Therapeutic Interventions for Age-related Muscle Wasting
Importance of Innervation and Exercise for Preventing Sarcopenia

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Introduction

Skeletal muscles constitute approximately 40% of the mass of the human body and are composed of very long multinucleated muscle cells (myofibres) filled with specialized contractile proteins. The myofibres are arranged in bundles and attached by connective tissue and tendons to bones of the skeleton. The muscles are under voluntary control and contract to move the bones only in response to signals sent by the nervous system. There are over 600 individual skeletal muscles that are essential for all aspects of movement such as breathing, eating, posture, walking and reflexes. With old age there is a marked loss of skeletal muscle mass, known as sarcopenia, resulting in decreased muscle function: this is due to the complex interaction of many factors including changes in metabolism and especially innervation. There is considerable interest in ways to reduce or reverse such muscle loss and an understanding of sarcopenia and evaluation of interventions is the topic of this review.

The review is divided into three sections. The first provides an overview of the situation in aging skeletal muscle with respect to the changes in muscle structure and function and the many factors that contribute to this. The second section focuses on the crucial role of effective motor neuron innervation in aging skeletal muscle. The final section discusses the benefits of exercise to prevent age-related loss of muscle mass and function. Throughout these discussions, therapeutic approaches to sarcopenia are critically commented upon.
Section 1. Sarcopenia: general overview

Age-related changes in skeletal muscle mass and strength

Aging in men and women is associated with significant changes in body composition, which involve a decrease in body mass and skeletal muscle mass with increased fat deposition [1]. Generally, between 50 and 80 years of age men lose 35–45% of their muscle strength; women start to loose strength earlier although their rate of the strength loss is slightly lower then in men [2]. This marked loss of muscle mass (sarcopenia) and strength with age is extremely complex and also highly variable depending on muscle type, gender, ethnicity, diet, health status and lifestyle. Two distinct phases are distinguished in the process of muscle loss. Slight loss occurs from 24 to 50 years and is associated with a 10% decrease of the total muscle cross-sectional area (indicating reduced diameter/size of individual muscle cells, called atrophy) and a 5% loss in the total number of myofibres. Significant sarcopenia takes place from 50 to 80 years of age, when about 30% of muscle mass and 35% of myofibres are lost. In humans, the decline of muscle strength is higher in the lower extremities than in the upper extremities [3] although this differs between the sexes [4]. How loss of the skeletal muscle mass translates into decreased functional capacity is poorly understood [5] and the loss of muscle strength with age is probably not fully explained by loss of muscle mass alone. A recent study carried out on 3075 black and white men and women using computing tomography revealed that changes in the composition of the muscle (decreased muscle density and increased fat tissue) is a better indicator of decreased muscle strength than is reduced cross sectional area [6].

Age related changes in skeletal muscles are very complex and at the morphological level involve atrophy and loss of myofibres [7, 8], changes in myofibre type composition, abnormal grouping of fibre types associated with cycles of denervation/reinnervation [9, 10], severe selective atrophy and loss of type 2 (fast) myofibres [11–13], disruption of Z bands and myofibrils [14], decrease in capillary to fibre ratio [3], increased endomysial fibrosis and increased perivascular fat [15]. Age related atrophy of individual myofibres and changes in fibretype composition of skeletal muscles have been studied mainly in limb muscles (e.g., vastus lateralis) where aging decreases the size of both type 1 and type 2 myofibres, however type 2 myofibres are more affected and there is a tendency for increased proportions of type 1 (slow) myofibres [reviewed in ref. 16]. It is important to note that such a shift in myofibre types does not apply to all muscles and the reverse trend can be seen for example in human masseter muscle [17]. The complexity of age-related changes in skeletal muscles is further underlined by a study carried out on senile rats [18], where the loss of muscle weight is greater in weight-bearing muscles such as gastrocnemius, plantaris and soleus and lower in non-weight bearing muscle, such as the extensor digitorum longus.

The deterioration of muscle mass and function with aging has two main consequences. It is widely recognized that muscle mass plays a very important role in thermoregulation and thus age-related sarcopenia results in increased mortality due to a decreased tolerance to high and low temperatures [19]. The major role for skeletal muscle relates to all aspects of posture and movement. Deterioration of the
physiological function of skeletal muscle with age (diminished strength and power-generating capacity) results in muscle weakness and tiredness with the impairment of daily functions such as walking, stair climbing and rising from a chair [20]. The selective loss of fast myofibres means that fast reflex responses are impaired and this presents major problems. For example, when elderly people trip over a stone they are unable to respond rapidly, often resulting in falls and broken bones. Recovery from such injury usually requires immobilization and bed rest and leads to disuse atrophy of skeletal muscle.

Undeniably, there are many general age-related changes such as increased fibrosis and decreased vascular supply, combined with systemic changes in hormones and neuronal function that have negative effects upon many tissues including skeletal muscles. The decline in overall body function further impacts upon the loss of muscle mass and much of this is due to a decreased capacity for exercise [reviewed in ref. 21]. The capacity for new muscle formation (myogenesis) does not seem to be a limiting factor in healthy individuals even in very old age, although problems with reinnervation can lead to impaired function of the regenerates [reviewed in ref. 21]. The main reasons for the age-related decrease in muscle mass and function are considered to be due to changes in endocrine and cytokine levels, a decline in food intake and energy balance (also related to altered metabolism and biochemistry), and especially decreased physical activity and impaired neuronal function. These main systemic and local changes relating to sarcopenia and potential therapeutic intervention are summarized in Tables 1 and 2 and discussed below.

Reasons for the decline in muscle function with age

Huge advances have recently been made in identifying genes associated with longevity (e.g., involved in cell cycle regulation, metabolism, oxidative stress) in lower organisms and mammals and current research focuses on revealing their human homologues [reviewed in refs. 22, 23]. The power of microarray analysis to identify changes in the expression of genes and gene clusters with age in different situations [24] has barely started to reveal the full potential of this approach and much new information should emerge in the next few years. Many attempts are being made to develop anti-aging interventions and extend the life-span in humans, and the current status of these was reviewed at a recent workshop [25]. Meanwhile, there exists a great deal of sophisticated insight into the complex interactions that result in the almost inexorable decline of muscle function with age and various therapeutic interventions have been explored.

Systemic changes in circulating hormones and other factors

Changes in body composition are commonly attributed to alterations of systemic endocrine function of aging individuals leading towards hormonal imbalance [26]. Age is associated with re-organization of the hypothalamic-pituitary-testicular axis resulting in decreased testosterone [27–29] and estradiol [27], and increased luteinizing and follicle-stimulating hormone levels [28]. In women, some studies report association of the decline of muscle strength with menopause [30, 31]. Beneficial effects of testosterone replacement on stimulation of muscle protein synthesis [32], increase of fat-free mass, muscle size [32, 33] and strength [33] have been shown in
**Table 1. Systemic age-related changes that adversely affect skeletal muscle function**

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Consequence</th>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
<td>Poor nutrition leading to anorexia of aging</td>
<td>Malnutrition and muscle wasting</td>
<td>Stimulation of appetite</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td></td>
<td>Improved nutrition</td>
</tr>
<tr>
<td>Changes in digestive system and dental problems</td>
<td>Note: Muscle is also a major source of heat and thus hypothermia can be a consequence of muscle loss</td>
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<tr>
<td>Improved nutrition</td>
<td></td>
<td></td>
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<tr>
<td>Social situation</td>
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<tr>
<td><strong>Organ/tissue</strong></td>
<td></td>
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<tr>
<td>Overall reduced cardiovascular function</td>
<td>Reduced mobility</td>
<td>Appropriate treatment for medical conditions</td>
</tr>
<tr>
<td>Cardiovascular problems</td>
<td>Reduced general capacity to exercise</td>
<td>Suitable exercise regime (e.g., aerobic exercise increases cardiovascular function)</td>
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<tr>
<td>Lung and breathing problems</td>
<td>Depression</td>
<td></td>
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<tr>
<td>Osteoporosis, joint stiffness and pain</td>
<td></td>
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<tr>
<td>Impaired CNS function</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hormones, cytokines and other factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased levels of</td>
<td>Altered metabolism</td>
<td>Hormone replacement</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Disruption of protein synthesis and degradation balance</td>
<td>Vitamin D supplementation</td>
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<tr>
<td>IGF-1</td>
<td>Muscle atrophy</td>
<td>Exercise</td>
</tr>
<tr>
<td>Sex steroids</td>
<td></td>
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<tr>
<td>Vitamin D</td>
<td></td>
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<tr>
<td><strong>Immune system</strong></td>
<td></td>
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</tr>
<tr>
<td>Altered inflammatory response</td>
<td>Inflammatory muscle wasting (cachexia)</td>
<td>Treatment with anti-inflammatory agents</td>
</tr>
<tr>
<td>Altered cytokine production (e.g., elevated TNF-α)</td>
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<td>Exercise</td>
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</tbody>
</table>
Table 2. Local (within skeletal muscle) age-related changes that adversely affect skeletal muscle function

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Consequence</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes in composition</td>
<td>Reduced nutrition from blood vessels</td>
<td>Use of anti-fibrogenic agents (?)</td>
</tr>
<tr>
<td>Increased interstitial connective</td>
<td>Reduced oxygen supply (hypoxia) from blood vessels</td>
<td></td>
</tr>
<tr>
<td>tissue (fibronectin and collagen)</td>
<td>Impaired re-innervation and synapse formation</td>
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<tr>
<td>Vascular supply</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced capillary network</td>
<td>Impaired oxygen supply</td>
<td>Exercise to maintain aerobic function</td>
</tr>
<tr>
<td></td>
<td>Impaired physical activity</td>
<td></td>
</tr>
<tr>
<td>Nerve supply</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of motor neurons</td>
<td>Denervation and myofibre atrophy</td>
<td>Exercise to stimulate production of trophic factors and maintain NMJs</td>
</tr>
<tr>
<td>Detachment of axonal endplates from myofibres</td>
<td>Changes in myofibre composition</td>
<td></td>
</tr>
<tr>
<td>Reduced re-innervation capacity</td>
<td>Lack of trophic signals from nerve</td>
<td>Administration of neurotrophic factors (?)</td>
</tr>
<tr>
<td>Demyelination of nerves</td>
<td>Myofibre atrophy due to denervation</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes in biochemistry and metabolism, increased protein breakdown, adverse changes in mitochondria</td>
<td>Increased oxidative stress contributing to increased damage of cell components</td>
<td>Exercise to increase production of muscle specific IGF-1 and other trophic factors</td>
</tr>
<tr>
<td>Reduced IGF-1 signalling</td>
<td>Loss of muscle mass and function</td>
<td></td>
</tr>
<tr>
<td>Reduced production of neurotrophic factors</td>
<td>Lack of trophic signals from muscle</td>
<td></td>
</tr>
<tr>
<td>Reduced Ca$^{2+}$ transport and contraction efficiency</td>
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</table>
hypogonadal men. However, results obtained from studies on healthy elderly men are controversial. For example, one study reported that injection of testosterone for 4 weeks, which produces serum concentrations equal to those of young men, increased muscle protein synthesis, elevated insulin like growth factor-1 (IGF-1) mRNA level in muscle and increased muscle strength [34]. However, in another study delivery of testosterone by means of patches (6 mg/day) for 36 months did not affect muscle strength, although resulted in decreased fat mass and increased lean mass [35]. Testosterone administration did not preserve muscle strength in bed rest confined subjects either [36]. With respect to hormone replacement therapy in women, a recent study carried out in post-menopausal women (50–55 years) reported increased muscle size and improvement of explosive-type muscle performance (jumping) following administration of oestradiol/noretisterone acetate for 1 year, although there was no significant improvement in muscle strength (judged by knee extension torque). The same study showed that hormone replacement had much more pronounced effects and increased muscle strength when combined with exercise [37].

Another steroid, which deserves major attention with respect to hormone replacement therapy, is dehydroepiandrosterone (DHEA). DHEA and DHEA-sulfate are produced in the adrenal gland and can be converted into testosterone and estradiol in peripheral tissues [reviewed in ref. 38]. Plasma levels of DHEA and DHEA-sulfate decline with age [39, 40]. Positive correlation between serum levels of DHEA-sulfate and quadriceps muscle strength and optimal shortening velocity has been shown in 66–84 old women, but not men [41]. Moreover, men and women appear to respond differently to DHEA replacement. Daily administration of 100 mg of DHEA for 6 months resulted in decreased fat mass and increased muscle strength in men but not in women, although in both genders DHEA replacement increased serum IGF-1 levels [42]. Commercial supplements containing sex steroid precursors are available to promote anabolic muscle growth in humans, but there is no strong evidence that they have major benefits and unfavorable side effects must be considered [reviewed in ref. 43].

Of particular interest is the decreased activity of the growth hormone (GH)/IGF-1 axis. It was widely held that sarcopenia is directly related to an age-related decline in GH secretion but this view is now contested and numerous studies in humans do not support a benefit of GH administration on muscle protein synthesis [reviewed in refs. 25, 43]. Administration of GH results in increased serum IGF-1 levels [44–46], however data on the beneficial effects of GH on skeletal muscle size and particularly strength are controversial. In senile rats administration of GH increased muscle mass and strength and decreased muscle fat content [44]. GH administration did not increase strength or enhance protein synthesis in skeletal muscles of exercising men [47] and diminished exercise-induced improvement in insulin sensitivity [48], although increased fat free mass was reported [47, 48]. A study on the effect of GH alone and in combination with resistance exercise on skeletal muscle was undertaken in elderly men and women subjected to recombinant human GH (rhGH) administration at a dose recommended by the manufacturer (15 μg/kg per day), however the investigators had to reduce this dose to 2.5 μg/kg per day because of side effects (arthralgia, edema and carpal tunnel syndrome) [45]. Administration of rhGH for 6
months did not significantly increase muscle strength or size. Exercise alone, and in combination with rhGH, significantly increased muscle strength but not myofibre cross-sectional area. Unfortunately, the authors did not elaborate on whether increased muscle strength was statistically significant between exercising groups with or without rhGH administration. Interestingly, rhGH administration increased the percentage of type 2 myofibres in elderly subjects [45]. One study showed that combination of GH administration with endurance exercise (which is different from strength training) increased skeletal muscle oxidative enzyme activity, however the effects on muscle strength were not reported [46].

Synthetic peptides that cause the release of GH (GH secretagogues, e.g., benzoazepines and their analogues) have been used clinically but their efficacy is unclear, as are the benefits of commercial hormone replacement therapies [reviewed in ref 43].

The growth factor IGF-1 seems to be particularly important for maintaining muscle mass and the complex effects of IGF-1 on muscle are mediated through several pathways as outlined in Figure 1. IGF-1 stimulates proliferation and differentiation of myoblasts and exerts pleiotropic anabolic (i.e., resulting in increased protein content) actions on skeletal muscle cells, which include stimulation of amino acid uptake and incorporation into proteins, uridine and thymidine incorporation into nucleic acids, glucose uptake and also suppression of protein degeneration [49, 50]. For detailed discussion of mechanisms by which IGF-1 can prevent age-related myofibre atrophy the reader is referred to several recent reviews [21, 43, 51–53]. With old age, serum levels of IGF-1 decrease [40], but remain highly variable among individuals [54]: for example serum IGF-1 levels might vary between 12.8 to 461.3 μg/litre among elderly women [54]. The same study showed that, after age-adjustment, IGF-1 levels were associated with knee extensor flex but not with grip strength or hip flexor strength [54]. A study carried out in centenarian men and women did not show any association of higher or lower IGF-1 content with body mass index, serum lipid levels, liver function and frequency of hip fractures [55]. However significantly more of the low IGF-1 subjects had dementia, which supports an important role for IGF-1 in maintenance of neural cell function (see Section 2). Previous studies, which claimed that the circulating liver-specific isoform of IGF-1 (expressed under GH control) has almost no effect on postnatal growth of mice [56, 57] resulted in underestimation of the contribution of endocrine function of IGF-1 in growth and development [reviewed in ref. 58]. The importance of the endocrine function of IGF-1 for somatic growth has clearly been demonstrated by Lupu et al. (2001) by comparing phenotypes of mice, which lack the gene for the growth hormone receptor, IGF-1, or both [59]. These authors estimated that body growth unrelated to the GH/IGF1 axis accounts for ~17% of body weight, the contribution of IGF-1 alone is more significant then that of GH (35% vs 14% of total body weight) and the combined GH/IGF1 action contributes 34% to total weight [59]. Overlapping effects of GH and IGF-1 account for the body growth mediated by systemic and local actions of IGF-1. However, with high levels of circulating IGF-1, major problems can arise in association to heart hypertrophy and increased rates of cancer especially of the prostate [51].
Figure 1. Putative signalling pathways regulated by IGF-1 in skeletal muscle cells. In a simplified scenario IGF-1 signalling can be broken down to three main pathways: Raf-MEK-MAPK pathway – critical for mitogenic response; Ca²⁺-dependent calcineurin pathway – responsible for hypertrophic growth of muscle; and PI3-kinase (PI3K) – Akt pathway mediating muscle cell hypertrophy and survival.
It is now recognized that there are several isoforms of IGF-1, some are present in the circulation, whereas others are expressed locally in skeletal muscle and other tissues [51]. It appears that the IGF-1 isoforms produced locally in response to muscle exercise and stretch are of particular importance for maintenance of muscle mass. Therefore, skeletal muscle specific over-expression of local IGF-1 deserves much attention with respect to developing strategies to decrease muscle wasting. Coleman and colleagues developed mice with skeletal muscle localized over-expression of the human IGF-1 driven by regulatory elements from the avian skeletal α-actin gene [60]. These mice maintained normal levels of circulating IGF-1 and exhibited hypertrophy of all types of myofibres. Another group of investigators introduced extrahepatic IGF-1 isoform (mIGF-1 or Exon 1-Ea combination) driven by myosin light chain (MLC) regulatory elements into mice [61] and produced the transgenic mouse model which over-expresses this local IGF-1 isoform only in skeletal muscles [62]. Skeletal muscle specific over-expression of the transgene resulted in the functional hypertrophy of young skeletal muscle and rescued old skeletal muscle from age-related atrophy [62]. It has been suggested that the hypertrophic action of mIGF-1 over-expression results partly through stimulation of myogenesis [61, 63] and partly through increased protein synthesis [63]. However not all studies support the anabolic effect of high levels of IGF-1 on aging muscle. For example, Welle et al. (2002) did not find a correlation between levels of IGF-1 and the rate of myofibrillar protein synthesis in vastus lateralis muscle of elderly people, and maintenance of higher levels of IGF-1 expression did not prevent the age related decline in myofibrillar protein expression and muscle mass [64]. There is tremendous interest in IGF-1 due to its central role in hypertrophy, but the situation is complex and the roles of the various isoforms need to be clarified.

It has long been known that some β₂-agonists (agents that stimulate the β₂ receptors of the sympathetic nervous system and that are widely used to treat asthma) have powerful anabolic effects on skeletal muscle. The application of β₂-agonists (and in particular clenbuterol) for increasing muscle mass and performance and their adverse effects is the subject of a recent excellent review [65]. Clenbuterol mediates hypertrophy of skeletal muscle [66, 67] and inhibits denervation-induced atrophy [66, 68] through its action on β₂-adrenergic receptors [66]. The ability of clenbuterol to increase muscle mass (through reducing muscle protein degradation) and especially to reduce the fat content has made it an attractive illegal anabolic drug for body builders. However, the use of clenbuterol is limited by numerous undesirable side effects including sweating, tachycardia, tremor, effects on the central nervous system and serious heart complications that may be lethal [65]. Clenbuterol administration results in heart hypertrophy [67, 69] and malfunction [69]. In addition clenbuterol has been shown to have myotoxic properties and induce necrosis in heart and skeletal muscle of rats when injected subcutaneously [70]. Clenbuterol is also toxic when ingested and may cause tachycardia, hypokalemia, hypophosphatemia and hypomagnesemia [71]. Whether the powerful anabolic effects of β-agonists are even translated into improved muscle strength and function is questionable. Studies in animals and humans show little or no effect of β₂-agonists on muscle performance and sometimes decreased muscle strength [discussed in ref. 65]. In young adult rats, administration
of clenbuterol did not increase force of soleus muscle [67] or diaphragm [72], but did increase maximal tetanic force in fast EDL muscle [67]. In old rats clenbuterol increased mass as well as strength of diaphragm [72]. Another β2-agonist, fenoterol was more efficient then clenbuterol in increasing both slow and fast muscle performance. Although adverse effects of fenoterol on cardiac function have not been studied, it does increase the heart mass even more then clenbuterol and such cardiac hypertrophy is clinically most undesirable [67]. Thus, for sarcopenia, the use of β2-agonists would seem to be very inappropriate. While the β2-agonists appear to have fewer adverse affects then β2-agonists, their efficacy for ameliorating muscle wasting has not been definitely studied [discussed in ref. 43].

Myostatin, a member of transforming growth factor-beta superfamily, is another circulating growth factor that has attracted much attention since the absence of myostatin results in huge muscles known as double muscling in mice and cattle [73, 74]. Myostatin is a negative regulator of myogenesis and suppresses myoblast proliferation [75] and myogenic differentiation [76]. High serum and muscular levels of myostatin are associated with muscle wasting (called cachexia) in HIV-infected men [77]: cachexia is seen in pathological inflammatory conditions caused by high levels of pro-inflammatory cytokines. Comparison of mRNA levels of myostatin in vastus lateralis muscles of young (21–31 years) and old (62–77 years) healthy men showed no significant effect of aging on the expression of myostatin mRNA [64]. Also myostatin gene polymorphism is not significantly associated with variability of muscle mass and strength in the elderly [78]. It is an attractive idea that reduced myostatin will result in increased muscle mass. Experiments in mice show that blocking myostatin with antibodies increased muscle mass and strength in dystrophic mice [79]. It is also noteworthy that in mice, silencing of the myostatin gene results in decreased fat content [80]. Whether strategies to decrease myostatin activity might reduce muscle sarcopenia in humans remains speculative.

The importance of circulating vitamin D in maintaining muscle mass and strength is often overlooked and vitamin D deficiency is extremely prevalent in the elderly. Vitamin D is formed from calciferol that is mainly produced in the skin in response to sunlight. With age there is a decreased ability to manufacture vitamin D, resulting in marked deficiency, leading to muscle atrophy with selective loss of type 2B myofibres and other disorders. Fifty per cent of subjects with vitamin D deficiency display hip fractures [81]. It is suggested that vitamin D may act by stimulating IGF-I signaling [reviewed in ref. 21]. Clearly vitamin D deficiency should be carefully monitored in the elderly, it is readily diagnosed and is reversed by administration of calcium and vitamin D supplements [81].

Another important circulating cytokine that severely affects skeletal muscle is the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α). Increased inflammation is associated with many age-related problems (e.g., arthritis, inflammatory myopathies, heart failure, cancer) and the associated elevated TNF-α and interleukin-6 (IL-6) can directly contribute to loss of skeletal muscle tissue in aging humans. The role of TNF-α in extensive muscle wasting (cachexia) associated with severe inflammatory situations like cancer and acquired immunodeficiency syndrome has attracted considerable attention [reviewed in ref. 82]. Increased plasma TNF-α
and IL-6 are often observed in healthy elderly people and higher levels of these cytokines are associated with lower muscle mass and strength in well-functioning older men and women [83]. TNF-α expression is also elevated locally in skeletal muscles of the elderly [84]. It has been suggested that the effects of TNF-α on muscle atrophy may also be mediated in part via interference with IGF-1 signaling [21]. Attempts to minimize cachexia have focused on anti-inflammatory drugs to block TNF-α action. This could be done by blocking the TNF-α protein with remicade (Infliximab®) or by blocking TNF-α receptor with etanercept (Enbrel®) [85]. Remicade is already used clinically in the treatment of immune mediated diseases, like rheumatoid arthritis and inflammatory bowel disease, but can have some side effects [86]. Remicade should be considered as one of the potential anti-inflammatory agents, which could be tested with respect to amelioration of inflammatory cytokine induced muscle loss with aging. Reduction of serum TNF-α by administration of L-carnitine has been proposed to prevent muscle loss secondary to heart failure [87], since L-carnitine reduces TNF-α serum levels in rats with experimentally induced chronic heart failure: however, such L-carnitine treatment did not significantly increase muscle size.

Metabolism, energy balance and nutrition
Aging is associated with dramatic changes in the biochemistry and enzymatic activity of skeletal muscle resulting (among other effects) in reduced capacity to synthesize new proteins, up-regulation of pathways leading to increased protein breakdown (catabolism), and increased oxidative cell damage [reviewed in ref. 88]. Whether these and other deleterious biochemical alterations in aging muscle are inevitable or reversible is debated, but it would seem that some might be amenable to intervention. In theory, therapeutic strategies might be directed at many of these biochemical events. Diverse interventions have been attempted and some are the subject of patents. The excellent review by Lynch [43] points out the shortcomings and the minimal amount of information available from many therapeutic studies and it appears that more rigorous testing is required. The overall conclusion seems that few interventions offer any real benefit at this stage.

Imbalance between energy intake and energy expenditure is considered to be one of the main reasons for decreased lean body mass. This aspect includes altered food intake, combined with changes in basic metabolic rate and biochemistry.

Aging is associated with reduced appetite and thus a decline of energy intake and malnutrition, which can lead to physiological anorexia of aging [reviewed in ref. 89]. The causes for the anorexia are unknown but may involve increased satiety factors (e.g., leptin) and a reduced feeding drive [89]. Whether increased leptin does suppress appetite is unclear, since the elevated plasma leptin with age is often associated with decreased expression of leptin receptor in the hypothalamus [90] and leptin resistance [91].

In acquired immunodeficiency syndrome (AIDS) or geriatric cachexia patients, high levels of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α can also contribute to decreased food intake directly by affecting the gastrointestinal system or indirectly by affecting the CNS [reviewed in ref. 92]. Thus much attention is paid to
the development of therapeutic strategies to reduce levels of pro-inflammatory cytokines and improve appetite in geriatric patients. Clinical use of agents with anti-inflammatory action (e.g., pentoxifylline, thalidomide, megestrol acetate, N-acetylcysteine, melatonin) and appetite stimulants (e.g., dronabinol, n-3 fatty acids) tested for HIV-infected patients may also be appropriate in cases of geriatric cachexia [reviewed in ref. 92].

Marijuana (*Cannabis sativa*) is widely recognized as an appetite stimulant. While use of marijuana is illegal in many countries there is some strong support for its use clinically in situations such as cancer related anorexia [93]. The use of marijuana to stimulate eating in addition to giving a sense of well being with minimal side effects [93] might also be considered for the treatment of anorexia associated with aging.

The use of specific amino acid supplements and their derivatives has recently attracted much attention to reduce muscle protein breakdown and increase muscle mass and strength in athletes and patients with cachexia. Supplementation with a nutrient mixture of β-hydroxy-β-methylbuturate (HMB), a metabolite of leucine, L-glutamine and L-arginine increases lean body mass in patients with AIDS [94] and cancer [95]. Beneficial effects of HMB supplementation for increased upper body strength has been shown in exercising young (between 20–40 years) individuals [96]. In elderly individuals involved in strength-training programmes, administration of HMB also resulted in muscle strength increase and significant decrease of fat mass [97]. However, in resistance-trained athletes supplementation with HMB did not have an additional effect to exercise on either strength gain or lean mass increase [98]. It is worth mentioning that no adverse reactions were reported by subjects consuming HMB [97]. Another nutritional supplement which has attracted attention for increasing muscle mass and strength is creatine. In active but not trained young individuals, supplementation with creatine alone and in combination with HMB resulted in additive strength increase following weight training [99]. However not all studies support beneficial effect of creatine on muscle strength gain [100].

Crude linoleic acid (CLA) is another substance, which has attracted attention for manipulation of body composition in humans. Studies in growing animals show that supplementation with CLA is beneficial for feeding efficiency and results in increased lean body mass and decreased fat mass. Trials carried out in humans do not conclusively report improved body composition by CLA, however more research should be undertaken to elucidate the potential benefits of CLA for humans [reviewed in ref. 101].

Rather perversely, one of the very few interventions that extend longevity in animal models is restricted food or caloric intake [102]. This extends life expectancy by 30–40% if initiated early in the animal’s life, and by about 20% if started in middle age [25]. It is proposed that the main mechanism responsible for such longevity is reduced reactive oxygen species formation and thus reduced oxidative damage to cellular structures [reviewed in ref. 103]. However is seems that the price paid for extended longevity might be reduced fertility as a result of caloric restriction [104] and this side effect should seriously be taken into account when considering therapeutic application of caloric restriction for humans.
Many potential therapies for sarcopenia are based upon interventions for body builders, athletes, pathological conditions and studies in animal nutrition. Overall, while many approaches have been investigated and some are appropriate for pathological clinical conditions related to muscle wasting, the same treatments may be difficult to justify for sarcopenia in healthy elderly people. There are many claims for success but few genuinely seem effective or well substantiated and many can have deleterious side effects. In general, this applies to replacement of hormones (with anabolic effects) such as testosterone, DHEA, GH, circulating IGF-1 and β2-agonists. Reduction of inflammatory cytokines such as TNF-α might be beneficial, whereas the case for altered myostatin levels is not strong. Good nutrition and high protein intake is clearly very important, and supplementary vitamin D is often essential and beneficial. Since anorexia of aging can present a major problem, the use of appetite stimulants may be useful. Other nutritional supplements such as amino acids (l-glutamine, l-arginine), HMB, creatine and antioxidants, do not seem justified or require further investigation. It is important to note that some of these treatments may prove to be more effective in combination with exercise (see section 3).

Section 2. Innervation and sarcopenia

One of the most crucial factors in aging muscle would seem to be the loss of effective innervation over time. Thus this section focuses on what is required to maintain healthy neuronal function. Skeletal muscle depends upon a signal (action potential) from a nerve (motor neuron) to become activated and contract. The axon of a motor neuron extends from the nerve cell body in the spinal cord or the brainstem, to the muscle, where the axon branches and each axon terminal is attached to the surface of a myofibre at a neuromuscular junction (NMJ) called the synapse (Figure 2). The motor nerve terminal has densely packed synaptic vesicles containing the transmitter acetylcholine (ACh) and the post-synaptic (myofibre) membrane has many ACh receptors (AChR) as well as unique specialized proteins: the synapse with closely associated myonuclei is a specialized area of the myofibre and effectively operates like a “mini cell” [105]. The entire complex including the motor neuron (with cell body) and the group of myofibres it innervates is referred to as a motor unit.

Age-related neuromuscular changes are very complex and involve progressive degeneration of NMJs, fragmentation of nerve terminals, reduced numbers of motor neurons and demyelination of nerve fibres [106]. The latter contributes to impaired propagation of electric signals and the slowness of movement in frail elderly people [107]. The loss (death) of motor neurons with aging, results in denervation of myofibres. For example, in extensor digitorum longus muscle of 27–29 month old rats, ~6% of the total myofibre cross sectional area is denervated compared with ~1% in young rats and this accounts for about 11% (out of a total 34%) of specific force differences between young and old muscles [108]. Although denervated myofibres can be re-innervated by collateral sprouting from adjacent axons, the sprouting ability of neurons declines with age leading to incomplete re-innervation and resultant muscle denervation-induced atrophy [106, 109]. There may be repeated
Figure 2. Innervation of skeletal muscle. An axon of a motor neuron extends from the spinal cord, branches and attaches to the muscle fibres forming neuromuscular junctions (synapses). Exercise upregulates production of trophic factors by muscle and nerves and increases neurotransmitter release by the nerve terminal at the synapse. These processes contribute to stabilization of neuromuscular junctions and maintenance of muscle innervation and function.
cycles of nerve retraction and compensatory re-innervation. Even where muscles are re-innervated, the reduced number of axons results in decreased muscle force [110]. Apart from the complete death of motor neurons, denervation of myofibres can be caused by lifting of the axonal terminal from the target muscle [111]. Re-establishment of muscle innervation after nerve damage is reduced in old animals [112] and the increased connective tissue in older muscles (e.g., fibronectin) probably plays a major role in such impaired synapse formation. With aging, the areas of axonal contact at the synapse become progressively more scarce, leading to a reduction of the effective area of synaptic contact in NMJ and many newly formed synaptic sites are unstable [reviewed in ref. 105]. Problems with maintaining and re-establishing neuromuscular function in aging muscles appear to be of central importance for sarcopenia. They also underlie the major problems with pronounced disuse atrophy and loss of muscle function that occur in the elderly after enforced bed rest (resulting from damage caused by a fall, or illness). Microarray analysis is revealing more about the genetic and molecular alterations involved in age-related changes at motor unit level. For example, an analysis of gene expression in skeletal muscles of old rhesus monkeys showed up-regulation of genes involved in neuronal death, remodelling and repair [113].

The formation and maintenance of NMJs depends on signals from both nerve and muscle and the exchange of trophic factors between these two cell types [reviewed in ref. 105]. Down-regulation of trophic factors (from muscle or nerve) or impairment of synapse-specific molecular signalling pathways can result in destabilization of the NMJ and subsequent detachment of the axon terminal from the muscle surface. The interdependency of healthy motor neurons on healthy myofibres (that provide the appropriate factors to maintain strong synaptic connections) is the subject of intense interest especially for aging. Much information about NMJ formation and maintenance comes from studies of muscle development where myofibres are initially innervated by several motor neurons, whereas most mature myofibres are innervated by a single motor neuron [114]. The mechanisms mediating the transition from multiple (polyneuronal) to single innervation, by death of selected motor neurons are broadly reviewed elsewhere [115]. During development, when there is low neurotransmitter release at a NMJ, the AChRs (on the muscle surface) may become depleted leading to decreased synaptic area and ultimately retraction of the axons from the NMJ. Even focal blocking of the AChRs results in disappearance of AChRs from the post-synaptic membrane followed by loss of pre-synaptic nerve terminals [116]. Many factors produced by both nerve and muscle that are crucial for maintenance of NMJs have now been identified [reviewed in ref. 105]. Factors which appear to be critical for development and function of the nervous system are the neurotropin family of growth factors [for review see ref. 117]. Neurotropins are essential for neuron survival [118, 119] and axonal growth [120–123; also for review see ref. 124] and are expressed in muscle [125–127] and in CNS [128]. Moreover, neurons have the ability to retrogradely transport neurotrophic factors produced by myofibres [129, 130].

There is a considerable interest in clinical administration of neurotrophic factors to maintain neuronal function and treat neuromuscular diseases [131, 132]. Reports
of success with respect to rescue of skeletal muscle function have been claimed, although many of these seem unsubstantiated [reviewed by ref. 43]. The ciliary neurotrophic factor (CNTF) has attracted particular attention in aging. Administration of CNTF has been shown to retard adverse progressive motor neuron dysfunction and improve muscle strength in wobbler mice (an animal model for motor neuron disease) [131, 133]. In rats, production of CNTF in sciatic nerve strongly correlates with muscle strength and a significant decrease of sciatic CNTF expression occurs with aging. Furthermore, administration of CNTF increases muscle strength in 24-month-old animals [134]. A study of the relationship between CNTF genotype and muscle strength in adult (20–90 years old) humans showed that individuals heterozygous for the CNTF null mutation have significantly greater muscle strength and quality then subjects homozygous for both alleles [135]. Leukemia inhibitory factor (LIF) was also considered a good candidate for treatment of neuromuscular disorders although the initial promise has not been realized for increasing muscle regenerative capacities following degeneration [136, 137].

Another factor of clinical interest for neurodegenerative diseases is IGF-1. An excellent review by Dore (1997) discusses the potential therapeutic use of IGF-1 either alone or in combination with neurotrophic factors to slow down the loss of neuronal cells, based on the ability of IGF-1 to increase neuronal survival, promote sprouting of axon terminals, accelerate neuronal outgrowth and peripheral nerve regeneration, facilitate myelination in the CNS and induce neuromuscular AChR formation [138]. Administration of IGF-1 prevents motor neuron death, improves axonal regeneration in neonatal rats after sciatic nerve crush [139] and stimulates adult intramuscular nerve sprouting [140]. IGF-1 also has a stimulatory effect on myelination. Over-expression of IGF-1 in the brain of transgenic mice resulted in higher number of myelinated axons and increased thickness of the myelin sheath [141]. Tissue-specific isoforms of IGF-1 are expressed in skeletal muscles [51, 142, 143]. However, in order for IGF-1 produced by myofibres to have an effect on nerve regeneration and neuron survival, it seems necessary that IGF-1 crosses the synapse and is transported by the axon. Anterograde as well as retrograde transport of IGF-1 has been shown in sciatic nerve of adult rats [144]. Yet high expression of local IGF-1 within myofibres (e.g., Ea-containing IGF-1 transcript [reviewed in ref. 51] may have mainly an autocrine effect (Shavlakadze, unpublished data). Therefore, high IGF-1 expression by myofibres might instead act indirectly to stimulate the production of other trophic factors (by myofibres) that then directly affect the neurons. Beneficial effects of systemic administration of human recombinant (circulating isoform) IGF-1 have been demonstrated on wobbler mice, which are characterized by severe muscular atrophy associated with degeneration of lower motor neurons [145]. IGF-1 treated mice showed a striking increase in body weight, muscle strength and myofibre diameter. While the muscle wasting was reversed there was no prevention of motor neuron loss, thus the effective innervation of myofibres is probably accounted for by IGF-1 stimulated sprouting of the remaining axons. In marked contrast, systemic addition of proteoglycans (that bind many growth factors) in conjunction with IGF-1 also prevented the motor neuron death and this synergistic effect was seen with much lower doses of IGF-1 [146, 147; also for review see ref.
This observation illustrates the crucial role played by extracellular matrix molecules although few studies have taken such interactions into account to date. The lower levels of IGF-1 needed to produce the desired effect is an important advance, since a major clinical complication with systemic administration of IGF-1 (and other factors) that cause hypertrophy of skeletal muscle is the concomitant hypertrophy of cardiac muscle.

Strategies to sustain muscle innervation are of central importance for preventing sarcopenia. Whether therapeutic administration of exogenous neurotrophins will prove to be useful clinically remains to be determined. However, there already exists a very effective, inexpensive (and simple) strategy to help maintain strong NMJs in aging muscle. This is the endogenous production of the appropriate trophic factors by regular exercise [130].

Section 3. Exercise prevents muscle loss

It is well recognized that age-related loss of muscle mass results in large part from decreased physical activities [106]. Age related disabilities can be quite dramatic and involve impairment of the simplest daily activities such as walking, balance, standing up from a chair, or putting on clothes [149]. Such adverse changes lead towards reduced involvement of the elderly person in physical activities, restriction of independence and even depression. Thus the importance of musculoskeletal fitness for the enhancement of health status and overall quality of life in the elderly population is beyond any doubt. Evans (2002) suggested that exercise should be a standard care for all elderly people and stated that it is of high importance to not only educate people about the benefits of regular exercise, but also increase their involvement in physical activities [150]. Beneficial effects of regular exercise for the elderly include not only the widely recognized effects on physiological functions, but also the influence on the physiological status of the person. For example exercise has been shown to have a protective effect against depression in individuals over 50 years [151]. Anabolic effects of exercise are considered to be the main mechanism for prevention of muscle wasting. It is now recognized that resistance exercise alleviates sarcopenia by improving nitrogen balance and lowering dietary protein requirements in elderly people, which becomes very important with respect to problem of undernutrition [reviewed in ref. 152].

A recent review by Sakamoto and Goodear [153] describes the intracellular signalling pathways triggered by physical activity in skeletal muscle cells and the effects on muscle physiology. Essentially, these pathways encompass the (1) MAP kinase signal transduction pathway involved in regulation of gene transcription and possibly regulation of glucose uptake; (2) AMP kinase signalling pathway involved in regulation of contraction-stimulated glucose transport, fatty acid oxidation and regulation of respiratory cycle; (3) Akt signalling pathway, important for regulation of glycogen synthesis, insulin sensitivity, protein synthesis and gene transcription. It has also been suggested that the Akt mediated pathway (Akt/mTOR/p70S6K pathway) is involved in prevention of myofibre atrophy following denervation (Figure 1), and specific blocking of the Akt/mTOR pathway with rapamycin in vivo almost
completely prevents hypertrophic growth of skeletal muscle [154]. Signalling pathways initiated by exercise in skeletal muscle cells largely coincide with those initiated following activation of the IGF-1 receptor (Figure 1). It is now recognized that both autocrine and paracrine systems of IGF-1 are upregulated in exercising muscle [53] which would imply that IGF-1 is one of the principal mediators of exercise-induced effects.

Another important therapeutic effect of exercise for maintenance of muscular fitness relates to muscle-nerve interactions. The fact that expression of neurotrophic factors in skeletal muscles and nerves is activity dependent and elevated with increased stimulation is well established [127]. Local blockade of neuromuscular transmission decreases levels of neurotrophic factor-4 (NT-4) mRNA in skeletal muscle, whereas levels of mRNA of NT-4 [127], brain-derived neurotrophic factor (BDNF) and neurotrophic factor-3 (NT-3) [130] increase with increased activity of skeletal muscle. Also, the possibility of retrograde transport of neurotropins from skeletal muscle to motor-neurons with increased physical activity has been suggested [130]. Another mechanism through which exercise might contribute to the maintenance of NMJs is increased probability of neurotransmitter release by presynaptic nerve terminal, which affects stability of the postsynaptic endplate structure, similar to what happens during development.

Another important systemic benefit of exercise is reduction of pro-inflammatory cytokine levels. Of particular interest is the finding that in elderly people resistance exercise training decreased muscle TNF-α protein levels [84]. Moreover, the same study showed that the level of TNF-α in exercising muscle was inversely correlated with the rate of muscle protein synthesis [84].

A recent paper by Singh [155] gives a broad overview of the rationale for using exercise as the preventive strategy for slowing down age-related adverse changes (e.g., decreased aerobic capacity, sarcopenia, osteopenia, increased adiposity, decreased flexibility etc.), reduction of disease risks (e.g., cardiovascular disease, diabetes mellitus, falls) and for targeting syndromes of disuse and disability occurring secondary to various pathologies. In the same review the author gives recommendations for exercise programmes for geriatric patients: these include aerobic exercise, resistance, flexibility and balance training and their combinations.

With increasing age, many healthy elderly people may gradually cease regular sporting activities due to frustration at loss of the specific skills required (e.g., golf, tennis). This removes the opportunity for exercise as well as social interactions. Other pleasurable activities such as walking, dancing and swimming are not limited by such specific skills but problems may arise with access and simple amenities (e.g., ability to easily get in and out of a pool or from the ocean, or availability of benches for resting while on walks): such issues need to be addressed to facilitate these activities for the elderly. It seems that the relative merits of different types of exercise in aging individuals require further formal study.

Combination of resistance training with aerobic, balance and flexibility training will improve mobility and cardiovascular performance and reduce risk of falls. Benefits of strength training for prevention of age-related loss of skeletal muscle volume and function have been extensively described [156–159] and are based on the
fact that (even at a very old age) skeletal muscle preserves plasticity and adapts to training by increasing in size and strength [160]. For example resistance training has been shown to considerably increase muscle strength in 65 year old men [161] and in frail elders (72–98 years) [14]. Such muscle strength increase is even higher when resistance training is combined with nutritional supplementation [14] and is not dependent on the source of the protein diet [161]. On the other hand, a study performed in 65 years old men showed that supplementation with amino acids seemed not to effect resistance training induced muscle strength and volume gain [162]. Strength training alone or in combination with aerobic training also significantly increases flexibility, measured for example by the sit-and-reach test [158, 159]. It is reported that there is no major difference between the improvements achieved by high or low intensity exercise regimens [163]: this is of interest since high intensity training might be of concern for elders with disabilities. Moreover, resistance training for even one day per week maintains muscle strength and size in elderly (e.g., 70 year old) people [164].

Aerobic training by itself does not have a significant impact on muscle strength increase in the elderly [158, 165]. Flexibility training is also less effective for strength and balance improvement than resistance training [166]. Long-term (at least 3–4 years) involvement in balance training, such as Tai Chi exercise, increases strength of the muscles involved in maintenance of postural stability [167] and reduces deterioration of bone mineral density in weight bearing bones [168]. Regular involvement in Tai Chi for a shorter time (6 months) also had a beneficial effect on daily physical activities (walking, bending, dressing, bathing etc) and on a more vigorous activity like running [169].

It seems that far more emphasis needs to be applied to understanding and developing suitable enjoyable exercise regimes for all the elderly. Even for those with little mobility, weight training appears to have remarkable benefits. The promotion of exercise and making available easy access to suitable exercise facilities, represents a healthy and relatively inexpensive intervention that promises many benefits for the elderly, beyond the important effect of reducing the age-associated loss of skeletal muscle mass and function.

References


