Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans

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Balagopal, P., Olav E. Rooyackers, Deborah B. Adey, Philip A. Ades, and K. Sreekumaran Nair. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E790–E800, 1997.—A decline in muscle mass and contractile function are prominent features of the sarcopenia of old age. Because myosin heavy chain is an important contractile protein, it was hypothesized that synthesis of this protein decreases in sarcopenia. The fractional synthesis rate of myosin heavy chain was measured simultaneously with rates of mixed muscle and sarcoplasmic proteins from the increment of [13C]leucine in these proteins purified from serial needle biopsy samples taken from 24 subjects (age: from 20 to 92 yr) during a primed continuous infusion of L-[1,13C]leucine. A decline in synthesis rate of mixed muscle protein (P < 0.01) and whole body protein (P < 0.01) was observed from young to middle age with no further change with advancing age. An age-related decline of myosin heavy-chain synthesis rate was also observed (P < 0.01), with progressive decline occurring from young, through middle, to old age. However, sarcoplasmic protein synthesis did not decline with age. Myosin heavy-chain synthesis rate was correlated with measures of muscle strength (P < 0.05), circulating insulin-like growth factor I (P < 0.01), and dehydroepiandrosterone sulfate (P < 0.05) in men and women and free testosterone levels in men (P < 0.01). A decline in the synthesis rate of myosin heavy chain implies a decreased ability to remodel this important muscle contractile protein and likely contributes to the declining muscle mass and contractile function in the elderly.

protein synthesis; sarcopenia; contractile function; mass spectrometry; stable isotope

Advancing age is associated with profound changes in body composition. Sarcopenia is characterized by an age-related loss of skeletal muscle mass, a decrease in muscle strength, and increased fatigability (7, 9). Sarcopenia causes a substantial decline in physical performance. The weakness of quadriceps muscle predisposes to frequent falls, which may cause fractures in the elderly. There are many metabolic changes that also occur with the loss of lean mass and reduced physical activities characteristic of sarcopenia. It is estimated that 20–30 billion dollars of health care costs in the United States are spent on problems related to sarcopenia (29).

Besides its locomotive and metabolic functions, muscle is the largest repository of proteins and amino acids in the body. The functional capacity of muscle depends on both the quality and quantity of muscle proteins. The quality of muscle in this paper is defined by the efficiency to perform its various functions per unit muscle mass. Both the quality and quantity of muscle protein are likely to be maintained through a continuous remodeling process involving protein synthesis and breakdown. A decline in the synthesis rate of a specific protein is an indication of a diminished remodeling process, with possible functional consequences. Muscle mass is determined by the balance between breakdown and synthesis. A decline in muscle mass thus could occur if the rate of muscle protein synthesis is lower than the rate of muscle protein breakdown. How far the efficiency of muscle functions (or quality of muscle mass) is determined by the muscle remodeling process remains to be defined.

There is continued controversy as to whether or not whole body protein synthesis declines with age (20, 35, 36, 38). Whole body protein synthesis is an average of the synthesis rates of various proteins in different tissues of the body. Several recent studies have demonstrated that synthetic rates of mixed muscle proteins (total muscle and total myofibrillar component) are lower in the elderly (age >65 yr) than in the young (age <30 yr) group (33, 36, 37). However, the synthesis rates of mixed muscle protein (comprised of different muscle protein components such as sarcoplasmic, myofibrillar, and mitochondrial proteins) and myofibrillar protein (comprised of individual proteins such as myosin, actin, titin, tropomyosin, troponin, protein C, and some components of mitochondrial proteins) represent an average measure of synthesis rates of several proteins. Different muscle proteins are synthesized at different rates (39) and regulated by different genes (17). Each of these proteins has different functions. For this reason, an insight into the pathophysiology of sarcopenia at the molecular level requires measurement of the synthesis rates of individual proteins. We chose to study myosin heavy-chain synthesis (3), since this protein is critical for muscle contractile functions, converting chemical energy (hydrolysis of ATP to ADP) to mechanical energy (14). We also sought to determine whether the synthesis rate of sarcoplasmic protein decreases with age because this muscle protein fraction is involved in the anaerobic ATP production, intracellular transport, and several other enzyme functions. These measurements were compared with those of mixed muscle protein and whole body proteins. A previous study demonstrated that synthesis rate of mitochondrial proteins (responsible for aerobic ATP production) declines with aging (26).

A critical issue is to know the timing of age-related changes such that interventions designed to prevent these changes can be initiated before their occurrence. All previous muscle protein synthesis studies were performed comparing young with elderly populations (33, 36, 37) to determine whether the synthesis rate of
proteins in the elderly are different from that of the young group. We extended these studies to determine whether age-related changes also occur in middle age. The present study provides the first report on the effect of age on the synthesis rate of myosin heavy chain and its correlation to muscle strength and hormones.

METHODS

Subjects

A total of 24 healthy volunteers, eight each from three age groups, young (23 ± 1 yr), middle (52 ± 1 yr), and old (77 ± 2 yr), were recruited for the study. Equal numbers of men and women were recruited in each group. The detailed subject data are given in Table 1. Subjects were carefully screened to exclude postmenopausal women on estrogen replacement, subjects taking β-adrenergic blockers or steroids, and subjects exercising ≥20 min two times per week or more. These subjects are included in a report describing the effect of aging on mitochondrial protein synthesis (26).

Materials

L-[1-13C]leucine (99 atom % excess) was purchased from Cambridge Isotope Laboratories (Woburn, MA). Solutions were prepared under sterile conditions and were tested to be bacterial and pyrogen free before use in human subjects. All electrophoresis reagents were purchased from Bio-Rad Laboratories (Richmond, CA). Trifluoroacetic acid was purchased from Pierce (Rockford, IL).

Study Protocol

The protocol was approved by the Institutional Review Board of the University of Vermont, and informed consent was obtained from the volunteers before the study. All subjects were given a weight-maintaining diet (protein-fat-carbohydrate 15:35:50) for 5 days before the study at the General Clinical Research Center of the University of Vermont (GCRC). Subjects were given a weight-maintaining diet (protein-fat-carbohydrate 15:35:50) for 5 days before the study at the General Clinical Research Center of the University of Vermont. They followed the diet for 2 days as outpatients, and they were admitted as inpatients on the evening of day 2. Twenty-four-hour urine samples were collected on days 4, 5, and 6 to determine the average 24-h urinary creatinine excretion. During this period, measurements of total and regional body composition using Lunar Dual Photon X-ray Densitometer (Lunar DPX-L, Madison, WI) (13) were also made. On the fifth day in the postabsorptive state an intravenous glucose tolerance test was performed (27). The study was performed on the 6th day, after an overnight fast. The last meals of the day were given at 10 PM on all 5 days, so that subjects were used to the meal regimen and to minimize the overnight fast on the day of the study. An intravenous line for infusion was inserted before 10 PM on day 5, and the line was kept patent by saline (0.9% NaCl) infusion. At 1:30 AM the infusion was changed over to the isotopes. The isotope administration was comprised of a primed (7.5 µmol·kg⁻¹·h⁻¹) continuous infusion of L-[1-13C]leucine (7.5 µmol·kg⁻¹·h⁻¹), as previously described (22, 23) for 10 h. A 10-h infusion was necessary to achieve sufficient isotope level in slowly turning over proteins, such as myosin heavy chain, to measure the enrichment with high precision by mass spectrometry. Blood and breath samples were obtained at baseline and at every hour from 5 to 10 h. The CO₂ production was measured by indirect calorimetry using Deltrac (Sensormedics, Yor- bolinda, CA) between 6 and 7.5 h. Muscle biopsies were performed at 5 (6 AM) and 10 h (11 AM) from different legs, as previously described (22, 23). Samples (~50 mg for mixed muscle protein and ~150 mg for myosin heavy chain and sarcoplasmic proteins) were immediately frozen in liquid nitrogen and kept at −80°C until analysis.

Analytic Methods

Mixed muscle protein. Mixed muscle protein in the biopsy sample (25–50 mg) was separated and hydrolyzed as described previously (4). The constituent amino acids in the hydrolysate were purified by cation exchange chromatography (Dowex 50 H⁺ form, Bio-Rad Laboratories, Hercules, CA), and amino acids were eluted with the use of 4 M ammonium hydroxide, as described previously (3).

Sarcoplasmic protein. Muscle biopsy samples (100–150 mg) were homogenized in a sucrose (0.25 M), EDTA (2 mM), and tris(hydroxymethyl)aminomethane (10 mM) buffer (pH 7.4) using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at low speed (600 g), and the pellet was collected for the purification of myosin heavy chain. The supernatant was again centrifuged at 7,000 g. The sarcoplasmic protein was obtained by centrifuging the 7,000 g supernatant from above at 100,000 g for 60 min. The soluble or sarcoplasmic protein contained in supernatant was precipitated using 1 M perchloric acid and centrifuged at 1,500 g. The pellet was washed twice with perchloric acid.

Specificity and purity of sarcoplasmic protein fraction. To confirm that the obtained fraction is the sarcoplasmic component we performed Western blot analysis (24) of the skeletal muscle sarcoplasmic protein fraction from pig and human for the presence of calasequestrin, the most prominent Ca²⁺-binding protein in the sarcoplasmic reticulum using a monoclonal (mouse) anticalasequestrin antibody (immunoglobulin G2b; catalog no. MA3–913 from Affinity Bioreagents, Golden, CO). The possible contamination in sarcoplasmic protein fractions is from myofibrillar protein and mitochondral proteins. The sarcoplasmic protein fraction was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 4–20% analytic gradient gels essentially as described by Laemmli for contamination from myofibrillar proteins, and proteins were visualized after silver staining (2). Cytochrome-c oxidase activity was measured for contamination from mitochondrial proteins, as previously described (26).

Myosin heavy chain. The pellet (predominantly myofibrillar fraction) collected at low speed (600 g) after separation of mitochondrial and sarcoplasmic proteins was used for the purification of myosin heavy chain by a preparative continu-

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Body Weight, kg</th>
<th>Body Mass Index, kg/m²</th>
<th>Body Fat Mass, kg</th>
<th>Fat-Free Mass (DPX), kg</th>
<th>Muscle Mass (Creatinine), kg</th>
<th>Muscle Mass/Height, kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>23 ± 1</td>
<td>165.1 ± 3.8</td>
<td>61.8 ± 4.2</td>
<td>22.5 ± 0.6</td>
<td>11.9 ± 1.0</td>
<td>45.2 ± 3.9</td>
<td>29.7 ± 2.2</td>
<td>18.0 ± 1.2</td>
</tr>
<tr>
<td>Middle</td>
<td>52 ± 1</td>
<td>170.5 ± 3.0</td>
<td>77.6 ± 6.1</td>
<td>26.6 ± 1.4</td>
<td>24.1 ± 2.0*</td>
<td>48.9 ± 4.6</td>
<td>26.5 ± 2.8</td>
<td>15.5 ± 1.2</td>
</tr>
<tr>
<td>Old</td>
<td>77 ± 2</td>
<td>167.0 ± 3.1</td>
<td>66.4 ± 3.6</td>
<td>23.7 ± 0.9</td>
<td>23.1 ± 3.5*</td>
<td>42.7 ± 2.9</td>
<td>17.0 ± 1.4*</td>
<td>10.2 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects/group. DPX, dual photon X ray. *Significant difference from young group P < 0.01. †Significant difference from middle group P < 0.01.
uous elution electrophoresis technique, as described previously (2, 3). The purity of the myosin heavy-chain fractions in each sample was established by analytic SDS-PAGE on a 5% gel followed by silver staining (3). Myosin heavy-chain samples were overloaded on the analytic gel to detect any contamination from other proteins. The purified myosin heavy-chain fractions were pooled together, and the protein was precipitated with trichloroacetic acid. The myosin heavy-chain and sarcoplasmic proteins were hydrolyzed with the use of 1.5 ml of 6 N hydrochloric acid, and amino acids were partially purified as described for mixed muscle protein (3).

Leucine from the hydrolyzate (both total mixed muscle protein and myosin heavy chain) was purified by high-performance liquid chromatography, without prior derivatization, using a reversed-phase C-18 column as previously described (4). The purified leucine was lyophilized for analysis of isotopic enrichment measurements.

Measurement of isotopic enrichment in muscle protein. The measurement of [13C]leucine enrichment in skeletal muscle proteins for both mixed muscle protein and myosin heavy chain was performed using a ninhydrin-gas chromatography (GC)-isotope ratio mass spectrometry (IRMS) method, as described in detail elsewhere (1), with the use of a DeltaS isotoperatio mass spectrometer (Finnigan MAT, Bremen, Germany). The [13C]leucine enrichment of sarcoplasmic protein was measured using a GC-combustion-IRMS technique described previously (1).

Measurement of enrichment in precursor pool. 13C enrichment of ketosaccharoprotein (KIC) in plasma was measured as its quinoninal-trimethylsilyl derivative under negative ion chemical ionization conditions (10). The enrichment was monitored using ions with a mass-to-charge ratio of 233/232 using an HP-MS Engine (Hewlett Packard, Avondale, PA).

Tissue fluid was separated as described previously (4), the amino acids in the tissue fluid were derivatized as their t-butyldimethylsilyl ester, and enrichments were measured using a GC-mass spectrometry (HP-MS Engine, Hewlett-Packard) (30) under electron ionization conditions.

The enrichment, [13C]CO2, of expired air was measured using a GC-isotope ratio mass spectrometer, as previously described (1).

Measurement of muscle strength. One repetition maximum (1RM) represents the maximum amount of weight that a subject can lift once (28). After a warm-up and instruction regarding proper techniques for completing the movement, subjects were asked to lift progressively heavier weights until reaching a weight they could not lift. The selection of weights and the rate of increase were chosen to insure that the maximum capacity of the subject was reached in four to six lifts. 1RM tests were performed in this fashion for bench press and leg extension.

Isokinetic and isodynamic strength was tested using a Ledo isokinetic dynamometer (28). Isodynamic strength was measured at constant speeds of 60 and 240°/s for flexion and extension. At each movement speed, the subjects were asked to complete five repetitions as forcefully as possible through 90° of motion. Peak strength was the highest torque monitored during the five repetitions.

Measurement of hormones. Plasma glucose concentrations were measured enzymatically with an auto analyzer (Beckman Instruments, Fullerton, CA). Hormone levels were measured using established assays. Insulin was measured by chemiluminescent sandwich assay (Sanofi Diagnostics, Chaska, MN) (15). Growth hormone was measured using a locally developed two-site chemiluminescent sandwich assay. Values for insulin-like growth factor I (IGF-I), both free and total, were measured using commercially available kits (Diagnostic Systems Laboratories, Webster, TX) (6). These analyses include a simple extraction step in which IGF-I is separated from its binding proteins in serum (19). Total testosterone was measured using a commercial radioimmunoassay kit (Incstar, Stillwater, MN). Free testosterone level was determined with the use of a kit from Diagnostic Systems Laboratories. The procedure follows the basic principles of radioimmunoassay using a 125I-labeled testosterone analog, which competes with the unbound testosterone in the sample for binding to specific antitestosterone polyclonal antibodies.

Calculations

Fractional synthesis rate. Fractional synthesis rate (FSR) of muscle protein for myosin heavy-chain, mixed muscle, and sarcoplasmic proteins was calculated using (22) the equation

\[
FSR = \left( \frac{(E_f - E_i)/(E_p \times t)}{100} \right)
\]

where E_f and E_i represent the enrichment as atom percent excess of 13C derived from decarboxylation of leucine from myosin heavy-chain and mixed muscle protein at the final and initial muscle biopsies. E_p is the precursor pool (plasma KIC or tissue fluid leucine) enrichment, and t represents the time interval (5 h) between initial and final biopsies in hours.

Leucine kinetics. Whole body leucine flux, which represents rate of protein breakdown, is calculated from the isotope dilution of the infused tracer during a steady state (18, 22, 30). For flux calculation plasma [13C]KIC enrichment was used, since this is the best available plasma surrogate measure of intracellular leucine enrichment (18, 22). Leucine oxidation was calculated from expired air 13CO2 and CO2 production at steady state using plasma KIC as the precursor pool (23). The nonoxidative portion of leucine flux representing protein synthesis rate was calculated by deducting leucine oxidation from leucine flux.

For the estimation of whole body muscle protein synthesis, we assumed the leucine content of protein in human muscle to be 7.8% (22). The whole body muscle protein synthesis rate was calculated from FSRs and muscle mass (22). For this we assumed that protein constitutes 20% of muscle mass (12, 22). Skeletal muscle mass was estimated from daily urinary creatinine (average of 3 days) (12, 13). Insulin sensitivity was estimated by minimum model technique (27).

Statistics

All data are presented as means ± SE. Spearman correlation was performed to determine statistically significant correlation between FSR and different measurements. In addition, we performed step-wise regression analysis to determine the independent effect of hormones and aging on FSR. Single-factor analysis of variance (ANOVA) and unpaired t-tests were performed to compute significant interactions between groups. P < 0.05 was considered as an indicator of significant difference.

RESULTS

Table 1 lists the characteristics of the subjects. There was no significant difference in the mean age of men and women in the three age groups. Female subjects had lower height and weight than male subjects (P < 0.01; data not shown). The body fat mass was higher in the middle and old age groups than in the young age group (P < 0.01). The body mass index values of subjects were not different between different age groups.
Table 2. 13C enrichment values of plasma KIC and leucine and [13C]leucine in tissue fluid, mixed muscle protein, myosin heavy chain, and sarcoplasmic protein

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma leucine</th>
<th>Plasma KIC</th>
<th>TF leucine</th>
<th>MHC</th>
<th>Sarcoplasmic</th>
<th>Mixed muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>7.4 ± 0.3</td>
<td>5.8 ± 0.2</td>
<td>4.5 ± 0.3</td>
<td>0.0101 ± 0.0004</td>
<td>0.0120 ± 0.0015</td>
<td>0.0129 ± 0.0017</td>
</tr>
<tr>
<td>Middle</td>
<td>9.6 ± 0.8</td>
<td>7.5 ± 0.4</td>
<td>4.7 ± 0.3</td>
<td>0.0088 ± 0.0013</td>
<td>0.0129 ± 0.0017</td>
<td>0.0120 ± 0.0019</td>
</tr>
<tr>
<td>Old</td>
<td>9.7 ± 0.4</td>
<td>7.6 ± 0.3</td>
<td>5.8 ± 0.5</td>
<td>0.0080 ± 0.0006</td>
<td>0.0184 ± 0.0015</td>
<td>0.0135 ± 0.0010</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects/group. MHC, myosin heavy chain. *Plasma [13C]leucine and [13C]ketosioacrylic acid (KIC) values represent means of 6 values (5–10 h) during plateau. Tissue fluid (TF) leucine enrichment represents mean of 5 and 10 h. †Enrichment of muscle proteins represents increment in enrichment from 5 to 10 h.

Muscle mass, calculated based on creatinine, showed a significant decrease from young to old age (P < 0.01).

Isotopic enrichment data used for calculations are given in Table 2. The whole body leucine kinetics data are given in Table 3. ANOVA demonstrated an age effect on leucine flux (P < 0.01), leucine oxidation (P < 0.05), and nonoxidative leucine flux (P < 0.01). Leucine flux was lower in middle- and old-age subjects than in young subjects irrespective of whether normalized for body weight (P < 0.01) or fat-free mass (P < 0.05). There was no change in leucine flux from middle to old age. Leucine oxidation was also lower in the middle-aged and old subjects when normalized for body weight (P < 0.05 and P < 0.01, respectively), but the decrease reached statistical significance (P < 0.05) only in the old subjects when normalized for fat-free mass. Nonoxidative leucine disposal, a putative measure of whole body protein synthesis, was lower in the middle- and old-age subjects than the young subjects when normalized for both body weight and fat-free mass (P < 0.05).

Calciquestrin (a 63-kDa protein), which is the most prominent and specific sarcoplasmic reticulum Ca2+-binding protein in skeletal muscle (24), was identified by Western blot analysis in the sarcoplasmic fraction obtained from human, pig, and mouse skeletal muscle samples (results not shown). In Fig. 1, myosin heavy-chain band (~200 kDa) is absent for the sarcoplasmic fraction obtained from muscle samples. This rules out any contamination from myofibrillar proteins in the sarcoplasmic fraction. We performed this analysis in five separate human samples, which shows the reproducibility of the purification of sarcoplasmic protein fraction. Also, cytochrome-c oxidase, a marker for mitochondrial protein, was not detectable again, implying that there was no contamination of sarcoplasmic fraction.

The FSRs of myosin heavy-chain and sarcoplasmic protein in the three age groups are shown in Fig. 2. FSR of myosin heavy chain, calculated using KIC as the precursor pool, on ANOVA demonstrated a significant age effect (P < 0.01). Young subjects had a higher myosin heavy-chain synthesis rate (0.0361 ± 0.0009) than the middle-aged (0.0247 ± 0.0034; P < 0.01) and old (0.0215 ± 0.0018; P < 0.01) subjects (Fig. 2A). It decreased by 31% from young to middle age and a further 13% decline from middle to old age. Myosin heavy-chain synthesis rate calculated using tissue fluid leucine as the precursor pool also decreased with age (ANOVA, P = 0.01), decreasing from young to old age (young, 0.0469 ± 0.0034; middle, 0.0385 ± 0.0038; and old, 0.0292 ± 0.0033). In the young and old age groups tissue fluid enrichments from only seven subjects were available. The FSR of sarcoplasmic protein component (Fig. 2A) did not change significantly, but the mean values tended to increase with age, whether calculated using KIC or tissue fluid as precursor pool (young, 0.0426 ± 0.0060; middle, 0.0370 ± 0.0068; and old, 0.0494 ± 0.0048 using KIC enrichment and young, 0.0539 ± 0.0084; middle, 0.0575 ± 0.0081; and old, 0.0632 ± 0.0094 using tissue fluid leucine enrichment). FSRs of mixed muscle also showed a decline with age on ANOVA (P < 0.01), when calculated using KIC as the precursor for protein synthesis. The decrease from young (0.0471 ± 0.0028) to middle age (0.0332 ± 0.0041; P = 0.02) was 29%, but there was no significant change from middle age to old age (0.0359 ± 0.0022). When calculations were performed using tissue fluid leucine enrichments, the respective values were young, 0.0596 ± 0.0045; middle, 0.0521 ± 0.0051; and old, 0.0502 ± 0.0066, which also showed the tendency toward, but did not reach, statistical significance (P = 0.1). In all subjects the myosin heavy-chain synthesis rate was lower than that of mixed muscle protein. The ratio of FRS of myosin heavy chain to that of mixed muscle protein (Fig. 2A) decreased from 0.78 ± 0.05 to 0.61 ± 0.05 from young to old age (P < 0.05), whereas the ratio of FSR of sarcoplasmic protein to total mixed muscle protein increased (P < 0.05) from young to old.

Table 3. Whole body leucine kinetics

<table>
<thead>
<tr>
<th>Group</th>
<th>Leucine Oxidation</th>
<th>Leucine Flux</th>
<th>Nonoxidative Leucine Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol·kg body wt−1·h−1</td>
<td>µmol·kg FFM−1·h−1</td>
<td>µmol·kg body wt−1·h−1</td>
</tr>
<tr>
<td>Young</td>
<td>27.6 ± 1.7</td>
<td>38.0 ± 2.0</td>
<td>108.4 ± 4.1</td>
</tr>
<tr>
<td>Middle</td>
<td>21.3 ± 1.4*</td>
<td>33.8 ± 1.6</td>
<td>83.3 ± 3.8†</td>
</tr>
<tr>
<td>Old</td>
<td>20.3 ± 1.0†</td>
<td>31.7 ± 1.6*</td>
<td>80.6 ± 3.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects/group. FFM, fat-free mass. Significant difference from young group: *P < 0.05 and †P < 0.01.
age subjects (Fig. 2B). The FSR of myosin heavy-chain, mixed muscle, and sarcoplasmic proteins did not show any significant gender effect in any age groups.

The whole body protein and whole body mixed muscle protein synthesis rates were lower in middle-aged (P < 0.05) and old subjects (P < 0.01) than in the young subjects whether the values are corrected for fat-free mass or body weight. The values shown in Fig. 3 are normalized for fat-free mass. This age-related decrease in whole body protein synthesis was obvious when expressed in absolute terms also (Table 4).

The effects of age on various parameters of muscle strength are summarized in Table 5. On ANOVA there was an age effect on all parameters of muscle strength (P < 0.05). All strength measurements were significantly lower in the old subjects than in young subjects. Muscle strength was correlated to muscle mass (r = 0.77; P < 0.01). When muscle strengths (e.g., quadri-

**Fig. 1.** SDS-polyacrylamide gel analysis of sarcoplasmic fractions separated from human muscle biopsy samples. Aliquots of these fractions (~4 µg) were analyzed on a 4–20% SDS-polyacrylamide mini gradient gel, and proteins were detected by silver staining. Lanes 1 and 8 represent molecular weight standards (std; broad range 14.4–200 kDa) from Bio-Rad. Lane 2 represents myofibrillar pellet. Lanes 3-7 represent sarcoplasmic protein fractions (~4 µg) from 5 separate human muscle biopsy samples. These lanes (3-7) show no band in 200-kDa region that represents myosin heavy chain (MHC).

**Fig. 2.** Fractional synthesis rate (FSR) of (A) MHC and (B) sarcoplasmic proteins in young, middle-aged, and old subjects calculated using ketoisocaproate (KIC) and tissue fluid (TF) as precursor for protein synthesis. MHC showed a progressive decline (P < 0.01), whereas sarcoplasmic protein did not change with age. Ratio of MHC and sarcoplasmic protein synthesis rates to mixed muscle protein (MMP) synthesis rate in young, middle-aged, and old subjects are also shown. These data indicate that contribution synthesis rate of MHC to mixed muscle protein decreased with age (P < 0.05), whereas contribution of synthesis rate of sarcoplasmic protein increased (P < 0.05).*Significant (P < 0.05) change from young age group; **significant (P < 0.01) change from young age group; †significant (P < 0.01) change from middle age group; §denotes change (P = 0.07) from young age group.
ceps isokinetic and leg extension) were normalized for leg fat-free mass (muscle), there was a decline in muscle strength (both for isokinetic and 1RM) per kilogram leg muscle mass (Fig. 4A) with age (P < 0.01), and this ratio correlated with the fractional myosin heavy-chain synthesis rate (Fig. 4B). Table 6 shows the correlation of parameters of muscle strength to FSRs of myosin heavy-chain and mixed muscle protein. Parameters of muscle strength [leg curl, representing the whole leg, quadriceps isokinetic strength (240°/s), representing the muscle from where biopsies were taken, and the combination of leg extension and bench press, representing whole body muscle strength] were correlated (P < 0.05) with FSR of myosin heavy chain. These correlations were stronger in women than in men (Table 5). No significant correlation between the parameters of muscle strength and FSR of mixed muscle protein or sarcoplasmic protein was observed (data for sarcoplasmic protein not shown).

Table 7 shows hormone levels. There was a significant age-related fall of free testosterone, IGF-I (total and free), dehydroepiandrosterone sulfate (DHEAS), and cortisol levels (P < 0.05). Total IGF-I, DHEAS, and cortisol were lower in the middle-aged and old subjects than in the young subjects (P < 0.01). Testosterone (free) and IGF-I (free) levels were significantly lower in the old age group than in young subjects, P < 0.05 for testosterone and P < 0.01 for IGF-I. Although growth hormone, insulin, and insulin sensitivity tended to decrease with aging, they were not statistically significant. Figure 5 shows correlations of FSR of myosin heavy chain with the age-related changes in the levels of IGF-I, testosterone, and DHEAS, respectively. Although the FSR of myosin heavy chain showed correlation with plasma levels of testosterone (n = 12; males only, r = 0.74; P = 0.006 for free testosterone levels and r = 0.67; P = 0.033 for total testosterone levels) and IGF-I (r = 0.66; P = 0.0008 for total IGF-I and r = 0.4; P = 0.06 for free IGF-I levels; n = 24), there was no such relationship between FSR of mixed muscle protein and sarcoplasmic protein. DHEA also showed a correlation (r = 0.45; P = 0.03) with the FSR of myosin heavy chain but not with mixed muscle protein (Fig. 5).

FSR of myosin heavy chain, IGF-I (free and total), DHEAS, testosterone (free), and parameters of muscle strength showed a decline with age and, as described above, showed significant correlations to certain hormones, parameters of muscle strength, and muscle mass. However, in multiple regression analysis, after control for age, myosin heavy-chain synthesis rate showed no correlation with the above factors.

DISCUSSION

The current study demonstrated an age-related decline in the synthesis rate of skeletal muscle myosin heavy chain, whereas no such change in the synthesis rate of sarcoplasmic protein was observed with age. Whole body and mixed muscle protein synthesis rates also declined with age. But these measurements representing the average synthesis rates of various proteins in whole body and muscle, respectively, did not reflect the changes or lack of changes in synthesis rates of individual proteins. It was also demonstrated that the age-related decline in the synthesis rates of muscle proteins occur by middle age. Age also has an effect on muscle strength independent of muscle mass. The synthesis rate of myosin heavy chain was correlated not only to muscle mass but also to muscle strength per unit muscle mass. No such correlations were observed for synthesis rates of mixed muscle protein and sarcoplasmic protein with that of muscle strength. Synthesis rate of myosin heavy chain was correlated to circulating levels of IGF-I, DHEAS, and testosterone, all of which are reported to have anabolic effect on muscle.

Table 4. Absolute values of WBPS, WBMPs, and WBNMPS along with WBNMPS values normalized for nonmuscle mass

<table>
<thead>
<tr>
<th>Group</th>
<th>WBPS, mg/h</th>
<th>WBMPs, mg/h</th>
<th>WBNMPS, mg/h</th>
<th>WBNMPS/Nonmuscle Mass, mg/kg nonmuscle mass</th>
<th>1·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>8,436 ± 588</td>
<td>2,809 ± 280</td>
<td>5,627 ± 486</td>
<td>321 ± 24</td>
<td>321 ± 24</td>
</tr>
<tr>
<td>Middle</td>
<td>8,149 ± 721</td>
<td>1,744 ± 224*</td>
<td>6,405 ± 676</td>
<td>323 ± 12</td>
<td>323 ± 12</td>
</tr>
<tr>
<td>Old</td>
<td>6,799 ± 493*</td>
<td>1,260 ± 123†</td>
<td>5,539 ± 539</td>
<td>299 ± 18</td>
<td>299 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SE. WBPS, whole body protein synthesis; WBMPs, whole body muscle protein synthesis; WBNMPS, whole body nonmuscle protein synthesis (WBPS – WBMPs). *Significant decrease (P < 0.05) from young group; † significant decrease (P < 0.01) from young group.
The decline in the synthesis rate of myosin heavy chain indicates a compromise in the ability of skeletal muscle to remodel and, perhaps, to maintain the quality and quantity of this important contractile protein, which hydrolyzes ATP to release energy for contractile functions (14). The decrease in synthesis of myosin heavy chain was correlated not only to muscle mass but also to muscle strength per unit muscle mass (Fig. 4).

Table 5. Effect of age on muscle strength

<table>
<thead>
<tr>
<th>Group</th>
<th>Leg Extension 1RM, kg</th>
<th>Leg Curl 1RM, kg</th>
<th>Bench Press, kg</th>
<th>Isometric Quad, Nm</th>
<th>Isometric Ham, Nm</th>
<th>Quadriceps 60°/s, Nm</th>
<th>Hamstring 60°/s, Nm</th>
<th>Quadriceps 240°/s, Nm</th>
<th>Hamstring 240°/s, Nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>103.6 ± 14.4</td>
<td>46.7 ± 6.4</td>
<td>104.5 ± 13.1</td>
<td>117.8 ± 12.0</td>
<td>85.1 ± 9.6</td>
<td>139.1 ± 12.0</td>
<td>70.4 ± 7.9</td>
<td>79.9 ± 7.3</td>
<td>49.3 ± 4.5</td>
</tr>
<tr>
<td>Middle</td>
<td>93.1 ± 16.7</td>
<td>34.6 ± 6.6</td>
<td>79.0 ± 14.0</td>
<td>118.8 ± 15.6</td>
<td>75.3 ± 10.1</td>
<td>100.8 ± 15.1</td>
<td>60.4 ± 8.9</td>
<td>59.8 ± 7.9</td>
<td>42.6 ± 5.2</td>
</tr>
<tr>
<td>Old</td>
<td>53.1 ± 5.3†</td>
<td>15.7 ± 3.7†‡</td>
<td>45.3 ± 4.4†‡</td>
<td>79.1 ± 9.5†‡</td>
<td>51.6 ± 5.7*</td>
<td>65.4 ± 8.7†</td>
<td>35.8 ± 3.9†‡</td>
<td>39.5 ± 4.8†‡</td>
<td>27.4 ± 1.3†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects/group. 1RM, one repetition maximum strength; Quad, quadriceps; Ham, hamstring. *Significant difference from young age group (P < 0.05); †significant difference from young age group (P < 0.01); ‡significant difference from middle age group (P < 0.05).

The decline in the synthesis rate of myosin heavy chain indicates a compromise in the ability of skeletal muscle to remodel and, perhaps, to maintain the quality and quantity of this important contractile protein, which hydrolyzes ATP to release energy for contractile functions (14). The decrease in synthesis of myosin heavy chain was correlated not only to muscle mass but also to muscle strength per unit muscle mass (Fig. 4).
The decline in synthesis rates of other proteins may also contribute to the declining muscle mass. Muscle strength also is likely to be influenced by multiple other factors, including vascular and neuronal factors. Of interest, the decline in the synthesis rate of mixed muscle protein was also fully evident by middle age. Previous studies were confined to the measurements of synthesis rates of mixed muscle proteins (total muscle protein and total myofibrillar protein) and the comparison between the young and the elderly (33, 34, 36, 37). These studies demonstrated that the average synthesis rate of the mixture of various muscle proteins was lower in the elderly than in the young. The current study demonstrated that the decline in synthesis rates of muscle proteins occurs by a much younger age (average 52 yr) than previously reported. This observation implies that interventions such as exercise programs or hormonal replacement may have to be initiated well before "old" age to prevent a decline in muscle mass and function that occurs with aging.

In addition to the decline in muscle protein synthesis, we found other parameters of whole body protein turnover that changed with aging. Whole body leucine flux (an index of protein breakdown), leucine oxidation, and nonoxidative leucine flux (an index of whole body protein synthesis), after normalization for fat-free mass, were also found to decrease with aging (Table 3). These studies demonstrated that the average synthesis rate of the mixture of various muscle proteins was lower in the elderly than in the young. The current study demonstrated that the decline in synthesis rates of muscle proteins occurs by a much younger age (average 52 yr) than previously reported. This observation implies that interventions such as exercise programs or hormonal replacement may have to be initiated well before "old" age to prevent a decline in muscle mass and function that occurs with aging.

Previous studies also have examined the effect of aging on whole body protein synthesis. These studies revealed conflicting results (20, 33, 35, 36, 38). Cross-sectional comparisons are fraught with the problems of normalizing the data to various body composition parameters (20, 33, 35, 36, 38). In the current study, we normalized the whole body protein turnover values to fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique. The age-related decline occurred whether the data were normalized for fat-free mass or when the data were normalized for fat-free mass measured by dual photon X-ray technique.
chain (6). In another study, testosterone has been shown to stimulate synthesis rate of mixed muscle protein in elderly men (32). IGF-I also stimulates muscle protein synthesis (11). No direct correlation of growth hormone levels and protein synthesis was observed. A growth hormone effect may not be evident because of the pulsatile nature of growth hormone secretion, and we measured growth hormone levels from single plasma samples. Although insulin is an anabolic hormone, its main effect on protein metabolism in humans seems to be by inhibition of protein breakdown (21). Our study showed no evidence of increased protein breakdown, indicating that insulin resistance probably played no role. Of note, almost all studies of insulin effect were done to demonstrate acute effect of insulin deprivation or insulin administration. It is possible that insulin resistance may play a role by its chronic effect on protein synthetic machinery. All these changes of hormones, muscle strength, and muscle protein synthesis rate occur with aging, and, on the basis of our data, it is not possible to delineate the effect of aging per se from that of these secondary events. It remains to be determined whether the aging-related decline in the synthesis rate of myosin heavy-chain and other proteins could be partially or completely reversed by replacement of hormones such as IGF-I, DHEA, and testosterone. Only such intervention studies will allow us to determine the relative roles of the decline in hormones and aging in the pathogenesis of sarcopenia.

Interestingly, the correlation between muscle strength measurements and myosin heavy-chain synthesis rate was stronger in females than in males, whereas hormonal levels correlated with myosin heavy-chain synthesis rate more strongly in males. These findings suggest that the mechanism of protein synthesis in sarcopenia could well be regulated by different parameters in male and female. This observation needs further investigation. Additional factors such as exercise status may confound this gender-related observation.

The results of this study provide support to the hypothesis that a decrease in myosin heavy-chain synthesis that occurs with physiological aging plays a key role in the pathogenesis of sarcopenia. There may be several underlying mechanisms for the declining synthesis rates of muscle proteins. Phenomena such as...
downregulation of transcriptional factors, due to a reduction in all types of RNAs with advancing age as reported by Shikama and Brack (31), and abnormal protein modification (25) may be involved in the age-related decline in protein synthesis. Protein synthesis being an energetically expensive biochemical process, a decrease in the availability of energy in the form of ATP (8) may be a potential explanation for the observed decline in the synthesis rates of mixed muscle protein and myosin heavy chain with advancing age. This decrease may in turn be due to a reduction in the capacity of muscle to generate ATP, as hypothesized by Fleming et al. (8). Recently, it has been shown that there is a decline in mitochondrial protein content and its synthesis rate occurring with advancing age (26). Mitochondria is the ATP-producing machinery, and decline in synthesis rate of mitochondrial protein suggests a decline in the ability to maintain mitochondrial proteins, such as cytochrome-c oxidase, of sufficient quantity and probably of sufficient quality to maintain the necessary ATP production. A crucial step to make chemical energy (in the form of ATP) available for contractile functions of skeletal muscle is the hydrolysis of ATP to ADP. Myosin and actin are responsible for this ATPase function. The decline in synthesis rate of myosin heavy chain could therefore affect the availability of energy readily available for mechanical functions.

In summary, a selective decline of the synthesis rate of certain muscle proteins such as myosin heavy chain occurs with aging. A decline in synthesis rate of myosin heavy chain observed in this study and a similar decline in the synthesis rate of mitochondrial protein as previously reported will provide a theoretical basis for the sarcopenia of aging. Declining muscle strength may result from a reduced synthesis rate of myosin heavy chain. This decline in myosin-heavy chain synthesis coupled with the decrease of mitochondrial protein synthesis may explain increased muscle fatigability, reduced endurance capacity, and muscle weakness in the elderly.

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REFERENCES


