Age effect on myocellular remodeling: response to exercise and nutrition in humans

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Abstract

Aging is associated with decline in muscle mass and muscle functions. Muscle strength declines disproportionate to the decline in muscle mass indicating that muscle quality or protein quality also declines with age. Human studies have shown a progressive decline in muscle protein synthesis including proteins in the contractile apparatus and mitochondria with age. However, the decline in muscle protein synthesis is disproportionate to the decline in muscle mass that occurs with age prompting to hypothesize that muscle protein degradation also declines with age. A decline in mitochondrial capacity to synthesize ATP is likely a limiting factor of both synthesis and degradation, which are ATP dependent processes. In support of the above hypothesis, several studies have shown a decline in whole body protein turnover (synthesis and degradation). The timely and efficient degradation of irreversibly damaged or modified proteins is critical to maintain the quality of protein. It is proposed that a failure to degrade the damaged proteins and replacing them with newly synthesized proteins contribute to age related decline in muscle mass and quality of muscle proteins. The underlying molecular mechanism of these age related changes in human muscle needs further investigation.

Keywords

protein; synthesis; breakdown; turnover; isotope tracer

1. Introduction

The world’s elderly population is rapidly expanding, exerting huge socioeconomic ramifications due in part to the concomitant increase in age-related comorbidities and the onset of many diseases that manifest with aging in people who may have genetic predisposition. It is well recognized that aging is associated with the progressive decline in skeletal muscle mass, strength, and function, collectively known as sarcopenia (Roubenoff, 2000). Sarcopenia is a major cause of frailty, physical disability, and loss of functional independence among the elderly (Morley et al., 2011).
Nematodes (Herndon et al., 2002), rodents (Altun et al., 2007), nonhuman primates (Nemoz-Bertholet and Aujard, 2003), and humans (Short et al., 2005) all demonstrate progressive age-related declines in physical activity. This age-related decline in physical activity is likely due to the progressive loss of skeletal muscle mass, muscle strength, and endurance. These age-related decreases in physical activity further contribute to the manifestation of sarcopenia and its related disorders. Specifically, sarcopenia likely contributes to the development of insulin resistance, type 2 diabetes, and cardiovascular disease. There are emerging data in various species ranging from nematodes (Herndon et al., 2002) to humans (Talbot et al., 2007) indicating that declining activity levels lead to increased mortality. Therefore, there is a critical need to develop therapeutic strategies to prevent or delay the onset of sarcopenia.

The primary aim of this review is to examine the underlying mechanisms that contribute to the development of sarcopenia. We will also examine the therapeutic impact that endurance and resistance training have on aged skeletal muscle. Since proteins are the main functional molecules and constitute the predominant non-fluid lean mass, we hypothesize that the age-related loss of skeletal muscle mass, strength, and function are due to impairments in overall skeletal muscle protein homeostasis (the balance between protein synthesis and protein breakdown). The functions of proteins are determined not only by their concentrations but also by their quality. Removal of damaged proteins by degradation and their replacement by newly synthesized proteins are critical for maintaining the quality of the proteins. We posit that both declines in skeletal muscle protein synthesis and degradation play equally important roles in the development of sarcopenia and age-related skeletal muscle dysfunction. Here we discuss the regulation of protein synthesis and degradation and how alterations in these processes may cause sarcopenia.

2. Skeletal muscle protein synthesis

2.1 Aging and postabsorptive

Postabsorptive skeletal muscle protein synthesis rates have been reported by many investigators to be lower in older adults than younger adults in some (Balagopal et al., 1997; Guillet et al., 2004; Henderson et al., 2009a; Rooyackers et al., 1996; Short et al., 2004; Welle et al., 1993; Yarasheski et al., 1993) but not all investigations (Paddon-Jones et al., 2004; Volpi et al., 2000; Volpi et al., 1999; Volpi et al., 2001). We have previously reported that skeletal mixed muscle protein synthesis rates decline at ~4% per decade between the ages of 20–80 years (Short et al., 2004). Furthermore, Figure 1D demonstrates that postabsorptive skeletal mixed muscle protein synthesis is significantly lower in elderly adults than young adults in a study that involved measurements of skeletal mixed muscle protein synthesis in 144 elderly and 62 young adults (Henderson et al., 2009a). These age-related declines in mixed muscle protein synthesis represent an average reduction in the synthesis of all soluble proteins within skeletal muscle. Finally, since protein synthesis is an ATP-dependent process it is likely that the age-related decline in protein synthesis is due in part to the age-related decline in mitochondrial capacity to synthesize ATP.

A number of potential mechanisms may explain why some studies show an age-related decline, while others do not. The possibilities include a type II error by studying a small number of participants when comparing mixed muscle protein synthesis, which has substantial biological variability (Smith et al., 2010). Other factors such as diet prior to study, activity levels, and the variability of the mass spectrometry are also possible explanations for discrepant findings of age effects on protein synthesis.

Aged skeletal muscle is associated with a progressive loss of muscle mass and strength (Frontera et al., 2000) as well as a decrease in mitochondrial content, capacity and function
It is likely that these age-related changes are due to impairments in specific skeletal muscle proteins that are responsible for various functions. Significantly lower postabsorptive myofibrillar protein synthesis rates (Welle et al., 1993; Welle et al., 1995) and that of myosin heavy chain (a key contractile protein) (Balagopal et al., 1997) have been observed with age (Figure 2A). It has been previously reported that type IIx and IIa fiber number and cross-sectional area decline with age (Larsson et al., 1978; Lexell et al., 1988), which is likely related to the age-related decline in mRNA abundance of myosin heavy chain (MHC) IIx and IIa with no decline in MHCI (Balagopal et al., 2001). Furthermore, it has been demonstrated that skeletal muscle mitochondrial protein synthesis declines with age (Guillet et al., 2004; Rooyackers et al., 1996), likely contributing to the age-related decline in mitochondrial protein abundance and skeletal muscle oxidative capacity (Lanza et al., 2008; Short et al., 2005). Of interest, the age-related decline in MHC and mitochondrial protein synthesis occurs without age-related changes in sarcoplasmic protein synthesis (Figure 2 (Balagopal et al., 1997; Rooyackers et al., 1996)). It is important to consider that even though examining the protein synthetic rate of skeletal muscle subfractions provides a greater degree of specificity than mixed muscle protein synthesis alone, these skeletal muscle subfractions are likely contaminated with proteins from other skeletal muscle subfractions. Future studies are warranted to examine the effect of aging on individual skeletal muscle proteins. A recently developed methodology for measuring the fractional synthesis rate of individual skeletal muscle proteins (Jaleel et al., 2008) hopefully will advance our understanding of age-related skeletal muscle remodeling. Finally, the combination of measures of individual skeletal muscle protein synthesis rates along with their gene transcripts and translational control will provide a comprehensive analysis of the regulation of protein synthesis.

### 2.2 Aging and postprandial

Undernutrition due to age-related anorexia has been implicated as a potential contributing factor for the development of sarcopenia. As a result, investigators have hypothesized that sarcopenia may be due to age-related impairments in whole-body and skeletal muscle protein metabolism in response to feeding (Volpi et al., 2000). Consistent with this notion, Pannemans and colleagues have advocated that protein requirements increase with age (Pannemans et al., 1998). Moreover, it has recently been advocated that the recommended daily allowance (RDA) for protein be increased from 0.8 g/kg to 1.2 g/kg (50% increase) (Gaffney-Stomberg et al., 2009). However, clinical investigations have produced conflicting results with respect to the effect of increased protein or amino acid intake on protein synthesis in elderly adults (Dillon et al., 2009; Walrand et al., 2008).

It also has been demonstrated that healthy elderly adults increased mixed-muscle protein synthesis and net amino acid balance across the leg in response to an intravenous infusion of amino acids (Volpi et al., 1998) and oral consumption of amino acids (Volpi et al., 1999). In agreement, Koopman and colleagues also reported no age-related decrease in postprandial skeletal muscle protein synthesis in response to ~35g of intact intrinsically labeled casein (Koopman et al., 2009). Taken together these studies demonstrated that healthy elderly adults responded appropriately to exogenous amino acid supplementation. However, Volpi and colleagues reported increased mixed-muscle protein synthesis in young but not old participants in response to an oral amino acid-glucose mixture [40 g glucose and 40 g amino acids (Figure 3)] prompting them to propose that the elderly adults have an anabolic resistance to mixed meal feeding (Volpi et al., 2000). Indeed, anabolic resistance with aging was shown across graded increases of oral essential amino consumption (Cuthbertson et al., 2004). Mechanistically, age-related insulin resistance may impair insulin’s ability to increase skeletal muscle blood flow following a meal and therefore concomitantly impair postprandial amino acid delivery. Indeed, recent data indicate that age-related reductions in
insulin-induced skeletal muscle blood flow are associated with reduced skeletal muscle amino acid delivery and skeletal muscle protein synthesis (Timmerman et al., 2010).

A recent study showed that 10 days of consuming a high protein diet (~2 g/kg) failed to increase post-absorptive mixed muscle protein synthesis compared to consuming usual protein diet (~1 g/kg) in healthy elderly (and young) adults (Walrand et al., 2008). However, this study demonstrated an increase in whole-body nitrogen balance indicating that post-prandial protein conservation occurred. The short-term nature of this study precluded a thorough examination of whether chronic high protein intake results in an accrual of lean mass, particularly with respect to skeletal muscle mass. It remains feasible that long-term intake of a high protein diet may be an effective strategy for maintaining or even increasing skeletal muscle mass among healthy elderly adults. Moreover, the protein quality in the usual protein diet could have been an improvement over the participants’ habitual dietary intake, and therefore maximal stimulation of post-absorptive mixed muscle protein synthesis may have already been achieved with the usual protein intake. Along this line, Dillon and colleagues (Dillon et al., 2009) recently reported that three months of essential amino acid supplementation (7.5 g twice per day) increased mixed muscle protein synthesis, while concomitantly increasing lean body mass in healthy elderly adults. The increase in mixed muscle protein synthesis observed in this study could potentially be attributed to the selective increase in the bioavailability of essential amino acids. Despite a 30% increase in mixed muscle protein synthesis and a concomitant increase in lean body mass, three months of essential amino acid supplementation failed to increase skeletal muscle strength (Dillon et al., 2009) suggesting that the accumulation of skeletal muscle proteins alone may not be sufficient to improve strength (Henderson et al., 2009b).

2.3 Aging and acute exercise

Both resistance and endurance exercise represent potent stimuli for skeletal muscle protein synthesis. Phenotypic changes in skeletal muscle structure and function in response to exercise training are likely due to an accumulation of acute changes in skeletal muscle protein turnover in response to a single bout of exercise. The effects of acute exercise on skeletal muscle protein synthesis in the postabsorptive and postprandial states have recently been extensively reviewed (Kumar et al., 2009a).

In the postabsorptive state, skeletal muscle protein synthesis is increased following both resistance (Biolo et al., 1995; Chesley et al., 1992; Dreyer et al., 2006; Dreyer et al.; Kim et al., 2005; Kumar et al., 2009b; Phillips et al., 1997; Phillips et al., 1999; Sheffield-Moore et al., 2005) and endurance exercise (Carraro et al., 1990; Durham et al., 2010; Harber et al.; Sheffield-Moore et al., 2004). Of interest, Sheffield-Moore and colleagues demonstrated that postabsorptive skeletal muscle protein synthesis is increased in response to moderate-intensity endurance and resistance exercise in both young and older adults, albeit following a different time course (Sheffield-Moore et al., 2005; Sheffield-Moore et al., 2004). For example, in response to the acute bout of resistance exercise the older participants had a significant elevation in mixed muscle protein synthesis 10-min post-exercise, which was no longer significantly elevated at 1-h or 3-h post-exercise. In contrast, in response to the acute bout of resistance exercise the younger participants did not have a significant elevation in mixed muscle protein synthesis until 3-h post-exercise (Sheffield-Moore et al., 2005). Kumar and colleagues (Kumar et al., 2009b) reported a blunting of the increase in post-absorptive skeletal muscle protein synthesis following resistance exercise in elderly compared to the young (Figure 4). Moreover, recent data indicate that protein synthesis efficiency in response to endurance exercise declines with aging, thus requiring substantially higher arterial and interstitial amino acid concentrations for elderly compared to younger adults for a given rate of protein synthesis (Durham et al., 2010).
Exogenous administration of amino acids with or without carbohydrates has been shown to augment skeletal muscle protein synthesis in response to an acute bout of resistance exercise (Biolo et al., 1997; Dreyer et al., 2008; Rasmussen et al., 2000a; Symons et al., 2011). Importantly, aging does not appear to attenuate the increase in skeletal muscle protein synthesis in response to the combination of an acute bout of resistance exercise plus exogenous amino acid or protein intake (Drummond et al., 2008; Symons et al., 2011). Taken together, these studies indicate that the combination of resistance exercise plus moderate to high protein intake may provide an effective countermeasure against sarcopenia.

Coingestion of a modest amount of carbohydrate plus protein following an acute bout of endurance exercise has been shown to increase skeletal muscle protein synthesis in young adults (Levenhagen et al., 2002). Moreover, Wilkinson and colleagues have demonstrated that an acute bout of single-legged endurance exercise in the fed state increases mitochondrial protein synthesis with no change in myofibrillar protein synthesis (Wilkinson et al., 2008). However, Harber and colleagues demonstrated that feeding does not enhance the increase in skeletal muscle protein synthesis that occurs in response to an acute bout of endurance exercise achieved in the postabsorptive state (Harber et al., 2010). Of interest, Sheffield-Moore and colleagues also demonstrated that both young and older men increase skeletal muscle protein synthesis to a similar degree in response to a single bout of moderate-intensity endurance exercise in the postabsorptive state (Sheffield-Moore et al., 2004). Finally, prior bouts of endurance exercise have been shown to augment the protein synthesis effect of feeding for up to 48 h after exercise (Harber et al., 2009a; Miller et al., 2005).

2. Aging and exercise training

Both resistance and endurance training are cornerstones for achieving a healthy lifestyle. Indeed, endurance and resistance training have well documented benefits on skeletal muscle across in both young and older adults, which occur largely in an exercise modality-dependent fashion. It is likely that the skeletal muscle adaptations to specific training modalities are due to differential responses in skeletal muscle protein synthesis. Resistance training has been shown to increase mixed muscle protein synthesis (Balagopal et al., 2001; Kim et al., 2005; Phillips et al., 1999). Importantly, we have demonstrated that three months of resistance training increases both mixed muscle as well as myosin heavy chain protein synthesis in both young and elderly adults [Figure 5A and 5B (Balagopal et al., 2001)]. Furthermore, postabsorptive skeletal muscle protein synthesis is elevated in both young and older adults following a four months of endurance training demonstrating no differences in increments of protein synthesis related to age [Figure 6A & 6B (Short et al., 2004)]. Taken together, these studies demonstrate that both resistance and endurance training are effective at increasing skeletal muscle protein synthesis although only resistance training increases muscle mass. However, limited data exist that examine the effects of these two divergent training modalities on the synthesis rate of different functionally important skeletal muscle subfractions and individual skeletal muscle proteins. It is also possible that endurance exercise increases muscle protein turnover with little effect on net muscle balance. Finally, we hypothesize that the improvement in mitochondrial capacity to synthesize ATP during endurance training contributes to the improvement in skeletal muscle protein synthesis and degradation, which are ATP-dependent processes.

3. Molecular regulation of protein synthesis

The exquisite regulation of skeletal muscle protein synthesis in response to hormonal, nutritional, and mechanical stimuli is quite remarkable and continually being unraveled (Kimball et al., 2002; Miyazaki and Esser, 2009). Acutely, protein synthesis is largely
regulated at the level of translational initiation (Kimball et al., 2002). In addition, increases in gene transcription can further augment protein synthesis by increasing the abundance and delivery of gene transcripts to active ribosomes (Kubica et al., 2005).

The mammalian target of rapamycin (mTOR) is a major regulatory pathway for synthesis of skeletal muscle proteins in response to growth factors, nutrition, and mechanical stimuli (Figure 7). Consistent with this notion, the mTOR inhibitor rapamycin has been shown to completely block changes in protein synthesis in human skeletal muscle in response to exercise (Drummond et al., 2009). Insulin, insulin-like growth factor-I (IGF-I), amino acids, and mechanical stimuli are all putative stimuli for the mTOR pathway. The molecular regulation of the mTOR signaling has been extensively reviewed elsewhere (Drummond et al., 2009; Kimball et al., 2002; Miyazaki and Esser, 2009). Classically, IGF-I (and insulin to a lesser extent) stimulates the IGF-I receptor, which initiates a phosphorylation cascade that ultimately results in the phosphorylation of mTOR, ribosomal protein S6 protein kinase (S6K1), and elf4E binding protein [4E-BP1 (Kimball et al., 2002)]. The phosphorylation of 4E-BP1 by mTOR results in the inhibition of 4E-BP1, which is a known repressor of translational initiation (Gingras et al., 1999; Gingras et al., 2001; Kimball et al., 2002). Specifically, the phosphorylation of 4E-BP1 allows it to dissociate from elf4E (Gingras et al., 2001). Furthermore, phosphorylation of S6K1 results in the stimulation of elf2 (Kimball et al., 2002). Taken together, the disinhibition of elf4E and the concomitant stimulation of elf2, leads to translational initiation (Kimball et al., 2002). Furthermore, translational elongation is promoted by elf2 in response to stimulation by both mTOR and S6K1 (Kimball et al., 2002).

In addition to IGF-I and insulin stimulation of the mTOR signaling pathway, recent studies have also indicated that the mitogen-activated kinase kinase (MEK) and extracellular regulated kinase (ERK)-dependent signaling pathway can stimulate protein synthesis by inhibiting tuberosclerosis complex-2 (TSC2) repression of mTOR (Ma et al., 2007) as well as stimulating ribosomal protein S6 (Miyazaki et al., 2011). Future studies are warranted to determine the role of MEK/ERK signaling on skeletal muscle protein synthesis in humans particularly in response to aging or dietary and exercise interventions.

The traditional role of IGF-I regulation of hypertrophy has recently been called into question (Baar et al., 2010; Hamilton et al., 2010). Indeed, elevations in systemic IGF-I concentrations in response to resistance exercise were not sufficient to enhance skeletal muscle hypertrophy in non-exercised muscle groups (West et al., 2010). In contrast, a splice variant of IGF-I [IGF-IEc also known as mechano-growth factor (MGF)] has been proposed to act in an autocrine and paracrine fashion to promote skeletal muscle hypertrophy (Goldspink, 2005). However, there is a lack of in vivo data in humans demonstrating the hypertrophic effect of MGF in response to exercise. Finally, the local bioavailability of IGF-I may be acutely increased by the activation of pregnancy associated plasma protein A (PAPP-A), which is a protease that cleaves IGF-I from its binding protein FIGFBP-3 (Lawrence et al., 1999). Further work will need to further consider protein synthesis in response to IGF-I and MGF signaling.

Recent data indicate that mechanical stimuli (e.g., muscle stretch or contraction) can stimulate skeletal muscle protein synthesis independent of IGF-I signaling (Hornberger et al., 2004). Phosphatidic acid (PA) has emerged as a leading second messenger for the IGF-independent activation of mTOR in response to mechanical stimuli (Hornberger et al., 2006). Eccentric contractions in Sprague-Dawley rats activated phospholipase D1 (PLD1), which leads to increased PA synthesis (O’Neil et al., 2009). Importantly, it has subsequently been demonstrated in cultured HEK293 cells that PLD1 is also essential for Rheb activation of mTOR signaling (Sun et al., 2008). Taken together, it appears that mechanical stimuli...
activate mTOR in a PLD1-dependent mechanism using PA as its second messenger. Future research is warranted to determine if PLD-1 dependent activation is essential for exercise induced skeletal muscle hypertrophy in humans.

3.1 Aging and mTOR Signaling

Numerous investigations have been conducted to determine if the activation of the mTOR signaling pathway in response to various stimuli is impaired with aging. Most studies have indicated that the phosphorylation status of mTOR and its downstream effectors appear to be relatively similar between untrained young and elderly adults in the resting, postabsorptive state (Drummond et al., 2008; Fujita et al., 2009; Guillet et al., 2004). The lack of evidence for an age-related decline in basal mTOR signaling is consistent with studies that reported no significant age-related differences in skeletal muscle protein synthesis. However, Guillet and colleagues (Guillet et al., 2004) failed to demonstrate an age-related decline in basal mTOR signaling despite observing a significantly lower basal mixed-muscle protein synthesis in the elderly compared to the young (~0.06 %/h vs. 0.08%/h). The results of Guillet and colleagues (Guillet et al., 2004) suggest that an alternative pathway may regulate age-related declines in skeletal muscle protein synthesis in the postabsorptive and rested state. Future investigations are necessary to uncover potential mechanisms that regulate the age-related decline in skeletal muscle protein synthesis.

In contrast to the lack of evidence for age-related differences in mTOR signaling in untrained adults during resting and postabsorptive conditions, there appears to be age-related impairments in mTOR signaling in response to various anabolic stimuli. For example, Fujita and colleagues demonstrated that a supraphysiological dose of insulin was necessary to stimulate the phosphorylation of S6K1 (Fujita et al., 2009), indicating that age-related insulin resistance is associated with the development of age-related impairments in mTOR signaling. Moreover, S6k1 phosphorylation following an acute bout of exercise and essential amino acid ingestion was slightly attenuated in elderly adults compared to younger adults (Drummond et al., 2008).

3.2 Molecular Regulation of Mitochondrial Protein Synthesis

The regulation of mitochondrial protein synthesis is complex and remains largely unknown. Indeed, mitochondrial proteins are encoded in nuclear and mitochondrial DNA and are coordinately expressed. Certainly adding to the complexity is the necessity to interleave transcriptional and post-transcriptional events, including mRNA translation, protein folding, respiratory chain assembly and import (Devaux et al., 2010). It is generally believed that mitochondrial biogenesis is partly transcriptionally regulated by peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator 1 alpha (PGC1-α) and its downstream transcription factors including nuclear respiratory factor 1 and 2 (NRF1 and NRF2), mitochondrial transcription factor A (TFAM) (Hock and Kralli, 2009; Lin et al., 2005), PPARα (Vega et al., 2000), PPARβ/δ (Wang et al., 2003), and PPARγ (Huss et al., 2002; Kamei et al., 2003). NRF1 and NRF2 are involved in the transcriptional regulation of nuclear encoded mitochondrial proteins (Evans and Scarpulla, 1989; Virbasius et al., 1993); while TFAM is involved in the transcriptional regulation of mitochondrial encoded proteins as well as regulating mitochondrial DNA replication (Fisher et al., 1987). PPARs play an integral role in the regulation of skeletal muscle fatty acid oxidation, particularly PPARβ/δ (Evans et al., 2004). Indeed, PPARβ/δ agonists have been proposed as potential therapeutic agent as a result of the recent finding that 4 weeks of treatment with a PPARβ/δ agonist (GW1516) increases the gene expression for proteins that regulate skeletal muscle fatty acid oxidation in mice (Narkar et al., 2008).
With respect to exercise, PGC1-α is activated by a number of intracellular signaling cascades including AMP activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase (CaMK), and p38 mitogen activating protein kinase [p38 MAPK (Jager et al., 2007; Puigserver et al., 2001; Wright et al., 2007)]. Recent evidence also indicates that PGC1-α may also be activated by sirtuin 1 (SIRT1) and sirtuin 3 (SIRT3) in response to exercise stimuli (Li et al., 2011; Palacios et al., 2009). Taken together, PGC1-α and its downstream transcription factors can initiate mitochondrial biogenesis largely through elevations in gene transcription in response to an acute bout of exercise. However, elevations in protein synthesis can occur independently of changes in gene transcription. Future investigations are necessary to gain a better understanding of the molecular events that regulate skeletal mitochondrial protein synthesis in response to exercise independent of changes in gene transcription.

4. Epigenetic Regulation of Skeletal Muscle Protein Synthesis

Over the last decade, researchers have become increasingly interested in the epigenetic regulation of gene expression. With respect to sarcopenia, researchers have just begun to scratch the surface of defining the role that epigenetic regulation has on skeletal muscle gene expression. Broadly, epigenetic modifications can induce heritable changes in gene expression (i.e., ‘imprinting’) in the absence of changes in the nuclear or mitochondrial genomes (Reik, 2007) as well as labile changes in chromosomal packing that allow for acute changes in transcriptional activity in response to environmental stimuli (Clayton et al., 2006). Epigenetic modifications alter skeletal muscle gene expression and include DNA methylation and histone modifications (methylation and acetylation) [reviewed by (Baar, 2010)]. The degree to which these epigenetic modifications affect human skeletal muscle gene expression across the lifespan remains to be determined.

One could ask 1) if changes in the methylation status of specific DNA promoter regions within the nuclear DNA contribute to age-related declines in skeletal muscle protein synthesis and 2) if exercise can reverse these age-related epigenetic modifications? To begin to address the first question, Ronn and colleagues demonstrated that skeletal muscle biopsy samples from older adults had significantly higher levels of DNA methylation in the promoter region of cytochrome c oxidase subunit 7A (COX7A1) in the presence of concomitantly lower mRNA abundance of COX7A1 in compared to younger adults (Ronn et al., 2008). Of interest, COX7A1 is a PGC1-α responsive gene. These findings are consistent with age-related decline in PGC1-α expression as well as skeletal muscle mitochondrial content (Lanza et al., 2008; Short et al., 2005). In a corollary, it has recently been reported that there is increased non-CpG methylation of the PGC1-α promoter in the presence of decreased mitochondrial content in patients with type-2 diabetes compared to healthy controls (Barres et al., 2009). However, it is unknown if hypermethylation of the PGC1-α promoter contributes to the age-related decrease in PGC1-α expression. Future studies are warranted to determine whether exercise can reverse age-related elevations in DNA methylation.

It is now well accepted that histone modification plays an important role in regulating the initiation of gene transcription to environment stimuli, yet data from humans in response to exercise are lacking. Histones are acetylated by histone acetyltransferase (HAT) leading to transient relaxation of the chromatin structure, which results in the initiation of gene transcription (Allfrey et al., 1964; McKinsey et al., 2001). Conversely, histone deacetylases (HDAC) repress transcription by deacetylation of histones and returning the chromatin structure to its compact native form (McKinsey et al., 2001). Of interest, McGee and colleagues recently reported that endurance exercise results acetylation of histone H3K36, which is indicative of enhanced transcriptional elongation (McGee et al., 2009). Moreover,
they also reported that HDAC4 and 5 were exported from the nucleus in response to the acute bout of endurance exercise, thereby decreasing the potential for histone deacetylation and transcriptional suppression (McGee et al., 2009). Together, these findings indicate that an acute bout of endurance exercise can promote an environment that facilitates transcription and therefore skeletal muscle adaptation. Future studies are warranted to determine whether exercise-induced changes in the acetylation status of histones contribute to the regulation of skeletal muscle protein synthesis in humans.

5. Post-translational damage

Aging leads to the accumulation of protein modifications across a variety of tissues and likely contributes to debilitating conditions including Alzheimer’s (Castegna et al., 2002), cataracts (Chellan and Nagaraj, 1999) and loss of muscle function (Haus et al., 2007). Compared to recently synthesized proteins, relatively older protein isoforms have greater accumulation of post-translational modifications during stressful conditions such as uncontrolled diabetes (Jaleel et al., 2010). Some modifications can be repaired (e.g. oxidative damage), while others are irreversible and require the protein to be degraded (e.g., glycation or extensive oxidative damage). Protein breakdown is a critical mechanism to remove damaged proteins and maintain cellular homeostasis during aging [reviewed by (Koga et al., 2011)].

6. Skeletal muscle protein breakdown

6.1 Aging and postabsorptive

It has been repeatedly shown that basal rates of protein synthesis are decreased with age. It is likely that aging leads to declines in both protein breakdown and synthesis, otherwise the loss of muscle with age would be greater than is observed. High rates of loss of muscle mass are possible in traumatic conditions (Gamrin et al., 1997), yet the loss with aging is more gradual (Janssen et al., 2000). The age effects on skeletal muscle protein breakdown rates are controversial and fraught with methodological challenges. In contrast to protein synthesis, the analysis of protein breakdown rates cannot differentiate between sub-fraction or individual proteins within a tissue. Furthermore, measures of whole-body protein turnover are not specific to skeletal muscle and represent the dilution of isotopically labeled tracer by unlabeled amino acids released from all protein sources. A-V difference across a limb (commonly leg) provides more specificity to muscle tissue (since blood in femoral vein primary drains from muscle), but includes fat, skin and other tissues and is highly dependent on accurate blood flow measures as discussed above. Accurate blood flow measures are inherently difficult to obtain and add much variability. Even with accurate measures, it is still difficult to compare aging effects with a cross-sectional study design due to inappropriate normalization between age groups. Leg volumes can be readily calculated, however changes to lean and fat mass with age occur independently to volume (Koopman and van Loon, 2009). Fat free mass is another choice but the water content of muscle changes with age, thus a given mass of the limb or whole body will have decreased protein content in older people (Proctor et al., 1999). The fractional breakdown rate of skeletal muscle proteins can be measured, yet still represents an average of muscle protein pools (Biolo et al., 1992; Zhang et al., 1996).

Pre-study dietary controls will also influence protein turnover measures. Human studies without standardized pre-study diet controls have not detected age effects on protein turnover under basal conditions (Volpi et al., 2001; Wilkes et al., 2009). In contrast, we provided 3-days of standardized diets and showed lower rates of whole body protein breakdown in older men and women compared to younger [Figures 1A and 2 (Balagopal et al., 1997; Henderson et al., 2009a)]. Pre-study dietary controls must be considered when
comparing protein turnover studies. Such methodological difficulties have lead to varied conclusions that skeletal muscle protein breakdown with aging is increased (Trappe et al., 2004) or not altered (Volpi et al., 2001). The increase in protein breakdown shown by Trappe and colleagues was measured following 3-days of dietary control and could be due to their localized measurement of 3-methylhistidine release as a measure of myofibrillar protein breakdown (Trappe et al., 2004). Such findings highlight the importance of assessing specific protein pools in aging studies. It is known that nutrient availability regulates protein breakdown, but it is not well known if nutrient availability alters individual protein breakdown rates.

Animal and cell culture studies have repeatedly shown that pathways regulating protein breakdown are decreased with age (Cuervo and Dice, 2000; Dice, 1982; Ferrington et al., 2005; Goldstein et al., 1976; Martinez-Vicente et al., 2005; Miquel et al., 1974). Decreased regulation of protein breakdown could contribute to sarcopenia. In support, transgenic mice with deficient skeletal muscle Atg7 signaling had disorganized sarcomere structure along with decreased muscle mass and strength compared to wild type controls (Masiero et al., 2009). The loss of protein quality in skeletal muscle contributes to systemic aging of other tissues as well (Demontis and Perrimon, 2010). Such results from animal studies highlight the importance of maintain protein quality with aging.

6.2 Aging and postprandial

The decrease in food intake with aging, termed the anorexia of aging, is important to consider with skeletal muscle protein breakdown (Di Francesco et al., 2006). Nutrient starvation is a potent stimulator of autophagy in cell and animal models (Ju et al., 2009). The ability for feeding to modulate autophagy or proteasome pathways will be necessary to further understand the regulation of skeletal muscle protein breakdown that may not be detectable using tracer based methods.

During resting and post-absorptive conditions, the rate of whole body and skeletal muscle protein breakdown is greater than synthesis and leads to a net negative protein balance [Figures 1A–C and 8 (Henderson et al., 2009a; Meek et al., 1998; Phillips et al., 1997; Rasmussen et al., 2000b)]. Skeletal muscle protein breakdown is inhibited following feeding in humans and the response appears to be blunted with aging (Cuthbertson et al., 2004). The inhibition of protein breakdown with feeding appears to be mediated by insulin and amino acids (Chow et al., 2006; Greenhaff et al., 2008; Meek et al., 1998; Nygren and Nair, 2003), specifically by essential amino acids such as leucine (Nair et al., 1992). Greenhaff et al. reported dissociation between rates of protein breakdown and proteasome subunit content during graded increases in circulating insulin (Greenhaff et al., 2008). Insulin resistance with age or diabetes may lead to dysregulation of protein turnover (Denne et al., 1991; Wilkes et al., 2009) although not fully supported by some studies in type 2 diabetes (Halvatsiotis et al., 2002a; Halvatsiotis et al., 2002b; Staten et al., 1986; Welle and Nair, 1990). An impaired ability for feeding to regulate protein turnover could contribute to the accumulation of damaged skeletal muscle proteins that is observed during aging or uncontrolled diabetes (Jaleel et al., 2010).

6.2.1 Protein breakdown and caloric restriction—Caloric restriction (CR) without malnutrition is known to extend lifespan in a variety of animal models and may be related to changes in protein turnover. The underlying mechanism of CR on aging remains to be fully determined. The ability for energy balance to alter whole body protein balance has been known (Cuthbertson et al., 1937; Todd et al., 1984) and can specifically alter tissue and organelle regulation of protein turnover (Baltzer et al., 2010). Caloric restriction was also shown to decrease leucine flux after exercise and indicates that energy restriction can
attenuate whole body protein metabolism in response to physical stress (Friedlander et al., 2005). Caloric restriction requires that cells maintain the amino acid pool while in an energetically deprived state. Yet, global protein degradation is an inefficient fuel source and may be detrimental over extended periods for survival. It is possible that selective degradation of specific proteins, such as electron transport chain complexes, could improve cell function during energetic stress by increasing the quality of specific protein fractions.

The breakdown of damaged proteins theoretically helps maintain protein quality and also provides a source of essential amino acids to support protein synthesis during nutrient starvation conditions (Onodera and Ohsumi, 2005). Human studies indicate that leucine released from muscle protein degradation is preferentially acylated to tRNA for protein synthesis (Ljungqvist et al., 1997) and suggest that increased protein degradation may automatically increase protein synthesis. Protein turnover is energetically costly and it is known that protein balance is dependent on energy status (Todd et al., 1984). Amino acids that are liberated from proteins can also be used as an energy source, albeit an inefficient source, and the degradation of proteins is linked with lipid and carbohydrate oxidation (Singh and Cuervo, 2011).

### 6.3 Aging and exercise

Tracer-based studies have revealed that skeletal muscle protein breakdown is increased following acute resistance exercise in younger people (Phillips et al., 1997; Staples et al., 2011). Aerobic exercise is recommended for aging populations, yet resistance exercise is commonly used to study protein turnover and the response of protein breakdown may be specific to each modality. The emphasis on resistance exercise may be rooted in a desire to maximize protein synthesis and mass, despite that aerobic exercise can increase protein turnover acutely and after training (Murphy and Miller, 2010; Short et al., 2004). Aerobic exercise can increase protein breakdown similarly between younger and older men immediately following exercise (Sheffield-Moore et al., 2004) and remain elevated for 10 days (Fielding et al., 1991). Four months of aerobic training increased protein synthesis with no change in whole body protein breakdown in young and older people (Short et al., 2004). The studies by Sheffield-Moore et al. (Sheffield-Moore et al., 2004), Fielding et al. (Fielding et al., 1991) and us (Short et al., 2004) had similar conclusions of no age effect on post-exercise protein breakdown, yet had vastly different study designs including exercise duration, intensity, post-exercise feeding and tracer model (skeletal muscle vs. whole body). A few studies have provided additional insight by reporting changes in mRNA and protein levels of markers for autophagy or proteasome pathways (Glynn et al., 2010a; Glynn et al., 2010b; MacKenzie et al., 2009; Raue et al., 2007; Wohlgemuth et al., 2011; Wohlgemuth et al., 2010). The interpretation of such results is limited and the regulation of skeletal muscle protein breakdown following exercise is still largely unknown. There is much opportunity for future investigations into skeletal muscle protein breakdown following exercise in aging populations.

The induction of skeletal muscle protein breakdown with aerobic exercise should be beneficial for improving muscle quality and mass because aerobic exercise may cause oxidative damage to proteins and their replacement with newly synthesized proteins is critical to maintain the functional quality of the proteins. Autophagosome formation is associated with enhanced tissue repair following strenuous exercise in mice (Salminen and Vihko, 1984). Despite the evidence that protein degradation pathways are beneficial, the interpretation of results can suggest that protein degradation is detrimental and should be protected against. A recent study concluded that exercise protects against the induction of autophagy during doxorubicin treatment (Smuder et al., 2011). Yet, it is apparent that the removal of damaged cellular components by autophagy is beneficial for cellular function (Zhang and Cuervo, 2008). It is likely that exercise increases autophagic flux and thus
provides protection from accumulation of damaged cellular components. In support, LC3II protein content can decrease in response to increased autophagic flux (Tanida et al., 2005) and was decreased following exercise in rodents and humans, suggesting increased autophagy (Glynn et al., 2010a; Smuder et al., 2011). The ability for exercise to stimulate skeletal muscle protein breakdown will need to be considered using techniques that determine pathway flux (Klionsky et al., 2008; Mizushima et al., 2010).

6.3.1 Exercise and feeding—The combination of exercise with food intake is a strong stimulus for protein turnover. Negative energy balance induced by exercise without feeding will increase breakdown to a greater extent than synthesis and lead to net negative protein balance during the several hours after exercise (Phillips et al., 1997). Over several weeks, increased amounts of exercise lead to attenuated nitrogen loss during negative energy balance and shows that exercise can have anabolic effects during an overall catabolic state (Todd et al., 1984).

The combination of exercise and feeding may be an effective lifestyle countermeasure to improve protein turnover with age. Consuming a meal with approximately 25 grams of protein after exercise will lead to higher synthesis rates than breakdown (Staples et al., 2011), likely due to insulin mediated blunting of protein breakdown (Greenhaff et al., 2008; Staples et al., 2011). The decline in insulin sensitivity with age could lead to an impaired ability to suppress protein breakdown under basal condition. Exercise can improve insulin sensitivity and may restore the regulation of protein breakdown by insulin. In support, similar rates of skeletal muscle protein breakdown were reported after light aerobic exercise and protein feeding in young and older people (Koopman et al., 2006). Additionally, a recent report by Durham et al. showed that younger and older people have a similar rates of skeletal muscle protein breakdown following light aerobic exercise and amino acid infusion (Durham et al., 2010). The timing of amino acid ingestion around exercise is also important since consuming protein immediately post-exercise can improve nitrogen retention (Jordan et al., 2010) and training adaptations (Esmarck et al., 2001). The combination of exercise and feeding may overcome the anabolic resistance with aging.

The loss of mitochondrial function with age and chronic disease may be attenuated with increased mitochondrial protein turnover. It is recognized that aerobic exercise can stimulate mitochondrial protein synthesis (Wilkinson et al., 2008), which could be accompanied by increased mitochondrial protein breakdown. Impaired mitochondrial protein breakdown has been implicated for age-related disease states such as Parkinson’s (Youle and Narendra, 2011), yet very little is known about mitophagy during aging and exercise. Oxidative damage to mitochondria during exercise could allow a mitochondrial specific signal for increased protein turnover to remove damaged proteins. Future work can consider whether aerobic exercise stimulates mitochondrial protein degradation and promote skeletal muscle health through improved mitochondrial function.

7. Methodology considerations

It is important to consider the methodologies that are used to assess skeletal muscle protein breakdown in the context of aging and exercise. We have included a brief discussion about common measurements of the autophagy-lysosome and ubiquitin-proteasome systems.

7.1 Autophagy

Autophagy is a catabolic process by which cellular components are engulfed by a membrane for subsequent degradation within a lysosome [see (Koga et al., 2011; Ravikumar et al., 2010) for detailed reviews]. Autophagy is distinguished into three pathways based on the size of degradation including macroautophagy (large volumes), microautophagy (smaller

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volumes), and chaperone mediated autophagy (individual proteins). A recent review identified limitations to autophagy measurements (Klionsky et al., 2008). Importantly, static measurements such as mRNA or protein content do not measure flux through autophagy pathways. For example, microtubule-associated protein 1 light chain 3 (LC3) is lipidated from LC3-I to LC3-II and incorporated into the membranes of pre-autophagosomes for eventual degradation within the lysosome. LC3-II content is influenced by autophagic flux, but does not distinguish between increased or decreased flux (Tanida et al., 2005). Increased LC3-II content could be due to either increased autophagy that requires more preautophagosomes or decreased autophagy that leads to a buildup of preautophagosomes (Mizushima and Yoshimori, 2007). Additional measures such as electron microscopy and immunofluorescence provide important insight by showing changes in the content and localization patterns of autophagy proteins (Mizushima et al., 2010). Measurements of autophagy in humans are often limited to mRNA and protein content (Glynn et al., 2010b; Wohlgemuth et al., 2011). Future investigations that can distinguish autophagic flux will provide much insight into skeletal muscle protein turnover in humans.

7.2 Proteasome

The ubiquitin-proteasome pathway allows for selective degradation of individual proteins [see (Glickman and Ciechanover, 2002) for detailed review]. Proteins are tagged for degradation by attachment to single or chains of ubiquitin then transported to the proteasome for degradation. Signaling occurs between autophagy and proteasome pathways and the attachment of a single ubiquitin is sufficient to target a protein for degradation through autophagy (Kim et al., 2008).

The ubiquitin-proteasome pathway is commonly analyzed using immunoblots for ubiquitination of proteins and can be complemented with proteasome enzyme content or activity. The ubiquitin content of proteins provides a general assessment of protein degradation, however the extent of ubiquitination is not directly related to proteasome activity. Indeed, as few as four ubiquitin molecules are a sufficient signal for degradation by the proteasome (Thrower et al., 2000). The presence of ubiquitinated proteins means that the protein has not been degraded, therefore the extent of ubiquitination gives an indication of overall protein degradation potential of a tissue sample. Additional measures such as proteasome content or activity provide further insight into the regulation of protein breakdown.

8. Protein storage vs. remodeling

Diet and exercise are modifiable lifestyle factors that alter protein turnover and protein quality, yet the effects of each are fundamentally different: consumption of dietary nutrients promotes storage in tissues while exercise promotes remodeling of tissues (Figure 8). Dietary protein does not have a storage depot as do lipids or carbohydrate, but instead can be “stored” as newly synthesized skeletal muscle proteins (Miller, 2007). It remains to be determined what specific protein concentrations increase following a meal. Post-prandial increases in insulin promote protein storage through decreasing protein breakdown (Chow et al., 2006; Meek et al., 1998; Nygren and Nair, 2003), an effect that is blunted with aging (Wilkes et al., 2009). In contrast, exercise leads to tissue remodeling through simultaneously increasing protein synthesis of new proteins and breakdown of existing proteins (Phillips et al., 1997). Combining exercise with feeding appears to overcome the anabolic resistance of aging (Pennings et al., 2011) and may be beneficial for tissue remodeling and improving skeletal muscle protein quality with aging (Figure 8).
9. Summary

The loss of skeletal muscle mass and quality with age is very apparent, yet the changes to skeletal muscle protein turnover remain incompletely understood. Data from animal models have repeatedly shown decreased protein breakdown with aging, which we propose is associated with accumulation of damaged proteins. Human studies have been less clear but it is likely that protein synthesis and breakdown are both decreased with aging. If synthesis and breakdown were not coordinately decreased, then the rate of muscle loss would be exacerbated and rapidly lead to muscle wasting, which does not occur. Moreover, there is increasing evidence to show that with age, decreases in muscle quality occur when the replacement of damaged proteins with newly synthesized proteins decays (Figure 10). The combination of exercise and nutrition seems to provide the stimulus and substrates for tissue remodeling to increase protein quality. Changes in protein quality during aging and comorbidities such as obesity and diabetes will need to be evaluated in future investigations.

Acknowledgments

The authors are greatly indebted to the skillful assistance of Maureen Bigelow, Jill Schimke, Katherine Klaus, Dawn Morse, Bushra Ali, Jane Kahl, Dan Jakaitis, Roberta Soderberg, Beth Will, Deborah Sheldon and Melissa Aakre. We are also grateful for support from the National Institutes of Health UL1-RR-024150-01, AG09531 (K.S.N.), R01-DK41973 (K.S.N.), KL2 RR024151 (B.A.I.), and 5T32DK007352-32 (M.M.R.). In addition, we are grateful for the support from the National Center for Research Resources (NCRR), a component of the NIH, and the NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on NCRR is available at http://www.ncrr.nih.gov/. Information on Reengineering the Clinical Research Enterprise can be obtained from http://commonfund.nih.gov/aboutroadmap.aspx. Additional support was provided by the Mayo Foundation and the Murdock-Dole Professorship (to K.S.N.).

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### Research highlights

- Decreased protein turnover with age may diminish protein quality
- Exercise training increases protein turnover and protein quality
- Decreased muscle mitochondrial capacity with age may decrease protein turnover
- Feeding enhances the anabolic effects of exercise independent of age
- Methods are needed to examine the turnover of individual proteins
Figure 1.
“Baseline protein metabolism before chronic administration of hormones or placebo. A) Phenylalanine Ra. B) Conversion of phenylalanine to tyrosine. C) Disposal of phenylalanine via protein synthesis. D) skeletal muscle protein fractional synthesis rate. Values are means ± se. Young men, n = 30; young women, n = 32; elderly men, n = 87, elderly women, n = 57. Main effect of sex: *P < 0.0001;Ω P < 0.001. Main effect of age: #P < 0.0001. P < 0.001. No significant sex X age interactions. Statistical analysis by ANOVA with Tukey’s HSD post hoc test.” Reproduced with permission (Henderson et al., 2009a).
Figure 2.
Fractional synthesis rate (FSR) of (A) myosin heavy chain (MHC), (B) mitochondrial and (C) sarcoplasmic proteins in young, middle-aged, and old subjects calculated using ketoisocaprate (KIC) and tissue fluid (TF) as precursor for protein synthesis. *Significant (P < 0.05) change from young age group; **significant (P < 0.01) change from young age group. Panels A and C are reproduced with permission from (Balagopal et al., 1997) and Panel B is reproduced with permission from (Rooyackers et al., 1996).
Figure 3.
“The fractional synthesis rate (FSR) of mixed-muscle proteins in healthy young (30±3 yr) and elderly (72±1 yr) subjects in the basal state and during the intake of an amino acid-glucose mixture. Values are the mean ± SE.* P<0.01 vs. basal; §, P<0.05 vs. young.”
Reproduced with permission from (Volpi et al., 2000). Permission pending.
“Dose–response relationship of myobrillar protein synthesis (FSR, fractional synthetic rate, % h⁻¹) measured at 1–2 h post-exercise for 5 young men and 5 older men at each intensity. The responses of the young men overall were greater than those of the older men (P < 0.04). The responses between 60 and 90% of 1 RM in young and old were indistinguishable from each other but those in the young were together significantly higher than in the older men (P < 0.01) for 15 subjects in each group.” Reproduced with permission from (Kumar et al., 2009b).
Figure 5.
“Effect of exercise (vs. control) on the fractional synthesis rate (FSR) of myosin heavy chain (MHC) and mixed muscle protein in 39 subjects (aged 46–79 yr). Exercise increased FSR of MHC (P < 0.05) and FSR of mixed muscle protein (P < 0.05). Measurements did not change in the control group.” Reproduced with permission (Balagopal et al., 2001).
Figure 6.

“Age and exercise effects on fractional synthesis rate of mixed muscle protein. A: at baseline there was a significant decline with age in muscle protein synthesis rate. B: exercise training resulted in an overall 22% increase in muscle protein synthesis, *P < 0.05, whereas there was no change in the control group. C: change in muscle protein synthesis rate (Δ value) in the exercise group did not vary with age in men or women.” Reproduced with permission (Short et al., 2004).
Figure 7.
A schematic representation of mammalian target of rapamycin (mTOR) signaling pathway for the regulation of skeletal muscle protein synthesis in response to growth factors, nutrition, and mechanical stimuli.
Figure 8.
A comparison of protein turnover in response to exercise and feeding relative to young-fasted state. In the fasted state, protein breakdown is greater than protein synthesis across ages, and exercise without feeding increases protein turnover while maintaining negative balance. Feeding supplies amino acids to shift protein balance from negative to positive, an effect that is enhanced following exercise. Overall energy balance is a strong regulator of protein turnover.
Proteins that are damaged during exercise can be degraded through a variety of pathways. Aging leads to down regulation of protein breakdown pathways and contributes to accumulation of damaged proteins.
Figure 10.
A proposed model of sedentary aging with low protein turnover and accumulation of damaged proteins. Exercise training can decrease the accumulation of damaged proteins through increased protein turnover.
A sampling of studies that investigated protein breakdown with aging and exercise. The variety of study designs and methodologies lead to difficulties interpreting the effects of exercise and feeding with age on muscle protein breakdown pathways. *Indicates standardized pre-study diet.

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Methods</th>
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<th>Feeding</th>
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<td>8MF 31–59 6MF</td>
<td>Whole body</td>
<td>Rested</td>
<td>Fasted*</td>
<td>Decreased FSR and whole body Ra with age</td>
<td>[1-13C]-Leu</td>
<td>GC-C-IRMS</td>
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<td>Whole body</td>
<td>Rested</td>
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<td>Decreased whole body Ra with aging</td>
<td>[15N]-Phe</td>
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<td>(Volpi et al., 2001)</td>
<td>26M 22M</td>
<td>2 &amp; 3-pool</td>
<td>Rested</td>
<td>Fasted</td>
<td>No difference FSR or Ra with aging</td>
<td>[3H]-Phe</td>
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<td>(Sheffield-Moore et al., 2004)</td>
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<td>2 &amp; 3-pool</td>
<td>Acute aerobic 60 min@ 40%</td>
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<td>Lower Ra with aging, Training increased FSR with no change whole body Ra in yg and old</td>
<td>[1-13C]-Leu [15N]-Phe</td>
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<td>(Glynn et al., 2011a)</td>
<td>13M 13M 13M 13M 13M</td>
<td>3-pool, western, PCR</td>
<td>Resistance</td>
<td>Lo vs. Hi EAA Feeding</td>
<td>Decreased Ra (p&lt;0.1), Decreased LC3II protein increased mRNA autophagy</td>
<td>[3H]- or [13C]-Phe</td>
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<td>(Fielding et al., 1991)</td>
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<td>Whole body leucine flux</td>
<td>Aerobic 45 min 80% eccentric</td>
<td>Fed</td>
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<td>(Staples et al., 2011)</td>
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