Aging muscle1–5

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ABSTRACT
Age causes structural and functional changes in skeletal muscle in a wide range of species, including humans. Muscle changes in humans start in the fourth decade of life and cause frailty and disabilities. Associated changes in body composition form the basis of many metabolic disorders, such as insulin resistance, type 2 diabetes, hypertension, and hyperlipidemia, which result in an increased incidence of cardiovascular death. Decreases in the synthesis rates of many muscle proteins, specifically of myosin heavy chain and mitochondrial proteins, occur with age. The underlying causes of the reduction in mitochondrial biogenesis and ATP production seem to be decreases in mitochondrial DNA and messenger RNA. Reduced ATP production could be the basis of reduced muscle protein turnover, which requires energy. Both aerobic exercise and resistance exercise enhance muscle protein synthesis and mitochondrial biogenesis. Insulin and amino acids have also been shown to enhance muscle mitochondrial biogenesis and mitochondrial protein synthesis. However, the insulin-induced increase in muscle mitochondrial ATP production is defective in type 2 diabetic patients with insulin resistance. Moreover, a dissociation between increases in muscle mitochondrial biogenesis and insulin sensitivity after exercise has been noted in older persons. It remains to be determined whether muscle mitochondrial dysfunction causes or results from insulin resistance. Exercise seems to enhance the efficiency of muscle mitochondrial DNA in rodents. Reduced physical activity as a contributor of age-related mitochondrial dysfunction remains to be determined. It is proposed that a reduction in tissue mitochondrial ATP production signals the hypothalamic centers to reduce spontaneous physical activities. Voluntary physical activity is regulated by cognitive centers and could attenuate the progressive decline in mitochondrial functions that occurs with age. Am J Clin Nutr 2005; 81:953–63.

KEY WORDS Aging, muscle, proteins, mitochondria, myosin, hypothalamus

INTRODUCTION
All species share the common destinies of birth, growth, aging, and death. We are born with a set of genes that largely determine our lives on the planet. The expression of genes is influenced by environmental factors, which can alter the rate of absolute growth, aging, and death. Environmental factors affect not only gene transcription but also gene translation (synthesis of proteins) and the posttranslational modification of proteins. The major determinants of alterations in body functions are proteins. Cellular functions are dependent on the ability to synthesize proteins with specific functions, interactions of proteins to proteins, proteins and genes, and proteins and metabolites. There are gradual and progressive alterations in body functions from birth to death. The changes are rapid during the growth phase, but the changes are rather slow during a rather prolonged period of aging—from ≈30 y to death. The aging process or changes related to secondary events or diseases cause rapid deterioration of body functions in most species in the last phase of life. The topic covered in this review is the aging of skeletal muscle; hypotheses that will hopefully stimulate new research in this area are discussed. Most of the presentation is based on results from our laboratory.

AGING IN HUMANS
Aging affects all species and substantially influences the scope of our activities and quality of life but what “aging” is exactly remains to be fully understood. The definition of aging is complicated by the occurrence of various diseases that modify body functions and tissue structures. The structural and functional changes related to diseases that are common in older persons are often hard to delineate from the aging process per se. Many disease processes and environmental factors profoundly influence the rate of aging. Moreover, the aging process occurs at different rates among different tissues, and the functional manifestations also vary. Aging-related changes in one organ might affect the functions of other organs. The aging process also differs substantially in different species. Aging in humans is vastly different from that in most other species because of the relatively long duration of life in humans after the genetic potential for growth is complete. All of these reasons make it difficult to study aging, and studies done in many other species cannot always be directly correlated to human aging.

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The current review focuses on aging in muscle. For the purposes of this discussion, I assume that the aging process in humans starts from the fourth decade of life (at 30 y of age). This concept is supported by studies performed in human skeletal muscle (1–3), although it is not well established in all tissues. In healthy persons who died in motor vehicle accidents, it was observed that both muscle area and fiber numbers decreased from as early as the fourth decade of life (1, 4). We recently measured the cross-sectional area of muscle in the midthigh using computed tomography imaging in healthy human subjects ranging in age from 18 to 88 y. We observed an age-related decrease in muscle area starting from the fourth decade of life (2, 5) (Figure 1). In addition, a parallel decrease in muscle strength (knee extension) occurred with the decrease in muscle mass. We also noted that muscle strength normalized for fat-free mass in the leg also decreases with age, indicating that not only muscle mass declines with age but the efficiency of muscle mass (quality) does as well. Another key performance measurement that declines with age is maximal oxygen uptake (2), as noted in several previous studies (6, 7). It is a common belief that the muscle of older persons also fatigues faster than does that of young persons, although this has not been well documented by objective measurements. However, age-related changes vary substantially among persons, depending on their activity levels and other environmental factors.

It is likely that the decrease in muscle mass and muscle strength, in combination with reduced endurance, causes reduced physical activity (Figure 2). A reduction in muscle mass (responsible for ≈30% of resting energy expenditure and protein turnover as well as 70% body cell mass) and physical activity levels (contributes variable components to daily energy expenditure: 10–60%) decrease total energy expenditure in older persons. This reduction in overall energy expenditure in elderly persons results in an increased prevalence of obesity, especially abdominal fat accumulation. These alterations in body composition cause insulin resistance, which contributes to the development of a high prevalence of type 2 diabetes, hyperlipidemia, and hypertension in a genetically susceptible population (Figure 2). The combined effect of these metabolic abnormalities is increased cardiovascular death and other morbidities.

MUSCLE CHANGES IN AGING

Of interest, sarcopenia of aging is noted in a wide range of species, from nematodes, flies, rodents, and nonhuman primates to humans. A recent study in Caenorhahditis elegans showed gradual, progressive deterioration of muscle in this short-lived nematode that resembled human sarcopenia from midlife (8). The observed muscle deterioration occurred not only in body wall muscle but also in myofibrils of pharyngeal muscles. The sarcomeres of older C. elegans progressively became disorganized and contained fewer myosin thick filaments. Thus, it appears that C. elegans, like humans, undergo progressive loss of muscle mass and function. This nematode, which has a short life span of 20–25 d and whose genome is completely sequenced, is extensively studied to define the aging process. A remarkable finding of Herndon et al (8) was that these nematodes have a preserved central nervous system, even at an advanced age, and that muscle changes, which appear to occur at the beginning of midlife, indicate a dissociation between neuronal and muscle...
It is also fascinating that locomotor activity was found to be a predictor of life expectancy; those nematodes that showed earlier locomotor dysfunction had lower life expectancies. They found that mutation of \textit{age-1} (hx 546), which enhances locomotor activity in the aging population, delayed the age-related muscle nuclear changes. This finding suggests that the \textit{age-1} phosphatidylinositol-3-OH kinase might exert its effects on longevity in part by prolonging muscle integrity (8). Of note, an age-related decline in the synthesis rate of skeletal muscle myosin heavy chain (MHC) has been reported in humans (1, 9-11) and may explain the decline in myosin content reported in \textit{C. elegans}. Because MHC is a key contractile protein, its reduction is likely to contribute to a decline in locomotor function.

The underlying mechanism that causes a decrease in muscle fibers, muscle mass, and muscle function—which are reported in human aging—has been extensively investigated. Many studies have reported an overall decrease in the skeletal muscle protein synthesis rate (2, 10, 12-14). Studies that did not normalize the activity levels and diets of the participants before measuring the fractional muscle protein synthesis rate failed to show any differences between young and older persons (15). There is substantial evidence that the muscle protein synthesis rate is responsive to exercise. In studies performed in young and old persons, resistance exercise stimulates the synthesis rate of mixed muscle proteins after 2 wk as well as after 3 mo, although the increment attenuates from 2 wk to 3 mo (16, 17). Resistance training for 3 mo also increased specific muscle proteins, such as MHC proteins, in older persons (10) (Figure 3). Studies have also shown that synthesis rates of mixed muscle proteins increase 3–4 h after resistance exercise (18). Mixed muscle protein synthesis has been shown to increase after 4 mo of aerobic exercise (2). Together, these results indicate that mixed muscle protein synthesis in both young and older persons responds to aerobic and resistance exercise programs. It is, however, important to appreciate that the effects of aerobic exercise and resistance exercise on muscle metabolism and muscle size are different. Aerobic exercise improves many metabolic functions, including insulin-induced glucose disposal and mitochondrial functions. It is likely
that the muscle functional changes induced by aerobic exercise are mediated by changes in specific proteins, especially the enzymes. Many of these proteins are likely to be of low concentration in muscle tissue and may not contribute much to the muscle mass. In contrast, resistance exercise mainly affects muscle strength and muscle mass, which may include many structural proteins. It is therefore likely that the proteins involved are different in response to resistance exercise from the aerobic exercise. Measurement of the fractional synthesis rate (FSR) of mixed muscle proteins represents an average of many proteins (low- and high-abundant proteins with low and high FSRs) and the changes in the FSR of mixed muscle protein synthesis rates may not translate into changes in muscle mass. Moreover, changes in muscle protein breakdown in response to different types of exercise also may occur at different rates for different muscle proteins. These issues are difficult to resolve but offer challenging opportunities for future research. Changes in the composition of meals or in substrate milieu have also been shown to affect muscle protein synthesis rates and protein turnover (19-21). The previous day’s meals can affect not only protein turnover but also insulin sensitivity (22), which in turn may affect muscle protein turnover. It is therefore critical to standardize the diet and exercise status in young and older persons before protein turnover studies to determine whether age per se has any effect on muscle protein turnover. Additional studies are needed to assess whether activity levels and diet have differential effects in young and older persons.

The important question then is: What causes the reduced baseline muscle protein synthesis rate? It is possible that younger persons are physically more active than are older persons, which by itself may affect muscle protein turnover. In our studies, we selected subjects who did not engage in any sport activities and did not deliberately exercise regularly. On the basis of a leisure-time activity questionnaire, the young and older subjects had similar activity levels. It remains to be determined whether younger persons are generally more physically active than are older persons on the basis of more objective measures and whether low activity levels in older persons caused the reduced muscle protein synthesis.

DOES AGE REDUCE ACTIVITY LEVELS?

There is substantial evidence from various species that activity levels decline with age (8, 23). Age-related declines in locomotor activity levels have been documented in C. elegans (8, 23), flies (24), and animals (25, 26). In humans, indirect measurements suggest an age-related decline in activity levels (27), and it is a common dogma that humans slow down with age. This general impression remains to be confirmed by reliable and objective measurements.

MITOCHONDRIAL DYSFUNCTION AS A DETERMINANT OF PHYSICAL ACTIVITY LEVELS

There is increasing evidence in rodents (28) and in humans (14) (Figure 4) that muscle mitochondrial dysfunction occurs with age. Changes in muscle mitochondrial function include decreases in mitochondrial DNA copy numbers, decreased mRNA concentrations in genes encoding muscle mitochondrial proteins (5, 28), reduced muscle mitochondrial oxidative enzyme activities, and reduced mitochondrial protein synthesis rates (5, 14) (Figure 4 and Figure 5). There are conflicting results on whether the actual muscle mitochondrial ATP production decreases with age (Table 1) (29-39). Our own preliminary results strongly indicate that there is an age-related decrease in muscle mitochondrial ATP production (40). A plausible hypothesis is that muscle mitochondrial ATP production is a determinant of physical activity levels (Figure 6). The rationale for the above hypothesis is that humans and other species need ATP for muscle contractile activities. The availability of ATP may signal, via an activity center, an alteration in activity levels. It is likely that the effects of mitochondrial ATP concentrations are spontaneous under the control of the hypothalamus. Evidence from animal studies supports the fact that activity levels are regulated by the hypothalamus, possibly in the paraventricular nucleus. Orexin A (hypocretin 1), when injected into the hypothalamic paraventricular nucleus, increased spontaneous activity levels (41) and food intake (42) in rodents. An intriguing possibility is that a neuropeptide that stimulates physical activity levels (needed for gathering food) also increases food intake. A recent review (43) outlines various catabolic neuronal pathways that reduce food intake and increase energy expenditure (eg, melanocortin neurons in hypothalamic arcuate nucleus) and are stimulated by leptin and insulin. In contrast, those anabolic pathways (eg, neurons containing neuropeptide Y) that enhance food intake and decrease energy expenditure appear to be inhibited by...
Insulin and leptin. It remains to be determined how these catabolic and anabolic regulatory centers are involved in the regulation of activity levels. Triiodothyronine administration increases activity levels in association with metabolic rate (44). It also remains to be determined whether neuronal or chemical mediators are involved in signaling hypothalamus from periphery to stimulate the center regulating activity levels. The different signals to the periphery from the activity regulating centers also remain to be determined. It is proposed that afferent sympathetic nerves signal the hypothalamic center (likely the paraventricular nucleus) about the status of muscle ATP production. The signal for changing activity level (spontaneous and possibly voluntary) is proposed to occur via efferent sympathetic nerves (Figure 6). It is also possible that the signals are mediated by chemicals.

Physical activity levels are likely regulated in 2 ways. Spontaneous physical activities are likely regulated by hypothalamic centers, and voluntary physical activities are regulated largely under cognitive control. It is proposed that spontaneous physical activity is regulated by hypothalamic centers in response to signals from the peripheral tissues, especially from skeletal muscle, and that spontaneous physical activity is reduced in older persons because of reduced muscle mitochondrial functions. The down-regulation of spontaneous activity may also influence the initiation of voluntary activities. Although voluntary activities are mostly under cognitive control, it is proposed that reduced spontaneous activity and other unknown regulatory factors interact with muscle mitochondrial functions to reduce the “motivation” of older persons to engage in voluntary physical activity. As a result, both spontaneous and voluntary physical activities decrease with age. Although it is a common dogma that humans “slow down” with age and are physically less active than are younger persons, hard supporting evidence is still not available.

**EFFECT OF REDUCED PHYSICAL ACTIVITY LEVEL ON MUSCLE CHANGES**

As discussed earlier in this review, synthesis rates of several muscle proteins are responsive to both aerobic and resistance exercise program (Figures 7 and 8) (2, 9). Substantial evidence indicates that mitochondrial biogenesis is stimulated by aerobic exercise (45). Muscle mitochondrial oxidative enzyme concentrations, such as cytochrome-c oxidase and citrate synthase, and mRNA concentrations of several genes encoding mitochondrial...
proteins and genes regulating mitochondrial transcription ([peroxisome proliferators–activated receptor co-activator 1 (PGC-1), nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (TFAM)] are enhanced by 4 mo of aerobic exercise (2). It was previously shown that the master regulator of mitochondrial biogenesis is PGC-1, a protein localized in nucleus (46, 47). PGC-1, a cold-inducible co-activator of nuclear receptors, stimulates mitochondrial biogenesis and respiration in muscle cells and through regulation of NRF-1 and NRF-2. In addition, PGC-1 also binds to and coactivates the transcriptional function of NRF-1 on the promoter for TFAM. TFAM is a direct regulator of mitochondrial DNA replication and transcription (48). Overexpression of PGC-1α has been shown to enhance slow twitch muscle fibers in a mouse model (49), thus favoring the oxidative phenotype. We previously showed that aerobic exercise enhances the mRNA expression of PGC-1 in human skeletal muscle (2), thus favoring the oxidative phenotype. Moreover, as discussed earlier, in human skeletal muscle mRNA concentrations of NRF-1 and TFAM along with those of NADH subunit 4 (NADH 4) and cytochrome-c oxidase subunit 4 mRNA concentrations are enhanced by exercise programs (5). Long-term aerobic exercise seems to favor the phenotype of type I fibers (50) in skeletal muscle. It is clear that there is a common pathway for regulating the phenotype of oxidative muscle fiber type.

Mitochondrial biogenesis and mitochondrial DNA replication are stimulated by overexpression of a constitutively active form of calcium/calmodulin-dependent protein kinase IV (CaMK IV) (51). This effect of CaMK is mediated through induction of PGC-1α expression (51). Chronic electrical stimulation of skeletal muscle causes a sustained elevation of intracellular calcium and activates calcium-regulated enzymes such as calcineurin and CaMK, both of which have been shown to synergistically activate slow and oxidative fiber-specific gene expression in myocytes (52). Muscle contractile activity through CaMK, AMPK, and calcineurin (51, 53–55) enhances mitochondrial biogenesis and oxidative phenotype of skeletal muscle. It appears that exercise favors oxidative muscle fiber type by well-defined regulatory pathways; CAMK and AMP kinase are key proteins in the cytoplasm of muscle cells that signal nuclear regulators involved in the biogenesis of mitochondria and expression of isoforms of MHC that determine the phenotype of muscle fibers. It remains to be determined whether this pathway of determining muscle phenotype is intact in aging. Future research may focus on the effect exercise on this regulatory pathway to determine the potential areas affected by aging.

Studies performed in rodents showed that mitochondrial DNA copy numbers are lower in skeletal muscle and liver (28) in older rats. The magnitude of the differences in mitochondrial copy numbers between young and older rats is related to the oxidative...
The values represent the percentage of the average value in young rats greater in older animals in tissues such as the soleus muscle and liver. The lateral (gastroc. lat.) tissues. mRNA expression per DNA copy number was activity), than in gastrocnemius medial (gastroc. med.) and gastrocnemius liver (but not in the heart, which is continuously engaged in contractile capacity of the tissues. For example, the lowering of mitochondrial copy numbers from young to older rats is greater in soleus (a highly oxidative tissue) than in gastrocnemius (Figure 9). However, DNA copy numbers in heart muscle are not different between young and older rats, despite the heart being a tissue with a high oxidative capacity. The potential explanation for this discrepancy is that heart muscle is in constant contractile activity and, as discussed in the previous paragraph, contractile activity may stimulate mitochondrial DNA replication and biogenesis through a CaMK-mediated pathway. Of note, in rodents, mRNA concentrations per DNA copy numbers were greater in older animals in tissues such as the soleus muscle and liver. The values represent the percentage of the average value in young rats, Cox 1 and Cox 3, cytochrome-c oxidase subunits 1 and 3.

**FIGURE 9.** Effect of age on mitochondrial (mt) DNA copy number in young and old rats and the ratio of messenger RNA (mRNA) to mtDNA in rats. *Significantly different from young animals, $P < 0.05$. Reductions in mtDNA were greater in the more oxidative tissues, such as the soleus and the liver (but not in the heart, which is continuously engaged in contractile activity), than in gastrocnemius medial (gastroc. med.) and gastrocnemius lateral (gastroc. lat.) tissues. mRNA expression per DNA copy number was greater in older animals in tissues such as the soleus and liver. The values represent the percentage of the average value in young rats, Cox 1 and Cox 3, cytochrome-c oxidase subunits 1 and 3.

**POTENTIAL MECHANISM OF AGING MUSCLE**

The underlying mechanism of functional and structural changes in aging muscle remains to be fully understood. There is a generalized decline in muscle protein synthesis in humans with aging (2, 9, 10, 12, 14, 56). However, studies have shown that whereas the FSR of muscle mitochondrial protein and MHC (10) are lower in older persons, the synthesis rate of sarcoplasmic proteins is relatively higher in older persons, which suggests that the age-related inhibition of muscle protein is not a global effect on all proteins but is selective for certain proteins. Almost all of the previous studies were performed during the postabsorptive state, whereas muscle protein balance becomes positive only after a meal (19, 20). Studies to understand the regulation of postprandial protein dynamics are difficult to perform and are fraught with theoretical problems when interpreting the results. One potential approach is to label the proteins in a meal with stable isotopes and measure their incorporation into multiple muscle proteins. Advances in protein purification techniques and mass spectrometry will soon enable us to perform such studies. Studies have shown that milk proteins can be labeled with stable isotope of amino acids by infusing the isotopes in lactating cows (57). However, many studies using the intravenous infusion of substrates and insulin have been conducted. Amino acids alone (20) and insulin with glucose (19) achieve a positive protein balance across muscle bed. The main antitrophic effect of insulin in the postabsorptive state is due to inhibition of muscle protein breakdown (19, 58), whereas the antitrophic effect of amino acids is due to stimulation of protein synthesis and inhibition of protein breakdown (20). Both insulin and amino acids are largely responsible for protein accretion in skeletal muscle after a meal. Acute studies indicate that the main effect of insulin and amino acids is on the stimulation of muscle mitochondrial protein synthesis (59, 60) (Figure 10). Insulin and amino acids also enhance mRNA abundance in genes encoding mitochondrial proteins (Figure 11). It remains to be fully elucidated whether the transcriptional regulation of insulin and amino acids is selective for specific genes involved in specific functions. Insulin and amino acids have been shown to act via the signaling pathways (mainly by PI3 kinase and mTOR) to facilitate the initiation of the translation of messages in transcripts, thus promoting protein synthesis. The enzyme activities of selective mitochondrial enzymes and ATP production are also enhanced by insulin and amino acids (59) (Figure 11). It is possible that the effect of insulin may be secondary to other events, such as substrate oxidation or changes in substrate availability. The role of substrate oxidation, especially the role of free fatty acids and glucose on mitochondrial function, remains to be determined. In our studies, we replaced glucose during an insulin infusion.
(achieving similar insulin concentrations) to prevent hypoglycemia and to maintain similar glucose concentrations in both diabetic and nondiabetic persons. The amount of glucose that was required to maintain similar blood glucose concentrations was lower in persons with type 2 diabetes. In type 2 diabetic patients, muscle mitochondrial ATP production was lower than in nondiabetic control subjects and insulin concentrations increased (59), although insulin concentrations were identical in both groups at low and high levels. The mechanism for the lack of stimulation of muscle mitochondrial ATP production in type 2 diabetic patients by insulin or amino acids remains to be determined. Reduced phosphorylation of signaling proteins and lower synthesis of mitochondrial proteins in type 2 diabetic patients may cause the lack of stimulation of muscle mitochondrial ATP production. Alternatively, a reduced delivery rate of glucose (and thus a reduced flux of glucose oxidation in muscle tissue) may affect muscle oxidative phosphorylation by an unknown mechanism. If insulin-induced signaling is important for muscle mitochondrial ATP production, insulin resistance in aging and type 2 diabetes could reduce muscle mitochondrial ATP production. Because insulin resistance occurs with aging, what remains to be determined is whether reduced insulin resistance causes a blunted increase in muscle mitochondrial ATP production after a meal or after an infusion of insulin with amino acids and glucose.

The reasons for the selective decrease in synthesis rates of certain muscle proteins in older persons also remain to be determined. The previous studies were based mainly on either the measurement of FSR of mixed muscle proteins (2, 13, 61) or selective fractions of muscle proteins, such as myofibrillar (56) or mitochondrial (14, 62) proteins. The FSRs of these mixed proteins represent the average of several proteins with a wide range of FSRs. Many of these proteins have different or even opposing functions. It is likely that, whereas aging inhibits the synthesis rate of some of these proteins, other proteins may not be affected by aging. In the case of mitochondrial proteins, the measurement of FSR of mixed proteins has definite functional importance because the main function of mitochondrial proteins is oxidative phosphorylation. However, >85% of these proteins (5 protein complexes with many subunits) are encoded by the nucleus, whereas others are encoded by mitochondrial genes. It is possible that if protein complexes involved at certain levels of electron transport are not available in sufficient quantities, ATP production may not occur. It is therefore important to study the abundances and FSRs of different mitochondrial proteins.

The interpretation of studies that show changes in muscle protein synthesis in response to interventions or in the basal steady state should be done with caution for a variety of reasons. One cannot assume that changes in the synthesis rate of a protein mixture can be translated to changes in the concentration of that protein mixture in the cell. First, a balance between protein synthesis and breakdown occurring in both the postabsorptive and postprandial states determines the change in protein content in a tissue. Second, the average synthesis rate of mixed muscle proteins measured in a tissue represents different proteins of a wide range of concentrations and turnover rates. For example, proteins that have a high turnover rate, such as enzymes, may make a larger contribution to the FSR but they make up a very small fraction of the concentration of mixed proteins. Proteins such as myosin, although constituting a major component of mixed muscle proteins, have a slow turnover rate and thus contribute only a small fraction to the synthesis rate of mixed muscle protein.

**FIGURE 11.** Effect of an infusion of insulin, while maintaining glucose and amino acid (AA) concentrations, on muscle mitochondrial ATP production (with the use of the substrates glutamate + malate and palmitoyl-L-carnitine + malate) and on ribosomal messenger RNA concentrations of genes encoding mitochondrial proteins [in the subunits NADH4 and cytochrome-c oxidase 3 and 4 (Cox 3 and Cox 4)].*Significantly different from saline, P < 0.05. AU, arbitrary units.
Moreover, the concentration and synthesis rate of proteins such as myosin are unlikely to change during a short intervention period—at least most techniques are not sufficiently precise to detect the change. In contrast, many proteins with a fast turnover but in low concentrations may change during short interventions. Because proteins in relatively high concentrations (but low turnover rates), such as myosin, contribute more to muscle protein mass than do proteins in low concentrations (with fast turnover), the changes in synthesis rates of mixed proteins may not necessarily equate with changes in total protein mass. Arteriovenous balance studies are extremely useful for determining changes in the balance of total proteins in the muscle bed. However, these measurements are not precise because the measurements of many factors, such as blood flow, are known to be widely variable. Moreover, because these measurements have to be normalized for lean tissue mass or area in the leg or forearm, cross-sectional comparisons are fraught with many problems. Older persons have a greater proportion of fibrous tissues in muscle mass and less metabolically active tissue with a higher water content than do younger persons (63). As a result, the normalization of flux values per unit mass introduces further inaccuracy in cross-sectional comparisons.

The measurement of the synthesis rate of MHC (9, 10, 64, 65) is an advance over previous techniques that measure the synthesis rate of mixed proteins. However, even MHC exists in at least 3 isoforms in human muscle, and the relative compositions of these isoforms have a substantial effect on muscle phenotypes. When the MHC isoform I is predominant, it favors the phenotype of oxidative-fatigue-resistant slow twitch type 1 fibers; MHCIIa and IIx favor type IIa and type IIb fibers, respectively, which are fast twitch and glycolytic. Currently, no techniques are available to measure the synthesis rates of these isoforms or other key proteins in skeletal muscle. There are many promising approaches for purifying and measuring synthesis rates and concentrations of multiple muscle proteins in humans (66, 67). Application of these novel approaches may, in combination with mRNA measurements, DNA studies, and studies to determine regulation of both transcription and translation, are likely to provide a new understanding of the underlying mechanism of aging muscle.

SUMMARY AND CONCLUSIONS

Structural and functional changes in muscle during aging occur in a wide range of species, ranging from C. elegans to humans. The structural changes include a reduction in muscle mass and muscle fibers, and a shift of muscle fibers toward type 1 fibers. These structural changes are associated with muscle weakness, reduced endurance capacity, and insulin resistance. Muscle weakness is largely related to reduced mass but the muscle strength per unit mass of muscle also declines. A reduction in the synthesis rate of MHC, the key protein in the contractile apparatus, is likely to contribute to the muscle weakness. Myosin is deficient in the muscle of C. elegans, which reduces its loco-motion. It remains to be determined whether the concentration of myosin and other key proteins involved in muscle contraction are reduced with aging in humans. In long-distance runners and in animal studies, type 1 fibers are rich in mitochondria and are relatively fatigue resistant. In contrast, the relative increase in type 1 fibers does not make older muscle fatigue resistant, perhaps because of a reduction in mitochondrial content with age. A reduction in mitochondrial ATP production could contribute to reduced endurance and muscle weakness. Increased mitochondrial DNA oxidative damage with aging and cumulative DNA damage could explain an overall reduction in mitochondrial DNA copy numbers in oxidative tissue, such as skeletal muscle (Figure 12) (68). A reduced mitochondrial DNA copy number may contribute to reduced mRNA abundance, which results in reduced mitochondrial protein synthesis and enzyme activity. The overall effect is a reduced capacity for oxidative phosphorylation. The reduced availability of ATP may contribute to an overall reduction in the remodeling process that involves the synthesis and breakdown of proteins, both of which are energy-consuming reactions in muscle.

Contractile muscle activity enhances muscle mitochondrial biogenesis through CaMK, AMP-activated kinase, and PGC-1α. Physical activity in the form of resistance and aerobic exercise stimulates muscle protein synthesis in both young and older persons. It remains to be determined whether different exercise programs have variable effects on different muscle proteins. Resistance exercise programs increase muscle mass; therefore, it is likely that the synthesis of structural proteins is enhanced by resistance exercise. In contrast, many metabolic changes occur with aerobic exercise with no increase in muscle mass. It is therefore likely that aerobic exercise stimulates the synthesis of many muscle proteins involved in metabolic processes. There is evidence in various species, from worms to rodents, that physical activity levels decrease with age. Although it is a common belief, supported by some experimental evidence, that physical activity levels decrease with age, further direct evidence is needed to verify this in humans. It is proposed that spontaneous activity levels in humans are regulated via the hypothalamus (possibly the paraventricular nucleus), and peripheral tissue mitochondrial ATP production is a determinant of hypothalamic control. In contrast, voluntary activities are likely to be mainly regulated by cognitive centers. Spontaneous activities decline with age in response to declining peripheral tissue mitochondrial function (Figure 13). This, together with a reduction in voluntary activities, further reduces mitochondrial biogenesis and functions as well as the synthesis rates of contractile proteins. Maintaining voluntary physical activities will partly prevent the age-related decline in muscle mitochondrial and contractile functions. Moreover, physical activities and related changes are also likely to delay or prevent insulin resistance. It remains to be determined whether age-related insulin resistance is due to or the cause of muscle mitochondrial dysfunction. It is likely that increases in

![Figure 12. Proposed mechanism for factors contributing to age-related reductions in maximal oxygen uptake (VO2,max), endurance, and impaired glucose tolerance (IGT). mRNA, messenger RNA.](https://academic.oup.com/ajcn/article-abstract/81/5/953/4649899)
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FIGURE 13. Hypothetical scheme for how mitochondrial damage and related changes can affect physical activity levels and how low activity levels can further reduce mitochondrial function. Reduced muscle function contributes further to metabolic disorders.

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