies using anabolic steroids as a means of improving the dystrophic pathology in DMD generally proved unsuccessful. However, a pilot trial examining the effects of the anabolic steroid oxandrolone (Oxandrin) on the muscle strength of 10 boys with DMD (Fenichel et al., 1997) led to a larger 6-month, randomized, double-blind, placebo-controlled study initiated by the Muscular Dystrophy Association (USA; Fenichel et al., 2001). Although oxandrolone did not produce a significant change in the average manual muscle strength score compared with placebo, the average of 4 quantitative muscle tests showed a significant improvement in the oxandrolone-treated boys compared with placebo. Furthermore, no adverse reactions attributable to oxandrolone were recorded. The study concluded that since oxandrolone had some beneficial effects in slowing the progression of muscle weakness, it may be useful before initiating corticosteroid therapy, the current mainstay of treatments for this very severe muscle disease (Fenichel et al., 2001). Other studies have suggested another benefit of oxandrolone treatment could be a reduction in muscle degeneration in DMD patients (Balagopal et al., 2006). In the US, oxandrolone is the only anabolic androgenic steroid that is US FDA-approved for restoring weight loss after severe trauma, major surgery or infections, malnutrition due to alcoholic cirrhosis, and muscular dystrophies (Orr & Fiatarone Singh, 2005). Oxandrolone treatment has been used for other muscle wasting disorders including burns (Przkora et al., 2005), catabolic disorders, HIV and AIDS-related wasting, neuromuscular and other disorders (Orr & Fiatarone Singh, 2005). It has been suggested that further assessment of the clinical efficacy of oxandrolone will require closer scrutiny of optimal risk/benefit ratios for oxandrolone and other anabolic steroids before they can be recommended for more widespread therapeutic use for other chronic wasting conditions, including sarcopenia (Orr & Fiatarone Singh, 2005).

Another class of compounds claimed beneficial for the treatment and prevention of muscle wasting are selective androgen receptor modulators (SARM) (Gao et al., 2006). These compounds are a series of nonsteroidal ligands that have been synthesized as second-generation agonists for the androgen receptor. Preclinical studies in rats have shown that some of these SARMs exhibit significant anabolic activity while having only moderate to minimal androgenic activity in vivo (Marhefka et al., 2004). It has been proposed that SARMs may provide muscle and bone enhancing qualities but minimize or eliminate the potential cardiovascular and prostate cancer risks often associated with testosterone therapy (Segal et al., 2006). Further preclinical studies are warranted to determine the efficacy of SARMs for attenuating muscle wasting across a range of disorders and importantly to assess whether SARMs can restore muscle function once significant wasting has occurred and where weakness is indicated.

3.5. Myostatin

Myostatin, originally termed growth and differentiating factor 8 (GDF-8) was identified during a search for novel mammalian members of the transforming growth factor-β (TGF-β) family in murine skeletal muscle, and was found to be highly expressed during the latter stages of development (McPherron et al., 1997; Lee, 2004). To determine the functional role of myostatin, they disrupted the myostatin gene in embryonic stem cells to generate myostatin null mice which were ~30% larger than their littermate controls, due to a 200–300% increase in skeletal muscle mass (McPherron et al., 1997). The muscular phenotype suggested that myostatin functioned as a negative regulator of muscle growth and development. Since that seminal study, the inhibition of myostatin has received a great deal of attention as a potential therapeutic target for muscle wasting and weakness (Wagner et al., 2002, 2005; Bogdanovich et al., 2005; Li et al., 2005; Jespersen et al., 2006).

Much research has focused on understanding the signaling pathways involved in the actions of myostatin in skeletal muscle (Thomas et al., 2000; Langley et al., 2002). Similar to TGF-β, myostatin is secreted as a latent complex that undergoes 2 proteolytic processes to form the active component (Lee et al., 2004). Following these proteolytic pathways, 3 distinct subunits are formed: (1) a 24-amino acid signal peptide, important for targeting the active complex to the secretory pathway; (2) an N-terminal fragment referred to as the ‘propeptide’, which acts to regulate the active myostatin fragment and is important for the correct folding and dimerization of the active fragment; and (3) the ‘active’ C-terminal fragment (Lee & McPherron, 2001).

Myostatin circulates as a latent complex and must undergo proteolytic cleavage and disassociate from the propeptide to become active. In addition to the propeptide, 2 other molecules have been found to attach to circulating myostatin, follistatin-related gene (FLRG), and growth and differentiating-associated factor associated factor associated serum protein-1 (GASP-1). Both FLRG and GASP-1 tightly bind to the active myostatin fragment and inhibit its activity (Hill et al., 2002, 2003). In contrast to the inhibitory actions of the myostatin propeptide, FLRG and GASP-1, members of the bone morphogenetic protein-1/tolloid (BMP-1/TLD) family of metalloproteinases cause the in vivo activation of myostatin through proteolytic cleavage of the propeptide (Wolfman et al., 2003).

Myostatin has been found to have effects on cell proliferation and differentiation (Fig. 4; Thomas et al., 2000; Langley et al., 2002; McCroeskey et al., 2003). During periods of growth and/or regeneration quiescent muscle precursor cells, largely composed of satellite cells are activated. These muscle precursor
cells adopt the myogenic lineage, proliferate, and differentiate into myotubes, which eventually mature into myofibers. In addition, myogenic precursor cells can fuse to existing myotubes to promote maturation and muscle hypertrophy (for review, see Charge & Rudnicki, 2004). Myostatin inhibits satellite cell proliferation through upregulation of p21, decreases in the level of both cyclin-dependent kinase 2 (Cdk2) and phosphorylated retinoblastoma protein (Rb; Langley et al., 2002). These changes act to inhibit the cell-cycle progression of myoblasts from the G1- to S-phase (Thomas et al., 2000). Myostatin-mediated inhibition of myoblast differentiation is believed to occur due to downregulation of specific muscle regulatory factors including MyoD and myogenin (Langley et al., 2002).

The active C-terminal fragment of myostatin is known to signal through a heterodimeric complex consisting of both type I and type II serine/threonine kinase receptors (Fig. 5, Lee & McPherron, 2001; Rebbapragada et al., 2003). Myostatin has been observed to bind to the activin type II receptors (ActRIIA and ActRIIB, with a greater affinity for the ActRIIB isoform; Lee & McPherron, 2001). The binding of myostatin to the ActRIIB allows for the transphosphorylation of a type I receptor (the activin receptor-like kinase 4 or 5, ALK4 or ALK5, Rebbapragada et al., 2003). This heterodimer of ActRIIB and ALK4 (and/or ALK5) has been found to phosphorylate Smad proteins, which appear to be key regulators of myostatin signaling. Phosphorylated Smad proteins (Smad2, Smad3 and/or Smad4) enter the nucleus and regulate the expression of downstream genes (Langley et al., 2002; Rebbapragada et al., 2003).

The direct involvement of myostatin in both proliferation and differentiation make it an attractive therapeutic target for conditions where muscle wasting and weakness are indicated. Over the past 10 years significant research has been focussed on the most effective way to block myostatin signaling in vivo and thus increase muscle size and strength. To this end, various potential therapies have emerged, including myostatin neutralizing monoclonal antibodies (JA16 and RK35; Whittemore et al., 2003; Holzbaur et al., 2006), a mutant form of the myostatin propeptide that is resistant to BMP-1/TLD induced cleavage (D76A; Wolfman et al., 2003), and a soluble form of the ActRIIB (Lee et al., 2005) which causes greater hypertrophy than inhibiting myostatin directly (Lee et al., 2005). These findings suggest that there are other molecules, likely related to myostatin, that signal through ActRIIB to inhibit muscle growth and development.

Results from animal studies which have inhibited or knocked out myostatin, combined with the recently documented case of an overly muscular child with a mutated form of myostatin (Schuelke et al., 2004) suggest that myostatin inhibition may be an effective treatment strategy for muscle wasting conditions. Although the role of myostatin in the regulation of skeletal muscle mass has received a great deal of attention, its potential role in other tissues is less well documented. For example, myostatin ameliorates the phenylephrine-induced cardiac hypertrophy through inhibition of p38 and Akt (Morissette et al., 2006). Myostatin inhibition is an attractive treatment for skeletal muscle wasting and weakness but further research is required to fully elucidate the role of myostatin in other tissues.

3.6. Leukemia inhibitory factor

Leukemia inhibitory factor (LIF) is a 180-amino acid single-chain protein, named after its effect on hematopoietic cells. LIF belongs to a group of cytokines that includes ciliary neurotrophic...
factor (CNTF), IL-6, IL-11, cardiotrophin-1, and oncostatin M (Kurek, 2000). In vitro and in vivo studies on axotomy and nerve crush models demonstrated a powerful effect of LIF in enhancing survival of motor and sensory neurons, while reducing denervation-induced muscle atrophy. In models of both axotomy induced neuronal death and in the wobbler mouse (a model of motor neuron disease), LIF was active at doses as low as 1 μg/kg when delivered systemically (Kurek, 2000). In muscle, LIF increased the rate of muscle regeneration in vivo when applied exogenously after injury and stimulated intrinsic muscle repair following its targeted release to muscles of dystrophic mdx mice (Kurek et al., 1997; Austin et al., 2000). LIF has also been reported to specifically enhance myoblast proliferation in vitro and increase the number and size of myotubes in regenerating skeletal muscle in vivo (White et al., 2001; Gregorevic et al., 2002a,b). Spangenburg and Booth (2002) determined the signaling mechanisms by which LIF induced satellite cell proliferation in culture by confirming that LIF induced proliferation of C2C12 immortalized myoblasts and cultured primary rat satellite cells. They showed that LIF induced satellite cell proliferation by activation of the Janus-activated kinase 2 (JAK2)-signal transducers and activators of transcription 3 (STAT3) signaling pathway, suggesting that this may be an important pathway in muscle growth and/or hypertrophy (Spangenburg & Booth, 2002).

Although LIF enhances skeletal muscle regeneration (Kurek et al., 1997; Austin et al., 2000; White et al., 2001; Gregorevic et al., 2002a,b), its role in skeletal muscle hypertrophy is less clear. Spangenburg and Booth (2006) examined the hypertrophic ability of the plantaris and soleus muscle mass after 7, 21, and 42 days of loading. In contrast, the LIF(−/−) mice had no increases in plantaris muscle mass at any time point, and the soleus muscle exhibited a delayed hypertrophic response. Systemic delivery of LIF to the LIF(−/−) mice restored the hypertrophic response to the same levels as WT mice after 21 days of functional overload, demonstrating that LIF expression in loaded skeletal muscle was critical for the development of skeletal muscle hypertrophy in this model of functional overload (Spangenburg & Booth, 2006).

3.7. Ciliary neurotrophic factor

There is no clear association between muscle mass, neurotransmission and levels of neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), brain-derived neurotrophic factor (BDNF), or glial-derived neurotrophic factor (GDNF) especially in relation to the progressive muscle wasting with sarcopenia and denervation atrophy (Frostick et al., 1998; Stuerenburg & Kunze, 1998; Keller-Peck et al., 2001). Local blockade of neuromuscular transmission decreases levels of NT-4 mRNA in skeletal muscle, whereas increased levels of mRNA of NT-4 (Funakoshi et al., 1995), NT-3, and BDNF (Gomez-Pinilla et al., 2001) occur in response to increased activity of skeletal muscle. Like IGF-I, CNTF has both myotropic and neurotrophic effects (Guillet et al., 1999) and in mammals, both show a decline in muscle mass.

![Proposed myostatin cell signaling pathways leading to inhibition of cell growth. The ‘active’ C-terminal fragment of myostatin binds to the ActIRIB (and to a lesser extent ActIR)(1), and initiates the intracellular signaling processes through the recruitment of a type I receptor (ALK-4 or -5) (2). The formation of this complex allows for the ActIRIB mediated transphosphorylation of the type I receptor (3), which then phosphorylates the intracellular Smad proteins (Smad-2 or -3) allowing them to interact with the co-Smad protein Smad-4 (4). This heterodimeric Smad complex is able to translocate into the nucleus (5) and regulate the transcription of myostatin target genes, including genes involved in the regulation of satellite cell proliferation and differentiation (6).](https://example.com/myostatin pathways.png)
synthesis with age (Bartlett et al., 2001; Roth et al., 2001). Although CNTF does have myotrophic properties (Peroulakis & Forger, 2000), it is most well known for its neurotrophic effects where it stimulates axonal sprouting and reinnervation of denervated muscle fibers (English, 2003), participates in the survival of motor neurons, and reduces denervation atrophy of skeletal muscles (Mousavi et al., 2002). CNTF levels in old rats were restored by exogenous CNTF administration with CNTF production and muscle performance being correlated strongly (Guillet et al., 1999). Follow-up studies in rats (Frayssé et al., 2000) and humans (Roth et al., 2001) supported the notion of a strong relationship between CNTF and age-related changes in muscle mass and function. Of interest to muscle wasting disorders, particularly denervation and sarcopenia, is neurotrophic factor administration to maintain neuronal function (Mitsumoto et al., 1994; Elliott & Snider, 1996).

Administration of CNTF can retard progressive motor neuron dysfunction and improved muscle strength in wobbler mice (Mitsumoto et al., 1994) and accelerated regeneration of transected sciatic nerve and muscle reinnervation in rats (Zhang et al., 2004). Although administration of recombinant human CNTF (rh-CNTF) was found to attenuate skeletal muscle wasting in rats after unilateral transection of the sciatic nerve (Helgren et al., 1994), follow-up studies in similarly denervated rats revealed CNTF administration had a significant catabolic effect with reductions in body weight gain and reduced food intake (Henderson et al., 1994, 1996; Martin et al., 1996). Furthermore, inhibitors of the cachectic cytokines, TNF and IL-1, did not significantly alter the wasting effects of rh-CNTF indicating that it caused skeletal muscle atrophy through anorexia and cachexia (Martin et al., 1996). Recent studies have shown that CNTF induces weight loss and improves glucose tolerance in humans and rodents by central mechanisms that induce hypothalamic neurogenesis to modulate food intake and peripheral mechanisms that alter hepatic gene expression, like that of leptin. Such properties indicate that CNTF has therapeutic potential for combating lipid-induced insulin resistance (Watt et al., 2006a). CNTF has been shown to signal through the CNTFRα-IL-6R-gp130β receptor complex to increase fatty acid oxidation and reduce insulin resistance in skeletal muscle by activating AMP-activated protein kinase (AMPK), independent of signaling through the brain (Watt et al., 2006b).

3.8. Interleukins

3.8.1. IL-6

Because LIF has been shown to increase myoblast proliferation and enhance muscle regeneration (Austin et al., 1992; Barnard et al., 1994), other members of the IL-6 family of cytokines have been suggested as modulators of muscle regeneration (Charge & Rudnicki, 2004). Indeed, transcript levels of IL-6, CNTF, and their receptors are upregulated at the site of injury in regenerating skeletal muscle (Kami et al., 2000). IL-6 has been shown to stimulate myoblast proliferation in vitro, albeit to a lesser degree than LIF (Austin & Burgess, 1991; Austin et al., 1992). However, unlike LIF, exogenous delivery of IL-6 did not enhance muscle regeneration (Kurek et al., 1997). Recently, it was demonstrated that IL-6 is induced during differentiation of C2C12 myoblasts in a p38- and NFκB-dependent manner (Baeza-Raja & Munoz-Canoves, 2004). Furthermore, downregulation of IL-6 during differentiation with siRNAs decreased the muscle differentiation specific genes myogenin and α-actin (Baeza-Raja & Munoz-Canoves, 2004). Thus, recent evidence implicates IL-6 in muscle differentiation, but there are still no data to support the notion that provision of exogenous IL-6 enhances muscle regeneration or postnatal growth. The interaction between the IL-6 family of cytokines and IGFs during skeletal muscle regeneration is largely unknown but could be of great importance. IL-6 is classified as a proinflammatory cytokine and similar to other proinflammatory cytokines such as TNF-α and IL-1β, IL-6 has been reported to increase levels of the inhibitory IGFBP-1 in blood serum (Samstein et al., 1996; Benbassat et al., 1999). Therefore, IL-6 may act to inhibit the actions of IGF signaling during myogenesis and muscle growth. Although speculative, IL-6 may also contribute to acquired IGF-1 resistance in a similar manner to other proinflammatory cytokines such as TNF-α and IL-1β (Broussard et al., 2003, 2004; Strle et al., 2004, 2006). The role of IL-6 in skeletal muscle wasting conditions is unclear based on the results to date, but little evidence supports it use as therapeutic.

3.8.2. IL-15

IL-15 was initially characterized as a T-cell growth factor that interacts with the beta and gamma subunits of the IL-2 receptor and was found to be highly expressed in skeletal muscle (Giri et al., 1994, 1995). The original characterization of IL-15 effects on skeletal muscle cultures showed that it did not alter myoblast proliferation or differentiation but significantly increased protein accretion (of contractile proteins such as MyHC) in differentiated myotubes (Quinn et al., 1995). Subsequently, IL-15 was shown to induce differentiation of clonal cells that were specifically engineered to inhibit the strong differentiation-inducing effects of IGF-I by overexpressing IGFBP4 in a C2 myoblast cell line (Quinn et al., 1997). IL-15 could thus affect differentiation in the absence of other factors such as IGF-I (Quinn et al., 1997). IL-15 has been shown to induce hypertrophy of C2 myotubes in vitro by increasing protein synthesis and reducing protein degradation, an effect independent of changes to myoblast proliferation or differentiation (Quinn et al., 2002). In addition to the muscle anabolic effects of IL-15 in vitro, administration of recombinant IL-15 protein has been shown to slow muscle wasting in rats with cancer cachexia in vivo (Quinn et al., 1995, 1997; Carbo et al., 2000; Busquets et al., 2005). IL-15 has received scant attention regarding its effects on skeletal muscle regeneration or other muscle wasting conditions, but our laboratory has recently demonstrated that exogenous IL-15 administration increased the CSA of murine myofibers during the early stages of muscle regeneration (Harcourt et al., 2005). In addition, results from our laboratory have shown that exogenous IL-15 administration enhanced diaphragm function in mdx mice, an effect associated with decreased fibrotic infiltration (Harcourt et al., 2005). Given
the ability of IL-15 to downregulate proteolysis by acting on specific components of the ubiquitin proteasome pathway and suppress DNA fragmentation during certain pathologic conditions (Figuera et al., 2004; Busquets et al., 2005), continued investigation of the effects of IL-15 in vivo during various muscle wasting conditions is warranted.

3.9. β2-Adrenergic agonists and sympathomimetics

Adrenergic receptors (adrenoceptors) are members of the guanine nucleotide-binding G-protein coupled receptor (GPCR) family, the largest group of cell-surface receptors in mammals and which comprise >1% of the human genome (Fredriksson et al., 2003). The most well-characterized family of GPCRs are the rhodopsin receptors, which include the dopaminergic, adenosine, histamine, and α- and β-adrenergic receptors (Strosberg, 1990; Fredriksson et al., 2003), and consist of a conserved structure of 7 transmembrane α-helices that couple to heterotrimeric guanine-nucleotide-binding regulatory proteins (G proteins).

Skeletal muscle contains a significant proportion of β-adrenoceptors, mostly of the β2-subtype, with approximately 7–10% β1-adrenoceptors present (Kim et al., 1991) and a sparse population of α-adrenoceptors, usually in higher proportions in slow-twitch muscles (Rattigan et al., 1986). Slow-twitch muscles have also been shown to have a greater density of β-adrenoceptors than fast-twitch muscles (Martin et al., 1989). Many synthetic adrenoceptor agonists and antagonists have been developed in the search for improved treatments for a number of diseases. β-Adrenoceptors have been the main focus of most investigations in relation to adrenergic receptors (O’Donnell, 1993), and information gathered from this receptor has helped develop a family of β2-agonist drugs for clinical purposes (Ball et al., 1991; Waldeck, 1996).

The primary clinical application of β-agonists has been in the treatment of asthma, where the agonists facilitate dilation of the bronchiolar smooth muscle for relief of bronchospasm (Ball et al., 1991). These agents have served as the principal acute drug to be used in the treatment of asthma and was given via nebulization (clenbuterol). Clenbuterol was developed initially in the 1980s as 1-(4-amino-3,5-dichlorophenyl)-2-ter-butylaminoethanol (clobutinol). Clobutinol was developed initially for the treatment of asthma, and it is a powerful bronchodilator; however, it is neither currently approved for human use in the US, nor does it have Federal Drug Administration approval. As a consequence of its potent muscle anabolic actions, the effects of clenbuterol administration have been examined in a number of animal models and human conditions of muscle wasting disorders in the hope of discovering a new therapeutic (Carter et al., 1991; Maltin et al., 1993; Kissel et al., 1998, 2001; Lynch et al., 2001; Lynch, 2002; Fowler et al., 2004).

A decrease in total body fat was also found to be associated with the β2-agonist-induced increase in muscle mass (Ricks et al., 1984; Bergen & Merkel, 1991), and it was the combination of these 2 effects that drew interest from the livestock industry as a means of improving meat quality and quantity (Sillence et al., 1991). Athletes involved in strength-related sports also began to use and abuse β2-agonists in the hope of improving athletic performance in strength- and power-related sports (Delbeke et al., 1995; Prather et al., 1995), with many claiming that they were asthmatics to justify use (Prather et al., 1995). The repartitioning characteristics and ability to modify body structure and function, through increasing muscle mass and reducing body fat, makes clenbuterol such an attractive anabolic agent for athletes (Prather et al., 1995; Lynch, 2002).

Whilst the importance of β-adrenergic signaling in the heart has been well documented over the last 50 years and continues to receive significant attention (Sham et al., 1991; Xiao et al., 1995, 1999; Kilts et al., 2000; Rockman et al., 2002), it is only more recently that we have begun to understand the importance of this system in skeletal muscle growth, development, and repair after injury (Hinkle et al., 2002; Beitzel et al., 2004). The biochemical mechanism of β2-agonist-induced skeletal muscle hypertrophy is not understood completely, but it is thought to be a result of an increased rate of protein synthesis and a reduced rate of protein degradation (for review, see Navegantes et al., 2002). A simplified diagram of the possible pathways involved in β2-adrenergic mediated skeletal muscle hypertrophy is presented in Fig. 6.

The β2-adrenoceptors couple primarily to the stimulatory G protein (Gs), but have also been shown to be linked to inhibitory G proteins (Gi) in the heart (Communal et al., 1999; Xiao et al., 1999; Kilts et al., 2000; Zhu et al., 2001) and skeletal muscle (Gosmanov et al., 2002). For a typical β2-agonist the binding or ‘active’ site is located in the transmembrane region between the 3rd and 5th domains of the adrenoceptor (Johnson, 1998). At this active site, an aspartate residue binds to the nitrogen molecule of the β2-agonist and 2 serine residues bind to the 2 hydroxy groups attached to the glycine ring of the agonist (Strader et al., 1989). Stimulation of the β2-adrenoceptor causes a heterotrimeric G protein to associate with the third intracellular loop of the β-adrenoceptor. The hydrolysis of GTP to GDP causes a conformational change in the Gs protein, splitting the heterotrimeric unit into Ga and Gβγ subunits. It is believed that the GDPase activity of Ga dictates the duration of each cycle of receptor activation, as the hydrolysis of GTP to GDP (Fig. 6) promotes the reconstitution and membrane localization of the Gaβγ trimer (Johnson & Druey, 2002).

Adenylyl cyclase (AC) has been described as the principal effector of Gaα signaling (Roberts & Summers, 1998), and the hydrolysis of GTP to GDP allows Gaα to increase AC activity (Murad et al., 1962). Nine different isoforms of AC are known to exist and are designated as types 1–9 (Premont, 1994; Simonds, 1999). Gaα activation of AC catalyses ATP to cAMP, which in turn binds to the regulatory subunits of the cAMP

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dependent protein kinase (protein kinase A, PKA). PKA is then thought to phosphorylate and regulate the activity of numerous proteins, including calpastatin and possibly certain SR regulatory proteins (Hawkins et al., 1995; Reiken et al., 2003). These act to inhibit the proteolytic action of calpains and thus reduce the level of Ca\(^{2+}\)-dependent proteolysis (Tidball & Spencer, 2000; Navegantes et al., 2001; Tidball & Spencer, 2002).

Calpains are concentrated in the Z-disc and are involved in myofibril protein degradation, specifically in the disassembly of the myofibril (Kunamoto et al., 1992). In conditions where muscle wasting predominates (e.g., muscular dystrophies, sepsis and cancer cachexia), calpains have been implicated in the level of protein turnover (Tidball & Spencer, 2000; Costelli et al., 2001).

It has also been suggested that the β\(_2\)-adrenocceptor may be linked to increased protein synthesis through multiple signaling mechanisms (Emery et al., 1984; Reeds et al., 1986; Maltin et al., 1987; Choo et al., 1992; Glass, 2003). One such pathway involves cAMP binding to PKA, changing its conformation such that its catalytic subunits are released and move into the cell nucleus. There they phosphorylate the transcription factor CREB at a conserved serine residue (Ser-133 in CREB1). Ser-133 phosphorylation then promotes activation of genes containing cAMP response element (CRE), of which there are \(>4000\) in the human genome (Pourquié, 2005; Zhang et al., 2005). An investigation of CREs in the promoters of genes may lead to the discovery of novel “hypertrophic” genes.

Whilst most β-adrenergic signaling studies have focussed on the G\(_{\alpha}\)-cAMP-PKA signaling pathway, other work has implicated the G\(_{\beta\gamma}\) subunits in various cell signaling processes (Fig. 6, Crespo et al., 1994; Ford et al., 1998; Diversé-Pierluissi et al., 2000; Dascal, 2001; Mirshah et al., 2002). Although originally believed to be involved solely in receptor selectivity (Birnbaumer, 1990; Kühn et al., 2002), it is possible that through G\(_{\beta\gamma}\) signaling, the PI3K-AKT1 pathway is activated (Fig. 6; Lopez-Illasaca et al., 1997; Murga et al., 1998, 2000; Schmidt et al., 2001). In support of this proposed pathway for a β-adrenocceptor mediated increase in protein synthesis is the finding of increased phosphorylation of eukaryotic translation initiation factor4E binding protein 1 (4E-BP1) and p70\(^{56K}\) activation following 3 days of clenbuterol treatment (Sneddon et al., 2001). Sneddon et al. (2001) hypothesised that these changes in 4E-BP1 and...
p70S6K were mediated through a transient increase in β2-agonist stimulated IGF-I and IGF-II. Further studies are required to determine the exact mechanism for β2-agonist mediated protein synthesis and/or protein degradation.

The anabolic effect of chronic β2-agonist treatment has been reported in both predominantly slow-twitch (soleus), and fast-twitch (EDL) skeletal muscles of rats (Zeman et al., 1987; Sneddon et al., 2000; Beitzel et al., 2004). However, it appears that this response is fiber type specific, since fast fibers exhibit a greater hypertrophic response than slow fibers (Gregorevic et al., 2002a,b; Ryall et al., 2002).

Although the β-adrenergic signaling pathway may represent a novel therapeutic target for the treatment of muscle wasting and weakness due to its involvement in skeletal muscle growth and fiber type modulation, it must be recognized that this pathway is highly susceptible to modification with chronic stimulation, and this may have a detrimental effect once exogenous stimulation is stopped. Also of concern is the population of β-adrenoceptors in tissues other than skeletal muscle. Thus, any approach involving the systemic administration of exogenous β-agonists must take into account effects in tissues other than skeletal muscle, particularly those related to the heart.

Clinical and preclinical studies have implicated β2-agonists in the development of hypertrophic myocardial pathology (Duncan et al., 2000; Burmiston et al., 2002; Leone et al., 2002; Sleeper et al., 2002; Gregorevic et al., 2005). Furthermore, recent reports suggest that chronic use of β2-agonists for prevention and relief of asthma symptoms may be associated with increased risk of adverse cardiovascular-related events (Salpeter et al., 2004). Thus, β2-agonist administration for the treatment of muscle wasting is currently limited by cardiac hypertrophy and potentially deleterious effects on cardiovascular function (Molenaar et al., 2006).

In the past 15–20 years, much research on asthma has focussed on extending the bronchodilating actions of β2-agonists, whilst maintaining or improving their safety profile (Ullman & Svedmyr, 1988; Löffdahl & Svedmyr, 1989; Ball et al., 1991; Guhan et al., 2000). To this end, 2 β2-agonists, formoterol fumarate and salmeterol xinafoate, have been approved recently in the US for the treatment of asthma. These β2-agonists have an extended duration of action in relaxing smooth muscle compared with traditional asthma medications, such as albuterol and terbutaline (Löffdahl & Svedmyr, 1989; Roux et al., 1996; Waldeck, 1996). We have shown that these newer generation β2-agonists, formoterol, and to a lesser extent salmeterol, are capable of producing skeletal muscle hypertrophy when administered daily to rats at microgram doses and that a greater margin of selectivity exists between skeletal muscle and cardiac muscle (Ryall et al., 2005). Other studies have identified anticachectic effects of formoterol and described its therapeutic potential for pathologic states where muscle protein hypercatabolism is indicated, especially in cancer cachexia and other wasting diseases (Busquets et al., 2004). Future studies examining β2-agonist administration for treating muscle wasting and weakness must be focussed on separating the beneficial effects on skeletal muscle from the potential detrimental effects on the heart.

4. Conclusions

Although exercise and nutrition can be effective for improving muscle function in some conditions and should be considered as the first therapeutic approach wherever realistically possible, unfortunately in many cases the severity of muscle wasting demands a drug intervention that can promote protein synthesis and/or reduce protein degradation.

Although there are many potential therapies that have been described for treating a variety of muscle wasting conditions, the preclinical testing of many emerging compounds for muscle wasting disorders are often incomplete due to inadequate testing of the drug efficacy to enhance skeletal muscle function. This is especially common in the evaluation of patents for potential drugs and treatments (Lynch, 2004a, b,c). In many cases, published evaluations of drugs and targets for muscle wasting fail to assess whether there is functional benefit of treatment such as enhanced muscle strength or power, or an improved resistance to muscle fatigue, which may be especially relevant in conditions such as the muscular dystrophies. For preclinical testing, provision of functional assessments would enhance the potential for new drugs to proceed rapidly to the next phase of development—ultimately for a safe, effective and realistic therapy for muscle wasting and weakness.

Given the range of muscle wasting disorders and the differences in their underlying etiologies, there remains significant work to elucidate more clearly the pathways regulating skeletal muscle mass and to identify novel therapeutic targets. In developing appropriate strategies for the different muscle wasting disorders, careful attention is needed for the evaluation of treatment efficacy, with an emphasis on whether a potential intervention can improve muscle function.

Acknowledgments

We are grateful for grant support from the Muscular Dystrophy Association (USA), the National Health and Medical Research Council (Australia), the Australian Research Council, the Rebecca L. Cooper Medical Research Foundation, and Pfizer Global Research and Development (USA).

References


