Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation

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Submitted 3 August 2004; accepted in final form 17 December 2004

Toth, Michael J., Dwight E. Matthews, Russell P. Tracy, and Michael J. Previs. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. Am J Physiol Endocrinol Metab 288:E883–E891, 2005. First published December 21, 2004; doi:10.1152/ajpendo.00353.2004.—Aging is associated with decreased skeletal muscle mass and function. These changes are thought to derive, in part, from a reduction in skeletal muscle protein synthesis. Although some studies have shown reduced postabsorptive muscle protein synthesis with age in humans, recent studies have failed to find an age effect. In addition to this disparity, few studies have attempted to characterize the hormonal factors that may contribute to changes in protein synthesis. Thus we examined the effect of age on skeletal muscle protein metabolism, with a specific emphasis on myosin heavy chain (MHC) protein, and the relationship of protein synthesis rates to plasma hormone levels. We measured body composition, muscle function, muscle protein synthesis, MHC and actin protein content, MHC isof orm distribution, and plasma concentrations of cytokines and insulin-like growth factor-I (IGF-I) in 7 young [29 ± 2 (SE) yr] and 15 old (72 ± 1 yr; P < 0.01) volunteers. Mixed-muscle (-19%; P = 0.11), MHC (-22%; P = 0.08), and nonmyofibrillar (-17%; P = 0.10) protein synthesis all tended to be lower in old volunteers. Old volunteers were characterized by increased circulating tumor necrosis factor-α receptor II (P < 0.05) and reduced IGF-I (P < 0.01). In addition, plasma C-reactive protein, interleukin-6, and tumor necrosis factor-α receptor II concentrations were negatively related to mixed-muscle and MHC protein synthesis rates (range of r values: −0.422 to −0.606; P < 0.05 to <0.01). No differences in MHC or actin protein content were found. Old volunteers showed reduced (P < 0.05) MHC IIx content compared with young volunteers but no differences in MHC I or Ila. Our data show strong trends toward reduced postabsorptive muscle protein synthesis with age. Moreover, reduced muscle protein synthesis rates were related to increased circulating concentrations of several markers of immune activation.

sarcopenia; muscle; aging; cytokine; stable isotope

AGING IS ASSOCIATED with reduced skeletal muscle mass and function, also termed sarcopenia (30). Because protein is the primary structural and functional macromolecule in muscle, it is commonly held that age-related changes in the processes that regulate muscle protein mass contribute to sarcopenia. At present, the alterations in muscle protein metabolism that cause sarcopenia, and the factors that potentiate these changes, have not been clearly defined.

Muscle protein content is determined by the balance between protein synthesis and breakdown, with changes in synthesis being the primary impetus for altered protein balance under most physiological conditions (25, 28). A number of studies in humans (1, 13, 29, 44, 46) have shown that postabsorptive muscle protein synthesis declines with age. In addition to reductions in mixed-muscle protein synthesis, which represents the average synthesis rate of all muscle proteins, these studies have observed reduced protein synthesis rates in specific subcellular protein compartments, such as myofibrils and mitochondria (1, 13, 29, 44). These findings have led to the hypothesis that reduced skeletal muscle protein synthesis contributes to sarcopenia. Recent studies showing no age effect on muscle protein synthesis, however, have challenged this notion (41). Thus there is some controversy as to whether human aging is associated with reduced postabsorptive muscle protein synthesis.

Age-related changes in the physiological systems that regulate skeletal muscle protein metabolism could contribute to sarcopenia. Of particular interest, recent findings have pointed to a role for cytokines and insulin-like growth factor-I (IGF-I) in the development of sarcopenia, specifically that increased circulating levels of cytokines and reduced IGF-I predict muscle loss and physical disability in the elderly (6, 26, 33, 40). The notion that these hormones might play a role in regulating muscle protein metabolism with age is well founded. Aging is associated with increased cytokine levels/production and reduced circulating IGF-I concentrations (5, 31). Moreover, studies in cultured myocytes and animal models have clearly demonstrated the catabolic effects of cytokines and anabolic effects of IGF-I on skeletal muscle (19, 22, 39). Perhaps more importantly, studies have shown that cytokines and IGF-I antagonize each other’s effects on skeletal muscle protein synthesis (8, 17). Thus the balance between the catabolic effect of cytokines and the anabolic effect of IGF-I may play an important role in the development of sarcopenia. The relationship of circulating cytokines and IGF-I to skeletal muscle protein metabolism, however, has not been examined extensively in humans.

Specific skeletal muscle proteins and groups of proteins, with important structural and functional roles, have different rates of metabolism. Because of this, mixed-muscle protein synthesis rates provide little information about how aging might affect distinct muscle characteristics and processes. From both quantitative and functional perspectives, myosin heavy chain (MHC) is the most important protein in skeletal muscle. It comprises ~25% of myocellular protein mass (47) and, together with actin and associated regulatory proteins, is responsible for the conversion of chemical energy into mechanical work. In this regard, MHC protein plays a key role in determining the quantity and functionality of muscle protein.

Several studies have shown that MHC protein synthesis is reduced with age (1, 13). Interestingly, the reduction in MHC

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protein synthesis in these studies was greater than that observed for mixed-muscle protein, suggesting the possibility that there could be a preferential reduction in MHC protein content with age. To date, however, no study has examined the effect of age on skeletal muscle MHC protein content. In addition to the overall mass of MHC protein, the type of MHC isoforms expressed has relevance for both the metabolism and functionality of aging muscle. Because the isoforms are synthesized at different rates (9), a change in MHC isoform distribution with age could contribute to altered MHC protein synthesis rates. Additionally, a shift in MHC isoform distribution can alter muscle performance given the different functional properties of each isoform (4, 21). At present, however, conflicting reports exist regarding the effect of age on skeletal muscle MHC isoform distribution (18, 24).

In the present study, we examined the effect of age on skeletal muscle protein synthesis and the relationship of muscle protein synthesis to circulating cytokine and IGF-I levels. To accomplish this objective, postabsorptive mixed-muscle and MHC protein synthesis rates and plasma hormone levels were measured in young and old volunteers. To study the primary effect of aging on protein synthesis, we performed these measurements after controlling experimentally for the effects of pretesting diet and exercise. In addition, we evaluated the effect of age on MHC protein content and isoform distribution. The goal of these MHC protein measurements was twofold. First, we wanted to characterize the effect of age on MHC protein content and isoform distribution. Second, we sought to further define the potential causes (e.g., age-related changes in MHC isoform expression could contribute to reductions in MHC protein synthesis rates) and consequences (e.g., reduced MHC protein synthesis rates could contribute to reduced MHC protein content) of age-related changes in skeletal muscle protein synthesis.

MATERIALS AND METHODS

Subjects. Seven young (29 ± 2 yr; 3 men, 4 women) and 15 old (72 ± 1 yr; 10 men, 5 women) volunteers were recruited. We did not restrict our recruitment approach on habitual activity patterns and accepted young and old volunteers with a wide range of physical activity patterns (i.e., from sedentary to physically active). Before study, a medical history was obtained, and physical examination and biochemical laboratory tests were performed. Additionally, an oral glucose tolerance test was conducted to ensure that all volunteers had normal glucose tolerance (glucose <7.77 mmol/l 2 h after 75 g oral glucose load). All volunteers were healthy and free of chronic disease with the exception of two old men with known coronary artery disease. Both of these volunteers had normal left ventricular contrac-
tile patterns, no exertional ischemia, as demonstrated by a normal electrocardiographic stress test to exhaustion, and reported no limitation in physical activity patterns. Both patients were treated with aspirin, one with a Ca2+ channel blocker and the other with a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor. An additional volunteer in the older group was taking aspirin (doses 81 or 325 mg/day). All patients on aspirin therapy discontinued use 5 days before study. Two women in the younger group were taking oral contraceptives, and one woman in the older group was taking estrogen replacement therapy. The nature, purpose, and possible risks of the study were explained to each subject before giving written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

Experimental protocol. Each volunteer was tested during outpatient and inpatient visits to the University of Vermont General Clinical Research Center (GCRC). Body composition, exercise capacity, and muscle strength were measured on an outpatient basis and were performed at least 1 wk before skeletal muscle protein synthesis measurement. Skeletal muscle protein synthesis was measured during an inpatient visit to the GCRC. For 3 days before admission, all subjects were provided a standardized, weight-maintenance diet [60% carbohydrate; 25% fat; 15% protein; protein content of the diet was at least 1 g/kg; young (Y): 1.66 ± 0.13 vs. old (O): 1.63 ± 0.06 g/kg]. The last meal of the standardized diet was consumed by 1900 the evening of admission, and subjects fasted until completion of the following day. Volunteers were instructed not to exercise the day before muscle protein synthesis measurements. These steps were taken to control for the possible confounding effects of diet and/or exercise on skeletal muscle protein metabolism.

The morning after the overnight visit, the subject was awakened, and Teflon catheters were placed in an antecubital vein and a dorsal hand vein. Baseline blood and breath samples were collected; a primed (4.8 μmol/kg), continuous (5.6 μmol·kg−1·min−1) infusion of [1,2-13C2]leucine was started; and the bicarbonate pool was primed (3.1 μmol/kg) with sodium [13C]bicarbonate (time 0 min). Muscle tissue (100–150 mg) was taken from the vastus lateralis under lidocaine anesthesia by percutaneous needle biopsy at 90 min and from the contralateral leg at 480 min. Biopsy samples were cleaned of adipose and connective tissue, frozen in liquid nitrogen, and stored at −70°C until analysis. Blood samples were drawn at 0, 165, 180, 195, and 210 min for measurement of whole body leucine kinetics and at 300, 360, 420, and 480 min for measurement of [13C]Ketosioacscuate enrichment, which was used as a surrogate of precursor pool enrichment for protein synthesis calculations. Oxygen consumption (VO2) and carbon dioxide production rates were determined at 60, 165, and 360 min for 15 min using the ventilated hood technique (DeltaTrac, Yorba Linda, CA).

Body composition. Fat mass and fat-free mass were measured by dual-energy X-ray absorptiometry using a Lunar DPX-L densitometer (DEXA; Lunar, Madison, WI). Appendicular skeletal muscle mass was measured using the region of interest option of the software and the skeletal landmarks of Heymsfield et al. (14). In addition, thigh and forearm muscle mass was measured. For the thigh, the proximal cut point was made at the most inferior point of the ischium and the distal cut point at the most inferior point of the medial and lateral condyles. For the forearm, the proximal cut point was made at the radial head and the coronoid process of the ulna and the distal cut point at the styloid processes of the radius and ulna. For forearm and thigh measurements, values represent the averages between left and right limbs. Regional measures of appendicular muscle mass by DEXA agree well with measurements of muscle size obtained by magnetic resonance imaging (42).

Exercise capacity. Peak VO2 was measured during a graded, treadmill test to volitional fatigue. Briefly, a comfortable initial walking speed was found for each volunteer and was maintained throughout the test. The grade was increased 2.5% every 2 min until volitional fatigue. Peak VO2 was defined as the highest 30-s average VO2 value measured during the last 2 min of the test. One young female volunteer did not complete the treadmill test.

Skeletal muscle strength. Isometric and isokinetic knee extensor strength was measured using a multijoint dynamometer (Lido Active; Loredan Biomedical, Sacramento, CA). The right leg was tested in all subjects. The volunteer was seated and positioned so that the lateral femoral epicondyle was aligned with the central axis of the dynamometer. Extraneous movement was restricted by a Velcro strap placed across the abdomen and a padded restraint at the distal point of the thigh. The lever arm of the dynamometer was attached just proximal to the lateral malleolus. Following instructions, volunteers were allowed to perform several practice trials for each condition at moderate intensity (~25%) to ensure familiarity with the procedure. For isometric measurements, the lever arm was fixed at 55°. Volunteers performed three brief (5-s) maximal voluntary contractions, each
separated by 1 min of rest. The highest torque (mN) value for each contraction was recorded. The average from the three trials was calculated. Isokinetic measurements were performed at 90°/sec. The range of motion was set from 0 to 90° flexion relative to full knee extension. Volunteers performed 15 consecutive contractions. The average of the three highest torque values from the first five contractions was recorded and averaged. Forearm muscle strength was measured using an isometric hand grip dynamometer (Lafayette Instruments, Lafayette, IN). Three trials were performed for each hand, each separated by 1 min of rest, and the average of all six trials was calculated as a measure of isometric forearm muscle strength. One young female volunteer did not complete strength testing.

**MHC content and isoform expression.** MHC and actin content were determined by SDS-PAGE, as described by Haddad et al. (11), with minor modifications. Muscle tissue was homogenized in extraction buffer (0.6 M KCl, 0.15 M potassium phosphate, 20 mM EDTA, 5 mM MgCl₂, and 3.3 mM ATP, pH 6.7) using a glass homogenizer and minor modifications. Muscle tissue was homogenized in extraction buffer, and the supernatant was decanted, and the pellet was extracted with 40 mM Na₄P₂O₇, 1 mM MgCl₂, and 1 mM EGTA, pH 9.5, and was then centrifuged at 12,000 g for 15 min. The homogenate was centrifuged at 100,000 g for 30 min, and the supernatant was discarded. The resulting homogenate was centrifuged, and the supernatant was discarded. The supernatant was allowed to precipitate overnight (at least 14 h) and then centrifuged. The precipitate was washed first with 10% TCA and then with distilled water and finally with petroleum ether. The ether was evaporated under N₂, 6 ml of 6N HCl were added, and the tube was capped and heated for 24 h at 110°C. Amino acids in the hydrolysate were isolated by ion exchange chromatography and derivatized to their N-(O,S)-ethoxycarbonyl ethyl ester (ECF) derivative (46). The ECF-derivatized amino acids were removed from the organic phase and ECF-leucine enrichment (atom percent excess) was measured by gas chromatography–-combustion-isotope ratio mass spectrometry (GC-C-IRMS) and converted to mle.

MHC and nonmyofibrillar protein synthesis measurements were performed according to the methods of Hasten et al. (12). Briefly, muscle tissue (~50 mg) was homogenized in sucrose buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM imidazole, pH 6.8), the homogenate was centrifuged, and the supernatant was discarded. The pellet was suspended in Triton X-100 buffer (175 mM KCl and 0.5% Triton X-100, pH 6.8), homogenized, and centrifuged. The supernatant, which contains mostly nonmyofibrillar proteins (36), was decanted. KCl buffer (150 mM KCl and 20 mM Tris base, pH 7.0) was added to the pellet, the pellet was homogenized, and the resulting supernatant was added to the nonmyofibrillar fraction. This process was repeated, and TCA was added (10% final concentration) to the nonmyofibrillar fraction; the sample was allowed to precipitate overnight (at least 14 h) and then centrifuged. Further processing and measurement of leucine enrichment in the nonmyofibrillar protein fraction was similar to that of mixed-muscle protein described above, excluding the petroleum ether wash. The pellet, which contains mostly myofibrillar proteins, was solubilized in loading buffer (2% SDS, 62.5 mM Tris, 10% glycerol, and 0.001% bromphenol blue, pH 8.8, with 5% β-mercaptoethanol), vortexed, and heated at 100°C for 5 min.

MHC was isolated by SDS-PAGE. The stacking gel contained 4% acrylamide-N,N’-methylene-bis-acrylamide (bis) and the resolving gel 10% acrylamide-bis. Gels were run at constant current (60 mA) for ~2.5 h at 22°C and then were stained with Coomassie blue. MHC and actin band intensity were determined by densitometry (Quantity One; Bio-Rad Laboratories) and were expressed as densitometric units per microgram of protein loaded.

The relative distribution of MHC isoforms was determined according to the method of Klitgaard et al. (18), with minor modifications. Briefly, ~15 mg muscle tissue were homogenized in buffer (20 mM KCl, 2 mM K₂HPO₄, and 1 mM EGTA, pH 6.8) and incubated on ice for 15 min. The homogenate was centrifuged briefly (6,500 g for 20 s) and analyzed for protein content using BSA as a standard (Bio-Rad Laboratories, Hercules, CA). An aliquot of the muscle homogenate was added to loading buffer (2% SDS, 62.5 mM Tris, 10% glycerol, and 0.001% bromphenol blue, pH 6.8, with 5% β-mercaptoethanol) and heated for 5 min at 100°C. For each muscle sample, 1 μg protein was loaded per lane, which was within the linear range of detection for both MHC and actin. Samples from young and old groups were analyzed together in a ratio of 1:2 to minimize gel-to-gel variation. The stacking gel contained 4% acrylamide-N,N’-methylene-bis-acrylamide (bis) and the resolving gel 10% acrylamide-bis. Gels were run at constant current (60 mA) for ~2.5 h at 22°C and then were stained with Coomassie blue. MHC and actin band intensity were determined by densitometry (Quantity One; Bio-Rad Laboratories) and were expressed as densitometric units per microgram of protein loaded.

**Whole body protein metabolism.** Plasma α-ketoisocaproate and breath CO₂ enrichments from 165, 180, 195, and 210 min were measured and used to calculate whole body leucine kinetics, as described previously (38), with the following minor modifications. Leucine oxidation (C) was calculated as

\[ C = \frac{F_{13C}}{1/E_2 - 1/E_1} \times 100 \tag{1} \]

where \( F_{13C} \) is the rate of \(^{13}\)CO₂ excretion in expired gas (μmol 13C·kg⁻¹·h⁻¹), \( E_2 \) and \( E_1 \) are the enrichment of the leucine tracer [mole percent excess (mpe)] in plasma and infusate, respectively, and the constant 2 accounts for the two 13C labels in the leucine molecule. \( F_{13C} \) was calculated as

\[ F_{13C} = \frac{(FCO₂ \times ECO₂) \times [W/(60 \times 44.6)](100 \times 0.81/(0.79))] \tag{2} \]

where \( FCO₂ \) is the CO₂ production rate (cm³/min), \( ECO₂ \) is the enrichment of expired CO₂ (mpe × 1,000), and W the subject’s fat-free mass (kg). The constants 60 (min/h) and 44.6 (μmol/cm³) convert FCO₂ to micromoles per hour, the factor 100 changes atom percent excess to a fraction, 0.81 accounts for the retention of \(^{13}\)CO₂ in the bicarbonate pool, and 0.79 accounts for the loss of the 2-13C label of leucine in tricarboxylic acid cycle exchange reactions (37).

**Skeletal muscle protein synthesis.** Mixed-muscle protein synthesis measurements were performed as described previously (27). Briefly, muscle tissue was homogenized in solubilization buffer (100 mM sodium pyrophosphate, 1% SDS, and 4 mM EGTA, pH 7.4), and the resulting homogenate was centrifuged, the supernatant was decanted, trichloroacetic acid (TCA) was added (10% final concentration), and the sample was allowed to precipitate overnight (at least 14 h) and then centrifuged. The precipitate was washed first with 10% TCA and then with distilled water and finally with petroleum ether. The ether was evaporated under N₂, 6 ml of 6N HCl were added, and the tube was capped and heated for 24 h at 110°C. Amino acids in the hydrolysate were isolated by ion exchange chromatography and derivatized to their N-(O,S)-ethoxycarbonyl ethyl ester (ECF) derivative (46). The ECF-derivatized amino acids were removed from the organic phase and ECF-leucine enrichment (atom percent excess) was measured by gas chromatography–combustion-isotope ratio mass spectrometry (GC-C-IRMS) and converted to mle.

**Hormone analysis.** C-reactive protein (CRP) was measured by ELISA (23) with an interassay coefficient of variation (CV) ranging from 2 to 4%. Tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) plasma concentrations and soluble receptors [TNF-α receptor II (RII) protein] were measured by ELISA (23) with a CV ranging from 2 to 4%.
and IL-6 soluble receptor (sR), respectively] were measured by ultrasensitive ELISA assays (R&D Systems, Minneapolis, MN) with interassay CVs of 16 and 6% for TNF-α and IL-6 concentration and 9 and 10% for their respective receptors. IGF-I was measured by RIA (ALPCO, Windham, NH). In this technique, IGF-I is dissociated from binding proteins by dilution in an acidic buffer. An antibody solution containing IGF-II is added to occupy IGF-binding sites, and free IGF-I was determined. The interassay CV is 5%.

Statistical analysis. Differences between groups were examined using ANOVA with group (young vs. old) as a factor. Because the age groups were not evenly matched for sex, we included sex (male vs. female) as a factor in the ANOVA model. If a significant sex effect was found for a given variable, the ANOVA model was adjusted for the effect of sex. In this adjustment, sex was defined as a categorical variable (1 = women, 2 = men). Those variables that were adjusted for the effect of sex are indicated. Data for these variables represent group adjusted means after the effect of sex has been removed statistically. Because some variables were not normally distributed, log transformations were performed before ANOVA procedures. Relationships between variables were assessed using Pearson correlation coefficients. For hormone measures that were not normally distributed, log_{10} transformed values were used for correlation analyses. All data are expressed as means ± SE.

RESULTS

Physical characteristics are shown in Table 1. All physical characteristic variables, with the exception of age, showed a sex effect (P < 0.05). Therefore, the ANOVA model was adjusted for sex before group comparisons. Of note, reduced whole body, arm, leg, thigh, and forearm fat-free mass were found in old compared with young volunteers (all P < 0.01). Although groups did not differ in absolute fat mass, percent fat tended (P = 0.09) to be greater in old volunteers (Y: 19 ± 3 vs. O: 25 ± 2%).

The effect of age on peak VO₂, isokinetic and isometric knee extensor strength, and isokinetic forearm muscle strength is shown in Table 2. All physical function variables showed a sex effect in the ANOVA model (P < 0.05). Thus each variable was adjusted for sex. All physical function measurements were reduced in old individuals, with the exception of isometric knee extensor strength. After statistically adjusting for fat-free mass, no differences in peak VO₂ were found (Y: 2.73 ± 0.16 vs. O: 2.40 ± 0.10 l/min; P = 0.22). Similarly, after statistical adjustment for thigh muscle mass, no differences in isokinetic (Y: 124 ± 8 vs. O: 107 ± 4 Nm; P = 0.17) or isometric (Y: 160 ± 10 vs. O: 178 ± 6 Nm; P = 0.22) knee extensor strength were noted. No difference in forearm isokinetic strength was found after adjustment for forearm muscle mass (Y: 125 ± 6 vs. O: 111 ± 4 Nm; P = 0.13).

No differences in whole body leucine rate of appearance (Y: 154 ± 13 vs. O: 138 ± 4 μmol·kg⁻¹·min⁻¹·min⁻¹; P = 0.89) or nonoxidative leucine disposal (Y: 130 ± 12 vs. O: 118 ± 4 μmol·kg⁻¹·fat-free mass⁻¹·min⁻¹; P = 0.95) were found. Leucine oxidation was lower (P < 0.05) in older volunteers (Y: 24 ± 2 vs. O: 19 ± 1 μmol·kg⁻¹·fat-free mass⁻¹·min⁻¹).

Differences in skeletal muscle protein synthesis are shown in Fig. 1. Although not statistically significant, trends toward reduced mixed-muscle (Y: 1.09 ± 0.12 vs. O: 0.89 ± 0.06%/day; P = 0.11), MHC (Y: 0.981 ± 0.087 vs. O: 0.769 ± 0.066%/day; P = 0.08), and nonmyofibrillar (Y: 1.51 ± 0.13 vs. O: 1.26 ± 0.08%/day; P = 0.10) protein synthesis were found. No difference was found in the ratio of MHC to mixed-muscle protein synthesis (Y: 0.919 ± 0.057 vs. O: 0.837 ± 0.051%/day). The statistical power to detect differences between groups was 43 and 35% for MHC and mixed-muscle protein synthesis, respectively. However, our observed differences in MHC and mixed-muscle protein synthesis would reach statistical significance if the younger group sample size was increased to n = 8 and n = 13, respectively. Exclusion of two old men with known coronary artery disease did not qualitatively alter these findings: mixed muscle (Y: 1.09 ± 0.12 vs. O: 0.86 ± 0.07%/day; P = 0.09), MHC (Y: 0.981 ± 0.087 vs. O: 0.762 ± 0.075%/day; P = 0.09) and nonmyofibrillar (Y: 1.51 ± 0.13 vs. O: 1.23 ± 0.08%/day; P = 0.08).

Table 1. Physical characteristics

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<td>Thigh muscle mass, kg</td>
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</table>

Data are means ± SE; n, no. of subjects. M, male; F, female. Forearm and thigh muscle mass represent average values from left and right arms and legs, respectively. Note: n = 6 for forearm muscle mass for young group. All variables were adjusted for the effect of sex, with the exception of age. *P < 0.01 vs. young.

Table 2. Peak aerobic capacity and muscle strength measures

<table>
<thead>
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<th></th>
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<th>Old</th>
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</thead>
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<td>2.10±0.13*</td>
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<td>Isokinetic knee extensor strength, Nm</td>
<td>136±9</td>
<td>97±6*</td>
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<tr>
<td>Isometric knee extensor strength, Nm</td>
<td>183±14</td>
<td>157±10</td>
</tr>
<tr>
<td>Isometric forearm strength, Nm</td>
<td>135±8</td>
<td>99±6*</td>
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Data are means ± SE. Peak VO₂, peak oxygen consumption. Note: n = 6 for young group for all measures. All variables were adjusted for the effect of sex. *P < 0.01 vs. young.

Fig. 1. Differences in skeletal muscle protein fractional synthesis rates between young (filled bars) and old (open bars) volunteers. MMP, mixed-muscle protein; MHC, myosin heavy chain. Note: n = 13 old volunteers for MMP measurement. Values are means ± SE. d, Day.
Age-related differences in circulating anabolic and catabolic hormone levels are shown in Table 3. A sex effect was found for TNF-α RII levels. Thus the effect of sex was statistically removed from the ANOVA model before age group comparisons. TNF-α RII levels were higher \((P < 0.05)\) and IGF-I levels lower \((P < 0.01)\) in old compared with young volunteers. There was a trend \((P = 0.06)\) toward increased IL-6 levels in old volunteers. No differences were noted in CRP \((P = 0.11)\), IL-6 sR \((P = 0.37)\), or TNF-α \((P = 0.73)\). Exclusion of two old men with coronary artery disease did not qualitatively alter differences in TNF-α RII \((Y: 2.125 \pm 151\) vs. O: \(2.665 \pm 110\) pg/ml; \(P < 0.05\), IGF-I \((Y: 2.28 \pm 0.06\) vs. O: \(2.06 \pm 0.04\) ng/ml; \(P < 0.01\), or IL-6 \((Y: 0.04 \pm 0.07\) vs. O: \(0.211 \pm 0.05\); \(P = 0.08\)). Moreover, no differences in cytokine levels were found in older individuals on aspirin therapy \((n = 3)\) vs. those not taking aspirin \((n = 12)\).

Data were pooled from young and old volunteers to examine the relationship between skeletal muscle protein measures and plasma hormone levels and markers of immune activation. Table 4 shows correlation coefficients between skeletal muscle protein synthesis measurements and circulating anabolic and catabolic factors. Most notable were the negative correlations of CRP and IL-6 to mixed-muscle protein and MHC synthesis rate. Scatterplots for the relationship of log IL-6 to mixed-muscle protein synthesis are shown in Fig. 2. Use of nonparametric statistical procedures, instead of log transformation, resulted in similarly significant relationships between IL-6 and both mixed muscle \((r = -0.450; P < 0.05)\) and MHC \((r = -0.552; P < 0.01)\). Exclusion of two old men with known coronary artery disease did not diminish the statistical significance of these correlations: log CRP vs. mixed-muscle and MHC protein synthesis: \(r = -0.535; P < 0.03\) and \(r = -0.602; P < 0.01\), respectively; log IL-6 vs. mixed-muscle and MHC protein synthesis: \(r = -0.614; P < 0.01\) and \(r = -0.606; P < 0.01\), respectively; TNF-α RII vs. mixed-muscle protein synthesis: \(r = -0.436; P = 0.07\). No correlations were found between plasma hormones and either myofibrillar protein content or MHC isoform distribution measures.

Skeletal muscle myofibrillar protein content data are shown in Fig. 3. No differences in MHC \((Y: 941 \pm 58\) vs. O: \(998 \pm 46\) arbitrary units (AU/μg protein; \(P = 0.48\)) or actin \((Y: 180 \pm 13\) vs. O: \(190 \pm 15\) AU/μg protein; \(P = 0.67\)) protein content were observed between groups. Similarly, no difference in the actin-to-MHC ratio was found \((Y: 0.196 \pm 0.018\) vs. O: \(0.191 \pm 0.012\); \(P = 0.81\)). Exclusion of two old men with known coronary artery disease did not qualitatively alter these findings: MHC \((Y: 941 \pm 58\) vs. O: \(986 \pm 49\) AU/μg protein; \(P = 0.57\)), actin \((Y: 180 \pm 13\) vs. O: \(185 \pm 16\) AU/μg protein; \(P = 0.83\)), and actin-to-MHC ratio \((Y: 0.196 \pm 0.018\) vs. O: \(0.188 \pm 0.014\); \(P = 0.74\)).

Figure 4 shows skeletal muscle MHC isoform expression. A representative gel showing the effect of age on MHC isoform expression is provided in Fig. 4A. Figure 4B shows group differences in MHC isoform expression. No differences in MHC I \((Y: 56 \pm 7\) vs. O: \(67 \pm 3\%\); \(P = 0.11\)) or MHC IIa \((Y: 37 \pm 5\) vs. O: \(31 \pm 3\%\); \(P = 0.20\)) were found, although MHC IIa was greater \((P < 0.05)\) in young vs. old volunteers \((Y: 7.5 \pm 2.7\) vs. O: \(2.8 \pm 1.2\%\)). Exclusion of two old men with known coronary artery disease did not qualitatively alter these findings: MHC I \((Y: 56 \pm 7\) vs. O: \(67 \pm 3\%\); \(P = 0.10\)), MHC IIa \((Y: 37 \pm 5\) vs. O: \(30 \pm 3\%\); \(P = 0.20\)), and MHC IIx \((Y: 7.5 \pm 2.7\) vs. O: \(2.8 \pm 1.4\%\); \(P = 0.10\)).
DISCUSSION

Our results support prior studies showing an age-related decline in skeletal muscle protein synthesis rates in humans (1, 12, 44, 46). Differences in protein synthesis rates between young and old volunteers in the present study, however, did not reach statistical significance ($P$ values: 0.08–0.11) because of a smaller magnitude reduction in protein synthesis rates with age. We found 22% lower MHC protein synthesis and 19% lower mixed-muscle protein synthesis in old compared with young volunteers. Prior studies have shown age-related differences on the order of 24–40%, depending on the protein compartment studied (1, 13, 29, 44, 46). A recent study by Short et al. (35) examining a large cohort of individuals aged 19–74 yr, however, has shown a similar 19% reduction in mixed-muscle protein synthesis between younger and older individuals. The reason for this variability among studies is not clear, since our study was similar to prior work with respect to the age range of the volunteers, dietary standardization procedures, and pretesting physical activity limitations. The diminished age difference in protein synthesis may relate to the fact that we did not restrict recruitment to sedentary volunteers. These inclusion criteria could diminish differences if the young group contained more sedentary volunteers and/or the old group contained more active volunteers. We feel that this scenario is unlikely, however, since the difference in peak VO$_2$ between young and old groups (30% lower in old group) predicts a per decade decline of 7.5%, which is in agreement with prior cross-sectional studies in sedentary populations (7, 15). Thus the habitual physical activity patterns of our cohort, as reflected by aerobic capacity measurements, are similar to large samples of sedentary individuals. Nonetheless, even if our comparisons are biased by the inclusion of physically active volunteers, we still observed a reduction in muscle protein synthesis with age, albeit to a lesser degree than some prior investigations. Although age differences did not reach statistical significance, it is difficult not to ascribe physiological significance to such marked reductions ($\sim$20%) in skeletal muscle protein synthesis.

The debate over age-related differences in muscle protein synthesis has focused on the potential modulating role of the pretesting environment and behavioral habits, specifically that controlling for diet and/or activity before muscle protein synthesis measurements can affect the ability to detect age-related differences. We chose to control for diet and exercise directly preceding muscle protein synthesis measurements. We feel that this is necessary to eliminate the confounding influence of diet and exercise habits on muscle protein synthesis measurements. In this approach, differences between groups are not related to antecedent diet or physical activity and are more reflective of primary aging effects. Parenthetically, protein intake (g/kg) was similar between young and old volunteers directly proceeding testing and was more than adequate to meet daily requirements. Thus it is unlikely that differences in protein synthesis measurements relate to protein deficiency in old volunteers. Similarly, it is unlikely that the excess protein provided (i.e., 1.6 g/kg average for both groups) affected skeletal muscle protein synthesis rates (45). Considering that age-related differences in protein synthesis are observed in studies that have rigorously controlled for traditional modulators of protein metabolism (1, 13, 29, 44, 46), such as dietary and exercise habits, we are left to conclude that other, as yet unidentified, factors contribute significantly to variability in muscle protein synthesis rates with age.

To explore those factors that may contribute to the age-related decline in skeletal muscle protein synthesis, we measured circulating concentrations of cytokines, markers

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**Fig. 3.** Differences in MHC and actin protein content between young (filled bars) and old (open bars) volunteers. MHC and actin protein content are expressed as AU/µg total muscle protein. Values are means ± SE.

**Fig. 4.** Differences in skeletal muscle MHC isoform expression. A: representative SDS-PAGE for the three isoforms of skeletal muscle MHC (I, IIa, and IIx) in young (Y) and old (O) volunteers. B: group mean data for young (filled bars) and old (open bars) volunteers. Data are expressed as a percentage of total MHC expression. Values are means ± SE. *$P$ < 0.05.
of immune activation (i.e., CRP) and IGF-I. These specific measures were chosen in light of recent studies suggesting a role for these hormones in the development of sarcopenia (6, 26, 33, 40). Old volunteers were characterized by increased circulating concentrations of TNF-α RII and IL-6 and reduced concentrations of IGF-I. These results are consistent with the notion that age is associated with immune activation (31) and reduced IGF-I levels (5). Perhaps more important than group differences, when data from young and old volunteers were pooled, concentrations of CRP, IL-6, and TNF-α RII were negatively correlated with mixed-muscle and MHC protein synthesis rates (Table 4 and Fig. 2). Although prior studies have shown that higher levels of IL-6 predict muscle atrophy and dysfunction in old volunteers (6, 26, 33), to our knowledge, this is the first study to demonstrate a relationship between circulating concentrations of cytokines and the biochemical processes that regulate muscle mass in humans. These correlations are in keeping with the well-established effect of cytokines to decrease muscle protein synthesis (19, 22). Our study cannot, however, discern whether circulating cytokines alter skeletal muscle protein synthesis in an endocrine fashion or if they are reflective of immune activation at the level of the skeletal muscle. In support of local immune activation controlling protein synthesis, Greiwe et al. (10) showed that skeletal muscle levels of TNF-α protein were negatively correlated with skeletal muscle protein synthesis rates in frail elderly volunteers. Regardless of the mode of action (i.e., endocrine vs. paracrine/autocrine), taken together, these findings suggest a role for immune activation in the regulation of skeletal muscle protein balance. Because the level of immune activation increases with age (Table 3 and Ref. 31), these results identify increased inflammatory tone as a potential mediator of depressed protein synthetic rates and, in turn, muscle atrophy. One problem inherent with such a conclusion is that the relationship between immune activation and protein synthesis is confounded by preexisting disease/frailty. This is particularly relevant to studies that have examined diseased or frail elderly (10). That the elderly volunteers in our study were largely free of pathologies and were fully functional, however, suggests that the effects of immune activation on skeletal muscle protein metabolism may be a more generalized phenomenon in the elderly population. Nonetheless, we should acknowledge that the relationships between cytokines and muscle protein synthesis measures do not delineate cause and effect. Further studies are needed to establish a mechanistic link between immune activation and sarcopenia.

Some studies have observed a greater age-related decline in MHC protein synthesis relative to mixed-muscle protein synthesis (1, 13). For example, in a recent study by Hasten et al. (13), a pronounced reduction in MHC protein synthesis (~32%) was found despite no decrease in mixed-muscle protein synthesis. Over time, this imbalance may contribute to a selective reduction in MHC protein relative to total muscle protein content. This type of selective MHC protein loss is not unprecedented. Studies in animal models and humans have shown MHC depletion under conditions of muscle disuse (11) and muscle disuse combined with increased catabolic hormone levels (20, 34). Because aging is characterized by changes in both catabolic/anabolic hormone balance (Table 3 and Ref. 32) and reduced physical activity, there is reason to believe that such a reduction in MHC protein could evolve. To our knowledge, however, no study has examined the effect of age on skeletal muscle MHC protein content. In the present study, we found no difference in either MHC or actin protein content expressed relative to total muscle protein content between age groups, suggesting that the stoichiometry of these myofibrillar proteins relative to each other (i.e., actin-to-MHC ratio) and to total muscle protein content are maintained with age. These protein content data agree with the fact that MHC synthesis (22% lower) was reduced in old volunteers in proportion to mixed-muscle protein synthesis (19% lower) in our population. Despite the fact that we did not observe age-related differences in MHC protein content, future studies that investigate the effect of age on MHC protein metabolism and/or muscle function should include measurements of MHC protein content.

MHC IIX levels were lower in old compared with young volunteers, whereas no differences in MHC I or IIA levels were found. Our findings differ from other reports in the literature (18, 24). Klitgaard et al. (18) found greater relative MHC I isoform content in older volunteers, whereas Marx et al. (24) found no age-related differences in MHC protein isoform distribution. Although studies differ in their ability to detect a statistically significant change in MHC isoform distribution with age, the pattern of differences in MHC isoforms is remarkably similar among reports; specifically, aging is associated with increased MHC I and reduced MHC II isoform expression. Variation among studies probably relates to differences in the populations studied. Although the degree of muscle atrophy in the studies cited above was similar, the age-related decline in muscle strength, as indicated by isometric knee extensor strength, was significantly greater in the old volunteers studied by Klitgaard et al. (18; 44% reduction) compared with Marx et al. (24; 26% reduction) and the present study (14% reduction). Thus MHC isoform distribution shifts with age may differ depending on the degree of functional impairment of the population studied. This would be consistent with the fact that the relative content of MHC is correlated to the functional demands/capabilities of the muscle (2). Whatever the reason for variation among studies, it is unlikely that changes in MHC isoform expression contribute to the age-related reduction in muscle protein synthesis rate. Although MHC isoforms are synthesized at different rates, with MHC I isoforms being synthesized at a faster rate than MHC II isoforms (9), aging is associated with minimal changes in MHC isoform distribution or a shift toward a greater relative content of MHC I isoform. Thus, if anything, we would predict that the observed shifts in MHC isoform distribution would increase, not decrease, muscle protein synthesis rates.

In conclusion, our results support the notion that skeletal muscle protein synthesis rates, including MHC protein synthesis rates, decline with age. Despite these changes in synthesis rates, MHC protein content was not altered with age, and minimal changes in MHC isoform distribution were noted. A novel finding in this study was the negative relationship between several markers of immune activation and skeletal muscle protein synthesis rates. Because the elderly are characterized by heightened inflammatory tone (31), these relationships provide further evidence to support the hypothesis that immune
activation may contribute to the development of sarcopenia (6, 10, 26, 33, 40).

ACKNOWLEDGMENTS
We are grateful to Ian Galbraith for skilled assistance and Kelly Begin for helpful technical suggestions.

GRANTS
This work was supported by National Institutes of Health Grants AG-17494, AM-02125, and RR-00109, the University of Vermont College of Medicine, and the American Federation for Aging Research.

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