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Review

Altered responses in skeletal muscle protein turnover during aging in anabolic and catabolic periods

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Abstract

One of the most important effects of aging is sarcopenia, which is associated with impaired locomotion and general weakness. In addition, there is increased susceptibility to illness in aging, which often results in muscle wasting episodes. In such instances, the mobilization of muscle proteins provides free amino acids that are used for energetic purpose, the synthesis of acute phase proteins, and the immune response. However, since muscle protein mass is already depleted, the ability of the aged organism to recover from stress is impaired. Therefore, elucidating the mechanisms that result in sarcopenia is of obvious importance. Age-related changes in protein synthesis and proteolysis are rather small and our current methodology does not enable one to establish unequivocally whether sarcopenia results from depressed protein synthesis, increased proteolysis or both. By contrast, in anabolic and catabolic periods, a number of dysregulations in muscle protein turnover became clearly apparent. The aim of this review is to provide an overview of such altered responses to nutrients and catabolic treatments, which may ultimately contribute to explain sarcopenia. This includes impaired recovery in catabolic states, impaired anabolic effects of nutrients, in particular leucine, and a lack of regulation of the ubiquitin-proteasome proteolytic system. These alterations are discussed with respect to modifications in the insulin/IGF-1 axis and glucocorticoid related effects.

Keywords: Hormones; Nutrients; Protein turnover; Sarcopenia; Skeletal muscle

Contents

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1. Introduction

One of the most important effects of aging is sarcopenia. A decline in muscle strength parallels muscle atrophy (Short & Nair, 2000). This results in impaired locomotion and general weakness. In addition, there is increased susceptibility to illness in aging. In such instances, muscle is the major reservoir of body proteins, and consequently of amino acids, which are used for energetic purpose and synthesis of acute phase proteins. Since muscle protein mass is already depleted, the ability of the aged organism to recover from stress is impaired. Therefore, elucidating the mechanisms that result in sarcopenia is of obvious importance.

Muscle mass declines in man during aging, while the total amount of lipid stores either remains constant or increases significantly (Cohn et al., 1980; Short & Nair, 2000). This phenomenon appears during the third decade in men, but is considerably delayed in women (between 50 and 70 years of age) (Hughes, Frontera, Roubenoff, Evans, & Fiatarone Singh, 2002). The reduced muscle mass mainly reflects a loss of myofibrillar proteins (Evans, 1997). In senescent rodents, although the whole-body protein mass is not reduced, wasting is apparent in white muscles containing mainly type II fibers (Holloszy, Chen, Cartee, & Young, 1991). This results from a reduction in fiber areas, a loss of myofibrillar proteins, and is associated with a conversion of type II (glycolytic) into type I (oxidative) fibers.

Proteins in skeletal muscle, as in other mammalian tissues, undergo a continuous process of degradation and synthesis (Waterlow, Garlick, & Millward, 1978). Rates of protein turnover regulate the levels of specific proteins. In addition, changes in skeletal muscle protein mass depend on the overall balance between rates of protein synthesis and breakdown. Muscle wasting may result from decreased protein synthesis, increased proteolysis, or simultaneous changes in both processes. These alterations are presumably multi-factorial. Major factors implicated in the etiology of sarcopenia include decreased physical activity, declines in α-motor neurons, proteino-energetic malnutrition, increased cytokine activity, oxidative stress, mitochondrial dysfunction, and abnormalities in growth hormone and sex steroid axes (Kamel, 2003; Morley & Baumgartner, 2004; Roubenoff, 2000). For example, the role of reactive oxygen species in sarcopenia has been discussed in several recent reviews (Carmeli, Coleman, & Reznick, 2002; Ji, 2001, 2002). The purpose of this review is to concentrate on alterations of protein turnover in anabolic and catabolic periods that may contribute to explain sarcopenia.

2. Age-related changes in muscle protein turnover in basal conditions

2.1. Changes in protein synthesis during aging

The concept of aging is strongly associated with the notion of a decrease in protein synthesis, which includes changes in genome integrity and gene expression (Hornsby, 1991; Papaconstantinou, Reisner, Liu, & Kuninger, 1996), and in translation and post-translational modifications of proteins (Rattan, 1996).

In vivo, muscle protein synthesis decreases rapidly from young growing to mature mammals (Attaix, Aurousseau, Bayle, Rosolowska-Huszcz, & Arnal, 1998; Lewis, Kelly, & Goldspink, 1984). By contrast, between the mature and the old organism, there is considerable uncertainty concerning several indicators of protein synthesis (El Haj, Lewis, Goldspink, Merry, & Holehan, 1986; Goldspink, El Haj, Lewis, Merry, & Holehan, 1987; Mays, McAnulty, & Laurent, 1991; Mosoni, Patureau Mirand, Houlier, & Arnal, 1993). For example, the same group reported that both the fractional rate of protein synthesis (Ks, in %/day) and the translational efficiency (kRNA, in mg protein synthesized/mg RNA/day) either decreased (Lewis et al., 1984) or increased (El Haj et al., 1986) in the rat tibialis anterior muscle between 1 and 2 years of age. By contrast, during the same period, protein synthesis was maintained when expressed in absolute terms and increased when expressed in fractional terms as a result of protein loss due to muscle atrophy (Mosoni et al., 1993b).

Surprisingly, more information is available on the mechanisms that are involved in reduced muscle protein synthesis rates in humans. In old (>60 years) compared with adult (<35 years) subjects, the decreased synthesis of mixed muscle proteins reflected a reduction in the synthesis rates of myofibrillar proteins (Welle, Thornton, Jozefowicz, & Statt, 1993), but not of sarcoplasmic proteins (Balagopal, Rooyackers, Adey, Ades, & Nair, 1997). The synthesis of myosin heavy chain was dramatically reduced (Balagopal et al., 1997), without any change in its rate of transcription (Welle, Bhatt, & Thornton, 1996). Aging is also characterized by a reduced rate of mitochondrial protein synthesis, which may contribute to the depressed aerobic capacity and muscle performance (Rooyackers, Adey, Ades, & Nair, 1996). However, it should be emphasized that all observations in humans have been obtained in a single muscle (e.g. the quadriceps). In addition, it is clear from both animal and human studies that the most important changes in protein synthesis rates occur relatively early. For example, the fractional rate of muscle mitochondrial protein synthesis rapidly declined in middle age (54 ± 1 years) compared with young humans (24 ± 1 years), but thereafter tended to increase with advancing age (73 ± 2 years) (Rooyackers et al., 1996). Therefore, age-related alterations in protein synthesis in the basal state may contribute to, but did not explain, the slow erosion of muscle protein mass.

2.2. Changes in protein breakdown in aging

The concept of aging is also strongly associated with the notion of increased proteolysis leading to muscle wasting. Alternatively, the accumulation of abnormal proteins during aging is widely believed to result from defects in protein breakdown. However, very few experimental data support these hypotheses.

The regulation of muscle proteolysis is still poorly understood. First, its study is fraught with methodological problems (Attaix & Taillandier, 1998). Myofibrillar proteins represent approximately 60–70% of the proteins in skeletal muscle and turn over very slowly in old animals and subjects (Waterlow et al., 1978). Thus, changes in protein turnover are small and difficult to quantitate reliably in old organisms. For example, only slightly higher basal rates of proteolysis were observed in old compared with healthy young men (Volpi, Sheffield-Moore, Rasmussen, & Wolfe, 2001). In addition, all techniques currently available to study skeletal muscle protein breakdown in vivo are indirect, opened to strong criticisms, and provide little if any information on intracellular regulatory mechanisms (Attaix & Taillandier, 1998). In vitro methods are available, but such preparations are always in a highly catabolic state. Therefore, observations in vitro may not necessarily reflect the in vivo situation, and caution must be exercised in interpreting such data.

Second, at least three major proteolytic pathways operate in skeletal muscle. The best known is the lysosomal pathway. However, skeletal muscle contains few lysosomes, and the major lysosomal proteases (cathepsins B, H, L and D) do not contribute significantly to overall protein breakdown in muscles incubated under optimal conditions (Attaix & Taillandier, 1998; Mitch & Goldberg, 1996). Furthermore, increased cathepsin activities and mRNA levels (except for cathepsin L, see below) have been reported in a few instances of muscle wasting (Attaix & Taillandier, 1998) and lysosomes are not involved in the degradation of myofibrillar proteins (Lowell, Ruderman, & Goodman, 1986; Tiao et al., 1994). This pathway is presumably responsible for the breakdown of some long-lived, membrane and endocytosed proteins (Mitch & Goldberg, 1996). Very little information is available on changes in the

lysosomal system in the aged muscle. However, recent studies have identified increased mRNA levels for cathepsin L as a marker of various muscle wasting conditions (Deval et al., 2001; Lecker et al., 2004). Interestingly, mRNA levels for cathepsin L (and cathepsin C) increased in the sarcopenic rat soleus muscle, but the induction was very limited compared with other catabolic models (Pattison, Folk, Madsen, Childs, & Booth, 2003).

The Ca^{2+}-dependent pathway comprise μ- and m-calpains, which are ubiquitous, and the muscle specific calpain p94 (Goll, Thompson, Li, Wei, & Cong, 2003). Calpains are believed to play a role in the disassembly of sarcomeric proteins (Huang & Forsberg, 1998; Williams et al., 1999), which is a rate-limiting step in the breakdown of myofibrillar proteins (Solomon & Goldberg, 1996). However, like cathepsins, calpains are not systematically activated in various muscle wasting conditions and are not directly responsible for the breakdown of actin and myosins (Attaix & Taillandier, 1998). To our knowledge, possible adaptations in the Ca^{2+}-dependent pathway have not been reported in aged muscles.

The third major proteolytic process is ubiquitin-proteasome-dependent. There are two major steps in this pathway. First, the substrates are polyubiquitinated in a process that is tightly controlled by ubiquitination enzymes (see Glickman & Ciechanover, 2002; Pickart, 2001 for recent reviews). Polyubiquitination requires the sequential involvement of the ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3), and is ultimately proof-read by deubiquitination enzymes. Second, polyubiquitinated substrates are selectively recognized and degraded by the 26S proteasome (Attaix, Combaret, Pouch, & Taillandier, 2001; Voges, Zwickl, & Baumeister, 1999). The 26S proteasome is formed by the binding of the proteolytic core (i.e. the 20S proteasome responsible for the proteolytic and peptidase activities) with two 19S regulatory complexes containing both ATPase and non-ATPase subunits.

In mammalian cells, the ubiquitin-proteasome-dependent proteolytic pathway catalyzes the selective breakdown of abnormal and short-lived proteins (e.g. oncoproteins, tumor suppressors, transcriptional factors, cell-cycle regulators, etc.) (Glickman & Ciechanover, 2002). In skeletal muscle, this pathway is also responsible for the breakdown of the long-lived myofibrillar proteins (reviewed in Attaix, Combaret, Kee, & Taillandier, 2003; Hasselgren & Fischer, 2001; Lecker, Solomon, Mitch, & Goldberg, 1999; Mitch & Goldberg, 1996). Table 1 summarizes the different adaptations in the ubiquitin-proteasome pathway that have been reported in various instances of muscle wasting. Increased expression or transcription of ubiquitin, muscle-specific E3s called Atrogin-1/MAFbx and MURF1, and 20S proteasome subunit genes has been consistently reported. Enhanced mRNA levels for at least one deubiquitination enzyme (Combaret et al., 2005) and for various ATPase and non-ATPase subunits of the 19S complex (Combaret et al., 2004; Lecker et al., 2004) have also been observed. Altogether, such adaptations correlated with increased rates of substrate ubiquitination and increased proteasome activities (Combaret et al., 2004).

Table 1
Alterations in proteolytic events within the ubiquitin-proteasome pathway in various instances of muscle wasting

Proteolytic events
Ubiquitination/deubiquitination
Increased ubiquitin expression or transcription
Increased expression of muscle-specific E3s (Atrogin-1/MAFbx and MURF1)
Accumulation of ubiquitin-conjugates
Increased rate of ubiquitination
Increased expression of deubiquitinating enzymes
Proteolysis
Increased rates of total and myofibrillar proteolysis blocked by proteasome inhibitors
Increased expression or transcription of most subunits of the 20S proteasome
Increased expression of some subunits of the19S complex
Increased proteasome activities

Unfortunately, information on the activity of the ubiquitin-proteasome pathway during aging is also limited. In skeletal muscles from old rats (Mosoni et al., 1999; Pattison et al., 2003) or humans (Welle, Brooks, Delehanty, Needler, & Thornton, 2003) there were no or small increases in mRNA levels encoding ubiquitin, the 14 kDa E2, E2B, and a few subunits of the 20S proteasome or the 19S complex. Similarly, the mRNA levels for the Atrogin-1/MAFbx E3, which are highly increased in numerous catabolic states (Bodine et al., 2001; Lecker et al., 2004), were either unchanged (Welle et al., 2003) or slightly increased (Pattison

et al., 2003) in human vastus lateralis biopsies and rat soleus muscle, respectively. In one study, the slightly higher expression of subunit C2 of the 20S proteasome correlated with enhanced 3-methylhistidine excretion per mg of creatinine (an index of myofibrillar proteolysis) possibly suggesting a modest activation of the pathway with aging (Mosoni et al., 1999). Recently, a large increase in ubiquitin protein levels has been reported in old rodent and human muscles (Cai, Lee, Li, Tang, & Chan, 2004). The protein content of several (but not all) subunits of the 20S proteasome was also increased in old rat muscle (Husom et al., 2004). Since the major peptidase activities of the proteasome (i.e. the chymotrypsin-, trypsin- and caspase-like activities) did not change with aging, there was a marked reduction in the proteasome specific activities (i.e. activities expressed per actual muscle content of proteasome) with aging (Husom et al., 2004). In addition, in these experiments, the protein content of 19S complexes relative to the 20S proteasome was also strongly reduced. Altogether, these data suggest a major impairment of the proteasome with aging (Husom et al., 2004). Unfortunately, these data are not in agreement with numerous studies performed in various tissues, in which the proteasome content actually decreases with aging (reviewed by Husom et al., 2004), including skeletal muscle. For example, in the LOU rat, there was a 27% decrease in muscle protein content only between 18 and 34 months of age. When age-related changes in proteasome-dependent proteolysis were examined (at 4, 18, 24, 29 and 34 months of age), the three major proteasome activities (see above) increased to 29 months, but then decreased in the very senescent animal when muscle protein loss was maximal (Bardag-Gorce, Farout, Veyrat-Durebex, Briand, & Briand, 1999). These variations in activity were accompanied by an identical change in the amount of 20S proteasome measured by Western blot, whereas the amounts of the S4 subunit of the 19S complex and of ubiquitin-conjugates remained constant. However, and in contrast, mRNA levels for proteasome subunits C3, C5, C9, and S4 actually increased in the very senescent animal. Thus, it remains totally unclear whether the ubiquitin-proteasome pathway is depressed or activated during aging. Indeed, myofibrillar protein turnover was found to be decreased, unchanged or increased in old rats (Makrides, 1983). In addition, the reduction in total muscle proteolysis that was reported in the elderly was abolished when data were expressed per g of excreted creatinine/day, suggesting that reduced protein breakdown mainly reflects muscle atrophy (Young, Munro, & Fukagawa, 1989).

3. Alterations in the regulation of muscle protein turnover during aging

Our current methodology is inappropriate to explain small differences in both components of protein turnover in basal conditions that may explain sarcopenia. Thus our strategy was to identify other factors that contribute to the catabolic response. Alterations in protein synthesis, protein breakdown, or both, have been described in anabolic and catabolic periods.

3.1. Control of protein turnover in anabolic conditions

Protein turnover in skeletal muscle is highly responsive to nutritional, hormonal and mechanical factors (Attaix et al., 2003; Mitch and Goldberg, 1996; Waterlow et al., 1978). We mainly discuss below alterations in protein turnover in response to nutrients and/or hormones that may contribute to explain sarcopenia.

3.1.1. Feeding

In young rats, muscle protein synthesis increases sharply within 1 h of feeding (Garlick, Fern, & Preedy, 1983). This stimulation of muscle protein synthesis is attenuated in adult animals compared with young rats (Baillie & Garlick, 1992). To determine whether a reduced sensitivity of muscle protein synthesis can be detected during aging, the in vivo response of protein synthesis to feeding was measured in the tibialis anterior muscle, which contains mainly type II fibers and is very sensitive to food intake manipulation (Mosoni et al., 1995). Protein synthesis (expressed in terms of either fractional or absolute rate, as well as kRNA) was stimulated during the post-prandial period in adult (12-month-old), but not in old (24-month-old) rats. Additional experiments in humans clearly support a differential effect of nutrients during aging. First, the response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia was impaired in the elderly (Volpi, Mittendorfer, Rasmussen, & Wolfe, 2000). Second, protein feed-

ing pattern does not affect protein retention in young women (Arnal, Mosoni, Boirie, Houlier, et al., 2000). In contrast, a protein pulse-feeding pattern was more efficient than was a protein spread-feeding pattern in improving, after 14 days, whole-body protein retention in elderly women (Arnal et al., 1999). Thus, a reduced post-prandial stimulation of muscle protein synthesis may contribute to a very progressive reduction in muscle protein mass in animals (Mosoni et al., 1995), and presumably in the elderly.

Feeding also inhibits the increased muscle proteolysis that normally occurs in the post-absorptive state. However, following a sustained catabolic state (i.e. 48 h of starvation in very young rats) the inhibition of the enhanced total and proteasome-dependent proteolysis seen in refed animals was delayed compared with the stimulation of protein synthesis (Kee et al., 2003). In fed elderly women infused with L-[1-C^{13}]leucine, the decrease in endogenous leucine production (an estimate of whole body protein breakdown) corrected for splanchnic extraction was much lower than in fed adults (Arnal, Mosoni, Boirie, Gachon, et al., 2000). To determine whether the post-prandial inhibition of muscle proteolysis is modified with aging, epitrochlearis muscles from adult and old rats were incubated in vitro. Feeding inhibited proteolysis in adult but not in old rats (Arnal et al., 2002). When similar experiments were performed without and with a proteasome inhibitor, proteasome-dependent proteolysis was also inhibited in the adult but not in old animals (Combaret, Dardevet, Grizard, & Attaix, unpublished data). Thus, the failure of feeding to depress muscle protein breakdown rates may also contribute to the slow rate of erosion of muscle proteins during aging.

3.1.2. Amino acids

Amino acids alone or with euglycemic insulin stimulate whole-body protein synthesis to a similar extent in postabsorptive young and elderly men (Fukagawa et al., 1989). Visceral tissues account presumably for a greater proportion of whole-body protein synthesis during aging, due to the reduction in muscle mass (Forbes & Halloran, 1976). In addition, in elderly men there was higher leucine extraction by the gut, liver, or both during feeding, which could lead to a lower peripheral availability of dietary leucine (Boirie, Gachon, & Beaufrère, 1997). However, although an increase with age in the first-pass splanchnic extraction of phenylalanine was also reported, this did not affect the increase in phenylalanine arterial concentration and delivery to the leg (Volpi, Mittendorfer, Wolf, & Wolfe, 1999).

The stimulatory effect of insulin on in vivo skeletal muscle protein synthesis, which is more marked in the presence of amino acids (Garlick & Grant, 1988), was reduced in adult rats (Baillie & Garlick, 1992). Therefore, the effects of both amino acids and insulin were studied on in vivo protein synthesis in the gastrocnemius muscle from 12-month-old adult, and 24-month-old aged rats (Mosoni, Houlier, Patureau Mirand, Bayle, & Grizard, 1993). Animals were infused with saline, amino acids, or amino acids with insulin and glucose. Amino acids significantly stimulated the muscle protein fractional synthesis rate to a similar extent (18–20%) in adult and old rats (when variability introduced by muscle atrophy was taken into account by a variance–covariance analysis). By contrast, insulin did not elicit any additional effect whatever the age. Exogenous amino acids were also demonstrated to stimulate net skeletal muscle protein synthesis in the elderly (Volpi, Ferrando, Yeckel, Tipton, & Wolfe, 1998). Thus, the capacity of muscle protein synthesis to be stimulated by amino acids was preserved during aging. However, in the absence of insulin (i.e. when secretion is blocked by diazoxide) the stimulatory effect of dietary amino acids on muscle protein synthesis was reduced in the old (Prod'homme et al., 2004).

Recently, a new role for amino acids as regulators of mRNA translation has been identified (Kimball & Jefferson, 2001). In this role, they modulate the phosphorylation state of proteins that represent important control points in translation initiation, including the translational repressor 4E-BP1 and the ribosomal protein S6 kinase S6K1. When administered orally to fasted rats the branched-chain amino acid leucine was particularly effective in stimulating translation initiation (Kimball & Jefferson, 2001). The effects of amino acids or leucine alone were recently assessed on protein synthesis in incubated epitrochlearis muscles from young, adult and old rats (Dardevet, Sornet, Balage, & Grizard, 2000). Amino acids, at physiologic concentrations, stimulated muscle protein synthesis and leucine reproduced this effect. The intracellular targets of amino acids were phosphatidylinositol 3′ kinase and the rapamycin-sensitive pathways mammalian target of rapamycin (mTOR)/p70S6 kinase. However, in old rats, the sensitivity of muscle protein synthesis to

leucine was lower than in adults and this paralleled the lesser ability of leucine to stimulate the rapamycin-sensitive pathways. Thus, aging is associated with a decreased leucine-induced stimulation of muscle protein synthesis (Dardevet et al., 2000). However, because aged rats are still able to respond normally to high leucine concentrations, it was hypothesized that a nutritional manipulation increasing the availability of this amino acid to muscle could be beneficial in maintaining the post-prandial stimulation of protein synthesis. A leucine-supplemented meal indeed restored a significant stimulation of muscle protein synthesis in old animals (Dardevet et al., 2002), and this effect was maintained for at least ten days (Rieu et al., 2003). In addition, leucine supplementation also restored the post-prandial inhibition of proteasome-dependent proteolysis (Combaret, Dardevet, Grizard, & Attaix, unpublished data) that is defective in aged animals (see above).

3.1.3. Insulin and insulin-like growth factor-1

Studies using the euglycemic clamp technique have demonstrated an impairment in insulin-induced glucose disposal in both old human subjects and animals (Nishimura et al., 1988). The potential role of insulin resistance in the development of sarcopenia has been recently reviewed (Volpi, Nazemi, & Fujita, 2004). Interestingly, a recent paper demonstrated that the impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans (Guillet et al., 2004). Insulin-like growth factor-1 (IGF-1), like insulin, stimulates glucose and amino acid transport and promotes a net positive protein balance by activation of protein synthesis and/or suppression of protein degradation. Insulin resistance and an impairment of IGF-1 action on glucose metabolism have been observed in non-insulin dependent diabetes mellitus and obesity. However, it was unknown whether IGF-1 and insulin resistance was always associated, and whether resistance affected only glucose, or protein metabolism as well. To answer these questions, IGF-1 and insulin actions on glucose and protein metabolism were analyzed in the epitrochlearis muscle from young (1-month-old), adult (6–8-month-old), and old rats (18–20-month-old) (Dardevet, Sornet, Attaix, Baracos, & Grizard, 1994). In young rats, IGF-1 was equipotent to insulin in stimulating 2-deoxy-glucose and aminoisobutyric acid transport, but more potent in increasing protein synthesis. Insulin and IGF-1 action on glucose transport decreased in adult compared with young rats. An insulin resistance of amino acid transport and protein synthesis was also observed in adults, but the stimulatory effect of IGF-1 on these processes was abolished. Thus the degree of resistance observed varied with the agonist and the metabolic process observed. Modifications of IGF-1 action in mature animals may result in part from the dramatic decrease in IGF-1 receptors (80%). However, no similar observation was observed for the insulin receptor. Since muscle IGF-1 receptor gene expression did not decrease in parallel with receptor number, an alteration in IGF-1 receptor mRNA translation or receptor degradation may be hypothesized. Thus, in contrast to glucose transport, intracellular IGF-1 and insulin post-receptor pathways leading to amino acid uptake and protein metabolism differ. In addition, modification in post-binding events might be involved in decreased insulin- and IGF-1-stimulated muscle metabolism during aging. Changes between adult and old animals must be further explored. However, it is noteworthy that the injection of a recombinant adeno-associated virus directing overexpression of IGF-1 in differentiated muscle fibers promoted an average increase of 15% in muscle mass and a 14% increase in strength in young adult mice, and remarkably, prevented age-related muscle alterations in old adult mice (Barton-Davis, Shoturma, Musaro, Rosenthal, & Sweeney, 1998).

3.2. Control of protein turnover in catabolic conditions

Evidence has also been provided that different adaptative mechanisms are responsible for the muscle wasting in acute catabolic conditions, and subsequent muscle recovery, in adult and old rats.

3.2.1. Starvation

The capacity of 5-day-refed 12- and 24-month-old rats to recover muscle mass lost after 10 days of food deprivation has been compared, by measuring in vivo protein synthesis, nitrogen balance, 3-methylhistidine excretion, and gene expression of components of the lysosomal, Ca^{2+}-dependent, and ubiquitin-proteasome-dependent proteolytic pathways (Mosoni et al., 1999). Twenty-four-month-old rats ex-

hibited an altered capacity to recover muscle proteins. Muscle protein synthesis, inhibited during starvation, returned to control values during refeeding in both age groups. In contrast, the lack of inhibition of muscle proteolysis during refeeding that was observed only in aged animals explained the delayed recovery of muscle mass. Accordingly, gene expression of components of the ubiquitin-proteasome pathway did not change in the muscles from starved or refed old animals. Thus, the ubiquitin-proteasome pathway was dysregulated in the muscles of old animals. Since protein breakdown is a major determinant of muscle protein deposition, the inability of old animals to modulate this process contributes to the muscle wasting.

3.2.2. Glucocorticoid treatment

The increased incidence of various disease states results in hypersecretion of glucocorticoids during aging (Dardevet et al., 1995; Savary et al., 1998). Glucocorticoids are well known to induce muscle wasting. When adult (7-month-old) and aged (22-month-old) rats received dexamethasone (approximately 500 μg/kg body weight/day) in their drinking water for 5–6 days, muscle wasting was much more rapid in aged animals (Dardevet et al., 1995). Animals were then allowed to recover, and rates of protein synthesis and breakdown were measured in vitro in the absence of insulin. The epitrochlearis muscle mass was normalized within 3 days in adults, but only within 7 days in aged rats. Thus, and again, muscle mass recovery following a catabolic state is delayed with aging. In addition, muscle wasting and recovery occurred by totally different mechanisms in both groups of dexamethasone-treated animals. In the adult, muscle wasting totally resulted from increased protein breakdown. By contrast, muscle atrophy in old rats was due to depressed protein synthesis. Accordingly, muscle mass was normalized in adults by a reduced rate of proteolysis (associated with an increased rate of protein synthesis), whereas this phenomenon only reflected increased protein synthesis in aged animals.

In vivo protein synthesis also decreased in fast-twitch glycolytic and oxidative glycolytic muscles (gastrocnemius, tibialis anterior, extensor digitorum longus) from rats treated with dexamethasone (Savary et al., 1998). The treatment affected mostly ribosomal efficiency, and depressed protein synthesis more rapidly in aged than in adult muscles. Further in vitro studies have shown that glucocorticoids significantly decreased the stimulatory effect of insulin and IGF-1 on muscle protein synthesis in adult rats by 26 and 58%, respectively. In old rats, this effect was even greater, being 49 and 100%, respectively (Dardevet et al., 1998). The role of p70 S6 kinase, p90 S6 kinase, and mitogen-activated protein (MAP) kinase pathways in the insulin resistance of muscle protein synthesis observed during glucocorticoid treatment has been investigated (Dardevet, Sornet, & Grizard, 1999). This resistance was associated with a total blockage of the stimulation of p70 S6 kinase by insulin without any significant decrease in the amount of the kinase. However, the effect of rapamycin (an inhibitor of several intracellular pathways, including p70 S6 kinase pathways) on rat muscle protein synthesis was not modified by dexamethasone. This suggests that rapamycin-sensitive pathways associated with the insulin stimulation of protein synthesis were not altered by glucocorticoids, and thus were not responsible for the insulin resistance observed. Incubation of muscles with a MAP kinase inhibitor (PD98059) did not modify the stimulation of protein synthesis by insulin. As glucocorticoids did not alter the effect of insulin on p90 S6 kinase activity, glucocorticoid-induced alterations in muscle protein synthesis regulation by insulin did not involve factors or kinases that are dependent on MAP kinase and/or p90 S6 kinase activity (Dardevet et al., 1999). Finally, glucocorticoids also induced a prolonged leucine resistance on muscle protein synthesis in old rats (Rieu, Sornet, Grizard, & Dardevet, 2004).

Glucocorticoid administration increases ubiquitin expression in rat skeletal muscle (Wing & Goldberg, 1993). Increased mRNA levels for ubiquitin were observed in the muscles of both dexamethasone-treated adult and old rats (Dardevet et al., 1995). By contrast, increased mRNA levels for the 14 kDa E2 and subunits of the 20S proteasome were only detected in dexamethasone-treated adults (i.e. when increased protein breakdown was observed), but not in the muscles from old animals (Dardevet et al., 1995). Furthermore, and in accordance with a major role of the ubiquitin-proteasome-dependent pathway in muscle, increased mRNA levels for ubiquitin, 14 kDa E2 and 20S proteasome subunits returned towards normal values in adult rats that were allowed to recover following dexamethasone treatment. The lack of effect of dexamethasone on mRNA levels for the 14 kDa E2 and subunits of the 20S

Table 2
Alterations in the control of protein turnover that contribute to muscle wasting in aged animal

Alteration	Effect	References
Reduced stimulatory effect of feeding on protein synthesis	Reduction in protein deposition	Mosoni et al. (1995)
Impaired sensitivity of muscle protein synthesis to leucine	Reduction in protein deposition	Dardevet et al. (2000), Rieu et al. (2003)
Defective inhibition of overall and ubiquitin-proteasome-dependent proteolysis in the post-prandial state	Reduction in protein deposition	Arnal et al. (2002), Combaret et al. (unpublished data)
Delayed recovery following a stress	Impairment in protein mass recovery	Dardevet et al. (1995), Mosoni et al. (1999)
Inability to regulate the ubiquitin-proteasome-dependent pathway in catabolic states and subsequent recovery	Impairment in protein mass recovery, since only changes in protein synthesis regulate this process	Dardevet et al. (1995), Mosoni et al. (1999)
Insulin- and IGF-1-resistance under glucocorticoids	Reduction in protein deposition	Dardevet et al. (1994, 1998)

proteasome in aged muscle further supported a lack of responsiveness of the ubiquitin-proteasome system during aging.

4. Conclusion

Although many studies have demonstrated or suggested abnormalities in either protein synthesis or breakdown in aged skeletal muscles, very little information explaining unequivocally sarcopenia is available. Recent experiments have identified a number of alterations in protein turnover that result in imbalances between protein synthesis and breakdown, and contribute to the slow erosion of muscle protein mass (Table 2). In particular, a defective regulation of the ubiquitin-proteasome-dependent proteolytic pathway, and an impaired ability to recover from stress, both resulted in a progressive or sometimes more marked irreversible loss of proteins. However, these changes are very subtle and difficult to demonstrate in vivo. An impairment in insulin, IGF-1, or glucocorticoid action or responsiveness may also contribute to explain age-related alterations in protein metabolism.

5. Future directions

Unfortunately, the characterization of the critical factors (physical activity, malnutrition, oxidative status, hormonal status, etc) that have pronounced effects on the establishment of sarcopenia remains elusive.

By contrast, recent studies in old rats and humans show decreased responsiveness of skeletal muscle to the anabolic effect of nutrients. Thus, we need more information on the alterations of the signaling pathways of some hormones and/or nutrients (i.e. amino acids) that control both components of protein turnover in the old. For example, experiments that aim to understand why ubiquitin-proteasome-dependent proteolysis in the aged muscle is not down-regulated in the post-prandial period are crucially needed. Direct experimentations showing that nutritional manipulation (i.e. leucine supplementation), which has positive effects in rodents, could be used to improve muscle protein deposition in old human volunteers are also needed. This possibility is currently being tested in our laboratory.

Theoretically such nutritional manipulations could be combined with other anabolic treatments (i.e. progressive resistance training and/or the utilization of trophic factors like testosterone and DHEA), which have a beneficial effect on muscle mass and/or strength in older adults (Kamel, 2003).

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