Assessment of biomarkers of protein anabolism in skeletal muscle during the life span of the rat: sarcopenia despite elevated protein synthesis

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Kimball, Scot R., James P. O’Malley, Joshua C. Anthony, Stephen J. Crozier, and Leonard S. Jefferson. Assessment of biomarkers of protein anabolism in skeletal muscle during the life span of the rat: sarcopenia despite elevated protein synthesis. Am J Physiol Endocrinol Metab 287: E772–E780, 2004. First published June 8, 2004; 10.1152/ajpendo.00535.2003.—Loss of muscle strength is a principal factor in the development of physical frailty, a condition clinically associated with increased risk of bone fractures, impairments in the activities of daily living, and loss of independence in older humans. A primary determinant in the decline in muscle strength that occurs during aging is a loss of muscle mass, which could occur through a reduction in the rate of protein synthesis, an elevation in protein degradation, or a combination of both. In the present study, rates of protein synthesis and the relative expression and function of various biomarkers involved in the initiation of mRNA translation in skeletal muscle were examined at different times throughout the life span of the rat. It was found that between 1 and 6 mo of age, body weight increased fourfold. However, by 6 mo, gastrocnemius protein synthesis and RNA content per gram of muscle were lower than values observed in 1-mo-old rats. Moreover, the relative expression of two proteins involved in the binding of initiator methionyl-tRNA to the 40S ribosomal subunit, eukaryotic initiation factors (eIF)2 and eIF2B, as well as the 70-kDa ribosomal protein S6 kinase, S6K1, was lower at 6 mo compared with 1 mo of age. Muscle mass, protein synthesis, and the aforementioned biomarkers remained unchanged until ~21 mo. Between 21 and 24 mo of age, muscle mass decreased precipitously. Surprisingly, during this period protein synthesis, relative RNA content, eIF2B activity, relative eIF2 expression, and S6K1 phosphorylation all increased. The results are consistent with a model wherein protein synthesis is enhanced during aging in a futile attempt to maintain muscle mass.

transcription initiation; eukaryotic initiation factor; ribosomal protein S6 kinase; aging

SARCOPENIA, the disproportionate loss of skeletal muscle that occurs during the last quartile of the life span, has been well documented in many species, including humans (4, 33). A number of physiological factors have been suggested to be involved in sarcopenia, including an age-related reduction of hormones such as growth hormone (22), thyroxine (30), and, in women and men, estrogen and testosterone (29), respectively. In addition to a disorganization of hormonal homeostasis, an elevation of inflammatory activity and TNF-α signaling (16) has also been suggested to contribute to sarcopenia. Furthermore, in humans and rodents, it has been observed that, with age, neuromuscular communication becomes dysfunctional, spinal cord motor neurons die, and motor units are lost (3, 6, 19). In rats this has been reported to lead to an increased incidence of denervation (28).

Although all of these physiological conditions could be expected to contribute to muscle mass loss, it remains to be determined which of these phenomena are primarily responsible for age-associated muscle loss. However, although the etiology of sarcopenia remains to be determined, it is reasonable to assume that it would affect the physiological environment of the muscle and thus would be reflected in the physiology of the muscle cells.

The molecular events that occur in muscle during sarcopenia are not well understood. Because muscle mass is determined by the net relationship between protein synthesis and breakdown, sarcopenia must be due to a relative decrease in protein synthesis, a relative increase in protein degradation, or a combination of both. The aim of this study was to assess the role of protein synthesis in sarcopenia by assessing the relative expression and function of cellular components involved in the initiation of mRNA translation, as well as components of the protein kinase B (PKB)/mammalian target of rapamycin (mTOR)-signaling pathway that plays a vital role in cell growth (7) (see Fig. 8). A key regulatory step in protein synthesis is the initiation of mRNA translation. This step is tightly regulated and requires the assembly of a translation initiation complex comprised of initiator methionyl-tRNA (met-tRNAi), mRNA, and various eukaryotic initiation factors (eIFs), the main function of which is to position the ribosome at the translation initiation start site on the mRNA. Assembly of the complex is regulated at two distinct points. One point is the exchange of GDP bound to eIF2 for GTP, a reaction that is mediated by the guanine nucleotide exchange protein eIF2B (see review in Ref. 31). The GDP-eIF2 binary complex generated after each round of initiation is incapable of interacting with met-tRNA, and therefore cannot participate in the first step in translation initiation, the binding of eIF2·GTP·met-tRNAi to the 40S ribosomal subunit. The activity of eIF2B is negatively regulated by glycogen synthase kinase-3 (GSK-3) through phosphorylation of the e-subunit of the protein on Ser535 and by phosphorylation of the α-subunit of eIF2 on Ser51. A second point of regulation is the phosphorylation of eIF4E-binding protein 4E-BP1 (see review in Ref. 12). Phosphorylation of 4E-BP1 releases it from...
elF4E, allowing the unbound elF4E to bind to elF4G to form the active complex termed elF4F, which is required for the binding of mRNA to the translation initiation complex. 

Changes in phosphorylation of ribosomal protein S6 have also been implicated in the control of mRNA translation (see review in Ref. 13). In this regard, activation of the 70-kDa S6 kinase S6K1 is associated with enhanced translation of mRNA with an oligopyrimidine tract preceded by a single cytidine, termed a TOP sequence, at the 5′-end of the mRNA. Examples of such mRNAs include those encoding the ribosomal proteins, translation elongation factors, and poly(A)-binding protein, in other words, proteins that participate in the protein biosynthetic process.

Several of the steps in the initiation of mRNA translation are regulated by the PKB/mTOR-signaling pathway. For example, mTOR positively regulates translation initiation by both phosphorylating 4E-BP1 and causing it to dissociate from elF4E, as well as by activating S6K1 via phosphorylation of Thr389 (see review in Ref. 13). Furthermore, PKB indirectly elevates the activity of elF2Be by inactivating GSK-3 (31) and reducing the level of Ser235 phosphorylation. Although the PKB/mTOR pathway can be upregulated by activation of the insulin receptor or IGF-I receptors with their appropriate agonists, it can also be activated by focal adhesion kinase (FAK) via stretch-induced activation of integrins (9, 15). Thus the PKB/mTOR pathway can be activated by hormonal inputs as well as by muscle use.

The hypothesis that was tested in the present study was that the decline in muscle mass during aging is due to a fall in the content and/or activity of proteins that regulate the process of translation initiation and/or a decrease in signaling through the PKB/mTOR or IGF-I receptors with their appropriate agonists, it can also be activated by focal adhesion kinase (FAK) via stretch-induced activation of integrins (9, 15). Thus the PKB/mTOR pathway can be activated by hormonal inputs as well as by muscle use.

The hypothesis that was tested in the present study was that the decline in muscle mass during aging is due to a fall in the content and/or activity of proteins that regulate the process of translation initiation and/or a decrease in signaling through the PKB/mTOR-signaling pathway. To test this hypothesis, various biomarkers of protein anabolism were examined in the gastrocnemius of male Sprague-Dawley rats, a muscle composed of a mixture of fast- and slow-twitch fibers that has been shown previously to exhibit sarcopenia with aging. The following biomarkers were examined: 1) the guanine nucleotide exchange activity of elF2B; 2) the relative expression of elF2Be, elF2a, elF4E, and elF4G; 3) the phosphorylation status of S6K1, elF2Be, and elF2a; and 4) the relative expression of S6K1 and mTOR. We also measured protein synthesis rates with a flooding dose of radiolabeled phenylalanine. Assays were made in 1-, 6-, 12-, 18-, 21-, 24-, and 27-mo-old male rats.

**MATERIALS AND METHODS**

**Animals.** The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine. Male Sprague-Dawley rats, ranging in age from 1 to 27 mo, were from a breeding colony maintained by Pfizer at Harlan Laboratories and were housed in a climate-controlled environment with a 12:12-h light-dark cycle with food and water provided ad libitum. Two separate studies were performed. In the first study, animals 1, 6, 12, 18, 24, and 27 mo of age were examined, whereas in the second study animals 12, 18, 21, 24, and 27 mo of age were used. Because the 12- and 18-mo age groups were common between the two studies, data for these groups were used to normalize results between the two studies. All animals were killed on the day of tissue harvesting, ~4 h after the start of the light cycle.

**Protein and RNA measurements.** The amount of protein and RNA in frozen muscle samples was determined according to the method of Schmidt and Thannhauser (34), as modified by Fleck and Munro (8) and Gauthier et al. (11). Frozen muscle samples (100 mg) were placed in 17 × 100-mm polypropylene tubes containing 4 ml of distilled water and were homogenized using a polytron (Kinematica, Lucerne, Switzerland) for 10 s at speed 5. The polytron tube was then rinsed with 4 ml of distilled water followed by 4 ml of 0.6 M HClO4, and the rinses were added to the original homogenate. The centrifuge tubes were placed on ice for 10 min to allow protein and RNA to precipitate and were then centrifuged at 6,000 g for 15 min. The supernatant was discarded, and the pellet was washed twice with 4 ml of 0.2 M HClO4. Next, the pellet was solubilized in 6 ml of 0.3 M KOH for 1 h at 37°C. The samples were then divided into three 2-ml portions. One portion was used to determine protein content in duplicate with the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA), with crystalline bovine serum albumin as a standard. The remaining portions were used to determine tissue RNA. An aliquot (1.2 ml) of 1.2 M HClO4 was added to the duplicate samples, and the tubes were placed on ice for 10 min and centrifuged at 6,000 g to precipitate DNA. The supernatant containing RNA was transferred into a new centrifuge tube. The pellet was washed with 3 ml of 0.2 M HClO4, and the supernatant was combined with the first one. The RNA concentration of the supernatant was calculated as follows:

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\mu g \RNA/ml = \left[ \frac{32.6(A_{260}) - 6.11(A_{232})}{3} \right] \times 6.2 \text{ (sample volume in ml)} \times 3 \text{ (dilution factor)}
\]

**Measurement of protein synthesis.** The rate of protein synthesis in fractions representing total mixed protein, myofibrillar, and soluble protein in the gastrocnemius was measured according to the procedures described previously (32, 37). Skeletal muscle (300 mg) was homogenized in 10 volumes of ice-cold buffer (250 mM sucrose, 2 mM EDTA, 10 or 0.5 mM cycloheximide, and 10 mM Tris-HCl, pH 7.4) using a motor-driven glass-on-glass homogenizer. A 500-μl aliquot of the homogenate was processed for the measurement of total mixed protein synthesis, and the remainder was used to separate myofibrillar and soluble proteins for the analysis of synthetic values in these fractions.

To determine the fractional rate of synthesis of total mixed protein, the 500-μl aliquot of homogenate was combined with 2.5 ml of 1.0 M HClO4 and boiled for 15 min. After cooling on ice, the samples were centrifuged at 1,400 g for 10 min at 4°C. The supernatant was discarded, and the pellet was washed twice with 0.5 M HClO4, twice with chloroform-ethanol-ether (1:2:1), once with ether, and air-dried overnight. The following day, the dried pellets were combined with 3.0 ml of 0.1 M NaOH and heated in boiling water until dissolved. To fractionate myofibrillar and soluble proteins, the remainder of the homogenate was centrifuged at 600 g for 10 min at 4°C to separate the nuclear-myofibrillar fraction (pellet) from the mitochondrial, microsomal, and soluble protein fractions (supernatant). The supernatant was centrifuged at 100,000 g for 60 min at 4°C, resulting in a supernatant that contained soluble protein. The pellet from the 600-g centrifugation step containing the myofibrillar proteins was washed twice with 3 ml of distilled water to release soluble and mitochondrial impurities. Myofibrillar proteins in the pellet were then solubilized in 3 ml of 600 mM KCl. The suspension was centrifuged for 33,000 g for 10 min at 4°C. The supernatant contained the dissolved myofibrillar proteins.

Rates of protein synthesis were estimated from the incorporation of radioactive phenylalanine into total mixed, myofibrillar, and soluble muscle protein fractions by using the specific radioactivity of serum.

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phenylalanine as representative of the precursor pool (27). The elapsed time from injection of the metabolic tracer until homogenization of the muscle was recorded as the actual time for incorporation of radiolabeled amino acid into protein. Aliquots were assayed for protein with the Bio-Rad Protein Assay (Bio-Rad Laboratories), with crystalline bovine serum albumin as standard. Another aliquot was assayed for radioactivity by liquid scintillation spectrometry. The specific radioactivity of serum phenylalanine was measured by HPLC analysis of supernatants from trichloroacetic acid extracts of plasma, as described previously (27). The rate of protein synthesis, expressed as nanomoles of phenylalanine incorporated into protein per hour per milligram of muscle protein (nmol Phe·mg protein\(^{-1}\)·h\(^{-1}\)), was determined by dividing the disintegrations per hour incorporated into protein in each fraction by the serum phenylalanine specific radioactivity (27).

Measurement of eIF2B and PKB activities. eIF2B activity in the muscle homogenate was measured by the exchange of \(^{3}H\)GDP bound to eIF2 for nonradioactively labeled GDP, as described previously (26). Activity was expressed as the rate of GDP exchange. PKB activity was measured using a kit from Upstate Biotechnology (Waltham, MA), as described previously (23).

Quantitation of the relative expression of eIF2B, eIF2α, S6K1, mTOR, eIF4E, and eIF4G. The relative expression of initiation factors and protein kinases in the gastrocnemius was measured by Western blot analysis. Briefly, samples containing equal amounts of protein were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), and proteins in the gel were electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were probed with primary antibody and subsequently developed using an enhanced chemiluminescence (ECL) Western blotting kit (Amersham Pharmacia Biotech). Because S6K1 normally resolves into multiple electrophoretic forms on the basis of its phosphorylation state, samples were treated with λ-phosphatase before electrophoresis to convert the phosphorylated forms into a single electrophoretic band. For this analysis, muscle was homogenized in buffer consisting of 50 mM Tris, pH 7.5, 0.1 mM EDTA, 5 mM dithiothreitol, 0.01% Brij, 2 mM manganese chloride, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.1 μg leupeptin, and 5.2 μl/ml aprotinin and then centrifuged at 1,000 × g for 3 min at 4°C. A 65-μl aliquot of the supernatant was incubated with 800 U of λ-phosphatase (New England Biolabs) for 15 min at 37°C before analysis of S6K1 content.

Phosphorylation of eIF2α on Ser\(^{\text{51}}\), eIF2Be on Ser\(^{\text{55}}\), and S6K1 at Thr\(^{\text{389}}\). Phosphorylation of eIF2α on Ser\(^{\text{51}}\) (25), eIF2Be on Ser\(^{\text{55}}\) (36), and S6K1 on Thr\(^{\text{389}}\) (1) was measured by Western blot analysis, as described previously, using antiphosphopeptide antibodies that specifically recognize eIF2α on Ser\(^{\text{51}}\) (BioSource International), eIF2Be on Ser\(^{\text{55}}\) (BioSource International), or S6K1 phosphorylated at Thr\(^{\text{389}}\) (New England Biolabs, Beverly, MA).

Quantitation eIF4G·eIF4E complexes. The amount of eIF4G present in a complex with eIF4E was quantitated as described previously (24). Briefly, eIF4E was immunoprecipitated from the muscle homogenate using a monoclonal anti-eIF4E antibody. Proteins in the immunoprecipitate were resolved by SDS-PAGE and then transferred to PVDF membranes. The membranes were probed with an anti-eIF4G antibody and developed with an ECL Western Blotting Kit (Amersham Pharmacia Biotech). The horseradish peroxidase conjugated to the anti-rabbit secondary antibody was then inactivated by incubating the blot in 15% hydrogen peroxide for 30 min at room temperature, and the membranes were reprobed with the monoclonal anti-eIF4E antibody. Values obtained for eIF4G content were normalized for the amount of eIF4E present in the sample.

Statistics. Results were analyzed by analysis of variance. When significant differences were detected, results were further analyzed using an unpaired, two-tailed t-test. For both analyses, \(P\) values <0.05 were considered to be statistically significant.

RESULTS

The rats used in the present study varied in age from 1 to 27 mo. Body weight increased almost fivefold between 1 and 6 mo of age and thereafter exhibited a slow, but not statistically significant, decline such that by 27 mo of age it was 88% of the value recorded at 12 mo (Fig. 1A). The weight of the gastrocnemius also declined with age (Fig 1B). Moreover, the decrease in gastrocnemius weight was much more dramatic than the change in body weight: the ratio of gastrocnemius to body weight fell from 0.0046 at 12 mo of age to 0.0023 at 27 mo of age, indicating that gastrocnemius muscle mass decreased approximately twice as fast as body weight. Interestingly, the amount of protein per unit wet weight of the gastrocnemius remained almost constant during development and aging, with the exception that at 24 and 27 mo of age it was significantly less than at 12 mo (Fig. 2A). In contrast to protein, the relative RNA content at 6 mo of age was <50% of the value at 1 mo (Fig. 2B). There was no significant change in RNA between 6 and 21 mo, but by 24 mo there was a significant increase. Because ~80% of total RNA is ribosomal, the increase in the relative RNA content suggests that the number of ribosomes was increased in 24-mo-old rats.
Protein synthesis was measured in the gastrocnemius muscle with the flooding dose method. As shown in Fig. 3A, changes in the rate of synthesis of total mixed muscle protein qualitatively mirrored those observed for relative RNA content. In this regard, the synthetic rate decreased between 1 and 6 mo of age but subsequently increased at 24 and 27 mo. However, changes in protein synthesis were quantitatively larger than changes in relative RNA content, such that the ratio of protein synthesis (nmol Phe/hr/mg protein) to RNA (mg/g muscle) decreased between 1 (0.75) and 6 mo of age (0.58) but thereafter remained unchanged. Therefore, alterations in eIF2B expression cannot account for the increase in eIF2B activity observed at 24 and 27 mo. Another potential mechanism for regulating eIF2B activity involves phosphorylation of eIF2B on Ser^535 (40). In this regard, the relative phosphorylation of eIF2B-Ser^535 was measured by protein immunoblot analysis with an antibody that recognizes eIF2B only when it is phosphorylated on Ser^535. It was found that the relative expression and function of translation initiation factors and protein kinases that are involved in the regulation of initiation of mRNA translation were examined. As shown in Fig. 4A, the guanine nucleotide exchange activity of eIF2B measured in extracts of gastrocnemius muscle fell between 1 and 18 mo of age, increased between 21 and 24 mo of age, and then remained constant until 27 mo. To establish whether or not the changes in eIF2B activity were a consequence of altered expression of the protein, the relative expression of the catalytic subunit of eIF2B, eIF2Be, was measured by protein immunoblot analysis. As shown in Fig. 4B, the relative expression of eIF2Be at 6 mo of age decreased to 59% of the value observed at 1 mo and thereafter remained unchanged. Therefore, alterations in eIF2Be expression cannot account for the increase in eIF2B activity observed at 24 and 27 mo.
that the relative phosphorylation of eIF2β-Ser535 gradually decreased as the animals aged, consistent with an increase in rates of protein synthesis.

To determine whether or not the content of the substrate for eIF2, eIF2, changed during development and aging, the relative expression of the eIF2 subunit of eIF2 was measured by protein immunoblot analysis. As shown in Fig. 5A, the relative expression of eIF2 decreased to 28% of the 1-mo value by 6 mo of age. Thereafter, eIF2 expression increased, reaching a maximum at 21 mo. The phosphorylation of Ser 51 on eIF2 was also assessed by protein immunoblot analysis with an antibody specific for eIF2/Ser51 when it is phosphorylated on Ser51, as described in MATERIALS AND METHODS. Values are expressed as the ratio of phosphorylated to total eIF2 e; n = 4–13 animals/group. Values not sharing a superscript are significantly different, P < 0.05.

As was observed for eIF2β, the relative expression of S6K1 in gastrocnemius muscle significantly decreased between 1 and 6 mo of age and thereafter remained constant (Fig. 6A). In contrast, the phosphorylation state of Thr389 on S6K1 relative to total S6K1 content exhibited a gradual, but sustained, increase beginning at 12 mo of age and continuing through 27 mo (Fig. 6B). In vitro, S6K1-Thr389 is phosphorylated by mTOR (5, 20). Therefore, the relative expression of mTOR in gastrocnemius was assessed by protein immunoblot analysis. It was found that the relative expression of mTOR gradually increased between 6 and 27 mo of age (Fig. 7A). In part, the activity of mTOR is controlled by PKB (35). However, as shown in Fig. 7B, no significant change in PKB activity occurred between 12 and 27 mo of age, indicating that the observed changes in S6K1 phosphorylation did not occur through activation of PKB.

Finally, the relative expression of two proteins that are absolutely required for the binding of the 40S ribosomal
that both young and old subjects have the same sensitivity to bed rest and fixed protein intake. In such studies, elderly subjects exhibited a reduced rate of both protein synthesis and protein degradation compared with young subjects (2, 39). Consequently, it has been proposed that the observed reduction in the protein synthetic rate with age is the primary stimulus behind age-related muscle loss and that reduced protein degradation is a compensatory response by the muscle in response to reduced anabolic activity. However, if older subjects are more sensitive than young ones to the effects of either the bed rest or the diets that are associated with preconditioning, then the preconditioning itself could influence the outcome of these measurements. Indeed, it has been suggested that variation in the preconditioning of subjects might underlie the conflicting results that were obtained in a more recent study in which amino acid incorporation measurements were made on elderly subjects who were instructed to maintain their normal dietary and physical activities before the measurement. In that study (38), instead of elderly subjects demonstrating a reduced protein synthetic rate, young and elderly subjects exhibited similar rates of synthesis.

DISCUSSION

Tracer amino acid methodology has been used extensively to estimate rates of protein synthesis in human skeletal muscle with aging. In early studies, the food intake and activity of subjects were standardized by admission to a hospital environment and feeding of a standard diet before infusion of tracer amino acid. Although this preconditioning would be expected to reduce variability between subjects due to differences in preexperimental activity, its success relies on the assumption that both young and old subjects have the same sensitivity to bed rest and fixed protein intake. In such studies, elderly subjects exhibited a reduced rate of both protein synthesis and protein degradation compared with young subjects (2, 39). Consequently, it has been proposed that the observed reduction in the protein synthetic rate with age is the primary stimulus behind age-related muscle loss and that reduced protein degradation is a compensatory response by the muscle in response to reduced anabolic activity. However, if older subjects are more sensitive than young ones to the effects of either the bed rest or the diets that are associated with preconditioning, then the preconditioning itself could influence the outcome of these measurements. Indeed, it has been suggested that variation in the preconditioning of subjects might underlie the conflicting results that were obtained in a more recent study in which amino acid incorporation measurements were made on elderly subjects who were instructed to maintain their normal dietary and physical activities before the measurement. In that study (38), instead of elderly subjects demonstrating a reduced protein synthetic rate, young and elderly subjects exhibited similar rates of synthesis.
In light of these conflicting observations, we sought to assess the influence of age on protein synthesis by using a different approach, specifically by monitoring the relative expression and function of key cellular components involved in the initiation of mRNA translation, as well as the activity of protein kinases involved in the PKB/mTOR-signaling pathway (Fig. 9). We have previously reported on the male Sprague-Dawley rats used in this study. These rats have a maximal life expectancy of 28 mo of age. At 18 mo of age they undergo an age-related decline in bone density and skeletal muscle mass, and the loss of muscle is accelerated after 21 mo of age (18).

By monitoring the relative expression and function of these cellular components during periods of high growth (1 mo), equilibrium (6–18 mo), and sarcopenia (21–27 mo), we were able to establish a temporal relationship between biomarkers for protein anabolism and muscle mass in the gastrocnemius muscle. Also, by comparing members of the same pathway at each time point, we were able to look for consistency between biomarkers with age. The observation that all translation initiation and PKB/mTOR-pathway biomarkers assessed were elevated during the extremely high growth seen at 1 mo of age is consistent with the high level of protein anabolism that occurs at this age. Likewise, as the rate of muscle growth declined and stabilized, so too did the protein anabolism biomarkers. All parameters remained statistically indistinguishable between 6 and 18 mo. Thus, as expected, protein synthetic rates and muscle growth are positively related during the period of the life of the rat, wherein muscle weight is proportional to body weight. However, this positive relationship was not maintained from 21 mo onward when sarcopenia first became evident. Surprisingly, while muscle mass was dropping, the biomarkers for anabolism as well as the protein synthetic rate either remained unchanged or began to rise. However, the concomitant observation of elevated protein synthesis in the 24th and 27th mo indicates that the muscle is fully capable of elevated rates of protein anabolism. Thus it is reasonable to assume that the elevated expression and/or function of these cellular components is related to increased protein synthetic potential. In addition, the enzymes that are elevated at 24 and 27 mo are exactly the same ones that were high at 1 mo. This implies that the muscle is using the same pathway for elevated protein synthesis that it used when the animals were young.

If protein synthesis is indeed rising with age while muscle size is dropping, then presumably protein degradation is elevated relative to synthesis. A confirmation that protein degradation rates are elevated in this time period still needs to be made. The elevation of protein synthetic potential in the face of sarcopenia observed in the present study indicates that a deficiency in the signals to initiate protein anabolism is not the primary cause of sarcopenia and that another stimulus is causing muscle wasting.

At this point it is not possible to categorically state the physiological cause of muscle wasting. However, the response of skeletal muscle to various wasting environments has been reported, and it has been observed that, depending on the wasting stimulus, the cellular response by the muscle cells can be quite different. Therefore, it is reasonable to assume that the molecular state of the muscle during sarcopenia would reflect the integrated inputs of all of the physiological conditions that could contribute to muscle wasting.

For example, in sepsis, skeletal muscle protein synthesis is suppressed, and these cells are resistant to the stimulation of protein synthesis by insulin or amino acids (21). Consistent with these observations, eIF2Be has been reported to be hyperphosphorylated in sepsis (19), and this hyperphosphorylation can be ameliorated with administration of TNF-α-binding protein (19). This would imply that TNF-α is necessary for increased eIF2Be phosphorylation in sepsis. It has been reported that TNF-α levels are elevated in muscle with aging. If sarcopenia was due to enhanced TNF-α signaling, then it would be expected that levels of phosphorylated eIF2Be would be elevated in aged muscle. Our observations that eIF2Be phosphorylation is not elevated with aging and that the GDP exchange rate of eIF2Be is actually increased would indicate that TNF-α does not play a dominant role in sarcopenia.

The age-related reduction in the hormones that have been ascribed an anabolic role in muscle, such as growth hormone...
(GH) and testosterone, would be expected to be associated with a reduction in markers for anabolism. For example, it has been suggested that the effect of GH on skeletal muscle may be mediated through elevation of IGF-I signaling. If reduced GH were responsible for sarcopenia, then it would be expected that IGF-I signaling and the PKB/mTOR pathway would be less active. This result was not observed in the present study, which suggests that reduced GH is also not responsible for sarcopenia.

An additional age-related phenomenon affecting muscle is spontaneous denervation. This is marked by loss of neuromuscular communication and neuromuscular junction remodeling as well as by loss of motor neuron soma from the spinal cord. The loss of neuromuscular communication results in an increased incidence of denervated muscles, which is evidenced by elevated levels of myogenin (28). It has been reported that the cellular response of skeletal muscle to denervation is both an elevation of protein synthesis (14) and protein degradation. Consistent with this, it has been reported that denervation of extensor digitorum longus muscles results in elevated phosphorylation of S6K1, a reduced phosphorylation of eIF2α, and elevation of elongation factor 2 (eIF2α) levels, all of which would be expected to contribute to increased rates of protein synthesis (17). It should be noted that these effects were confined to fast-twitch muscle and were not observed in hindlimb unloading (17).

Of the reported conditions of sepsis or inflammation, reduced GH levels, disuse, and denervation, which all occur during aging, the results presented here are most consistent with a cellular response to denervation. The possibility that denervation plays a major role in sarcopenia in rodents has not been established. However, the similarity of the results of this study and those of others suggests to us that spontaneous denervation could possibly play a significant role in sarcopenia.

In summary, we have measured the relative expression and function of a number of biomarkers of protein synthesis in rats throughout their life span, including the period of disproportionate muscle loss characterized in the last quartile. Surprisingly, we have found that, whereas in young animals these markers change in proportion to the rate of muscle growth, the relationship becomes disassociated at the onset of sarcopenia. Instead, protein anabolism appears to be elevated at the same time that muscle mass is dropping. This is different from other wasting environments, such as cachexia, in which protein synthesis is suppressed. Presumably it represents a response by the muscle cells to attempt to maintain mass in the face of a catabolic stimulus that is driving muscle mass down. Further studies will be needed that determine the relationship between protein synthesis and degradation in aged animals to establish the primary cause of age-related muscle loss.
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