Distribution of Protein Turnover Changes with Age in Humans as Assessed by Whole-Body Magnetic Resonance Image Analysis to Quantify Tissue Volumes

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ABSTRACT We tested the hypothesis that nonmuscle lean tissue mass and its rate of protein catabolism remain constant with aging despite changes in the proportional contribution of these tissues to whole-body protein metabolism. Whole-body protein kinetics, using the 60-h oral [15 N]glycine method, and muscle and nonmuscle protein catabolism, based on protein kinetic data, urinary N\(^{-}\)methylhistidine excretion and lean tissue volumes defined by whole-body magnetic resonance imaging, from eight healthy elderly subjects (5 females and 3 males, mean age 71.5 y) were compared with those of seven young persons (3 females and 4 males, mean age 28 y). There were no significant age or gender effects on rates of protein kinetics per L total lean tissue. There was a lower \(P < 0.004\) rate of muscle protein catabolism in the elderly (1.8 ± 0.2 vs. 2.6 ± 0.1 g \(\cdot\) L\(^{-1}\) \(\cdot\) d\(^{-1}\)) and a trend \(P = 0.08\) for lower muscle volume (19.7 ± 1.5 vs. 25.0 ± 2.4 L). This contrasted with intraabdominal lean tissue, where the rate of protein catabolism (13.8 ± 0.6 vs. 13.2 ± 0.9 g \(\cdot\) L\(^{-1}\) \(\cdot\) d\(^{-1}\)) and volume (7.5 ± 0.3 vs 8.0 ± 0.5 L) did not differ between age groups. Thus, the decrease in the contribution by muscle to whole-body protein metabolism with age is associated with an increase from 62 to 74% \((P < 0.001)\) in the contribution by nonmuscle lean tissues. These findings have potential implications for the nutrition of both normal and sick elderly persons. J. Nutr. 130: 784–791, 2000.

KEY WORDS: • protein turnover • aging • \(N^{-}\)methylhistidine • magnetic resonance imaging • elderly humans

Aging is associated with many changes that may affect body protein metabolism. Total energy expenditure declines due to a reduction in resting metabolic rate (RMR) and an even greater reduction in physical activity (McCandl et al. 1966, Roberts et al. 1995), resulting in decreased total energy requirement to maintain equilibrium. This fall in RMR is attributable to a loss of lean tissue with aging since there is little or no change when RMR is expressed relative to lean body mass (Visser et al. 1995). Body composition studies suggest that the decrease in lean tissues is primarily related to loss of muscle mass with age (Cohn et al. 1980, Forbes and Reina 1970). The skeletal muscle mass plays an active role in whole-body amino acid metabolism and is especially important during restricted energy and protein intakes (Young 1990). Although rates of whole-body protein turnover per kg of body weight (BW) have been reported to decrease with age (Golden and Waterlow 1977, Young et al. 1975), most studies (Fukagawa et al. 1989, Roberts et al. 1984, Uauy et al. 1978), including our own (Morais et al. 1997) found no differences when rates of protein kinetics were expressed per kg lean tissue. The finding that whole-body protein kinetics are unchanged per unit of lean body mass with aging does not preclude the possibility of changes in the turnover within individual lean tissues and thereby their relative contributions to whole-body turnover. Indeed, we and others have shown that the contribution of muscle protein breakdown to whole-body protein catabolism is decreased, with a proportional increase in the visceral organ protein catabolism (Morais et al. 1997, Uauy et al. 1978). Furthermore, muscle fractional protein synthesis has also been found to be decreased with age (Welle et al. 1993, Yarasheski et al. 1993). On the other hand, the effect of aging on visceral protein turnover has not been studied directly in humans because it requires invasive techniques that are difficult to perform in older individuals. We

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Abbreviations used: AT, adipose tissue; B-mB, nonmuscle protein breakdown; BMI, body mass index; BW, body weight; CIU, Clinical Investigation Unit; LT, lean muscle; 3MH, \(N^{-}\)methylhistidine; MRI, magnetic resonance imaging; mB, muscle protein breakdown; RMR, resting metabolic rate.
therefore tested the hypothesis that nonmuscle lean tissue mass and its rate of protein catabolism remain constant with aging in the presence of unchanged whole-body protein turnover per unit of lean tissue and therefore, a reduced contribution and turnover of muscle protein mass. The aim of this study was thus to determine the effects of aging on rates of protein turnover of the whole-body and of muscle and nonmuscle lean tissues in a noninvasive fashion. To achieve this, we concurrently applied the stable isotope technique using the oral 60-h [15N]glycine method to study whole-body protein metabolism, measurements of urinary N-methylhistidine excretion to estimate total body myofibrillar protein catabolism, and state-of-the-art whole-body magnetic resonance imaging (MRI) analysis to define muscle, nonmuscle lean tissue and adipose tissue volumes. We compared results in healthy elderly to those of young adult persons.

MATERIALS AND METHODS

Subjects. Eight healthy, moderately active elderly persons (5 women and 3 men), classified as demonstrating characteristics of "usual aging" (Rowe and Khan 1987) and seven lean young persons (3 women and 4 men) were recruited through advertisements in local newspapers. They were screened by history, physical examination and cognitive status using the Mini Mental State Examination (Folstein et al. 1975) (for older persons only), a laboratory investigation which included fasting blood sampling for complete blood count, serum electrolytes, plasma glucose, renal and liver function tests, total proteins and albumin, lipid profile, thyroid hormones, a urine analysis, a chest X-ray and an electrocardiogram (except for the young persons). Two elderly persons were on thyroid hormone replacement therapy, and two others were on treatment for mild arterial hypertension with drugs not known to interfere with the metabolic measurements. Subjects were admitted to the Royal Victoria Hospital Clinical Investigation Unit (CIU) and gave written informed consent. The elderly group was studied first. Following the in-patient stay, each group underwent total body MRI. The protocol was approved by the Human Ethics Review Committee of the Hospital. Subject characteristics are shown in Table 1.

Tissue measurement by MRI. MR images were obtained with a GE Signa Advantage, 1.5 Tesla scanner using software version 5.4.2, (Madison, WI). A T1-weighted, spin-echo pulse sequence with a 210 ms repetition time, 17 ms echo time and a rectangular field of view (48 cm x 36 cm) was used to acquire the MRI data. The MR images obtained in the abdomen and thorax regions were acquired using a 1/2 Fourier transformation-pulse sequence (1/2 NEX), meaning that a set of 7 images could be obtained in 26 s. During this 26 s the subjects were asked to take a normal inspiration and hold their breath. For the appendicular regions, the same pulse sequence was used with the exception that a single NEX was employed, resulting in a 43-s acquisition time for a set of 7 images. The total time required to obtain all MRI data (41 images) for each subject was ~25 min. During this time, the subjects lay in the magnet in a prone position. All image data were transferred onto an Indigo 2 computer (Silicon Graphics, Mountain View, CA) for analysis using specialized computer software (Tomovision, Montreal, QC).

Calculation of lean and adipose tissue area and volume. The segmentation method used to determine tissue areas is described in detail elsewhere (Ross et al. 1996). Briefly, the method is based on image morphology and employs a combination of edge detection filters and Watershed techniques. Initially a filter is used to distinguish between different gray level regions on the image. Once the edges are determined, lines are drawn on the image using a Watershed algorithm. If the regions (i.e., a group of voxels) are too small, they can be merged using statistical parameters inherent to the image. Once the regions representing the various tissues (i.e., skeletal muscle and adipose tissue) are identified, the observer uses a mouse pointer to identify each tissue using color codes. Each image is then reviewed using an interactive slice editor program which allows for verification and, where necessary, correction of the segmentation result (Ross et al. 1996). This operation is facilitated by superimposing the original gray level image on the binary segmented image using a transparency mode (Ross et al. 1996). To calculate tissue area (cm²), the respective tissue regions in each slice are computed automatically by summing the given tissues' pixels and multiplying by the pixel surface area. The tissue volume (cm³) for each slice is calculated by multiplying the tissue area (cm²) by slice thickness (10 mm). Adipose tissue (AT) and lean tissue (LT) volumes were calculated by adding the volumes of truncated pyramids defined by pairs of consecutive slices (Ross et al. 1996). Whole-body tissue volumes were calculated using all 41 slices. Intraabdominal LT volume was derived using four abdominal images extending from L4-L5 to three images above.

Validation and reliability of MRI. We have recently reported that the arm and leg AT-free skeletal muscle cross-sectional areas in cm² (n = 119) using MRI were not different from cadaveric estimates (38.9 ± 22.3 vs. 39.5 ± 23.0 cm²; P < 0.001). Similar good results were observed between MRI-measured and cadaver-measured interstitial and subcutaneous adipose tissue (Mitsiopoulos et al. 1998). Whole-body LT volume measurements on two obese men. For each subject, a complete data set was acquired (41 images) on two separate occasions during the same day. The mean difference between test 1 and 2 for MRI-LT (L) was < 2%. The MRI-LT calculations were thus determined by a single individual and thus represent the intraindividual error associated with repeated LT-volume calculations. In previous studies we have reported that for total AT volume (L), the mean difference between tests 1 and 2 was 2.6% with a range of 0.9 to 4.3% (Ross et al. 1992). The repeatability of whole-body LT volume measurements (in liters) was assessed from repeated measurements on two obese men. For each subject, a complete data set was acquired (41 images) on two separate occasions during the same day. The mean difference between test 1 and 2 for MRI-LT (L) was < 2%. The MRI-LT calculations were thus determined by a single individual and thus represent the intraindividual error associated with repeated LT-volume calculations.

Anthropometric measurements. Daily morning body weights of each subject were measured in the fasted state after voiding, and wearing the same light clothing, to the nearest 100 g on a Scale-Tronix digital scale (Ingram & Bell-Meditron, Le Groupe, Don Mills, ON). Body height without shoes was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kg/height in m², using the average daily weight from the 2 days onward.

Diets. The elderly subjects received an individualized diet based on a 6-d food diary record analyzed by the CBORD Diet Analyzer V 3.0.3 (the CBORD Group, Natural Software Limited, 1988, Ithaca, NY). On the basis of this assessment, the average protein intake was

![Table 1](https://example.com/table1.png)

**Table 1** Characteristics of subjects participating in the study to determine the changes in the distribution of protein turnover with age

<table>
<thead>
<tr>
<th></th>
<th>Elderly</th>
<th>Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8 (5F, 3M)</td>
<td>7 (3F, 4M)</td>
</tr>
<tr>
<td>Age, y</td>
<td>71.5 ± 1.0</td>
<td>72.9 ± 1.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.2 ± 3.6‡</td>
<td>63.5 ± 4.0‡</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.61 ± 0.03§</td>
<td>1.72 ± 0.04§</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8 ± 1.1*</td>
<td>21.4 ± 0.8</td>
</tr>
<tr>
<td>Urinary creatinine, mmol/d</td>
<td>8.5 ± 0.9†‡</td>
<td>12.6 ± 1.01</td>
</tr>
<tr>
<td>Urinary 3MH, μmol/d</td>
<td>154.5 ± 23.7†§</td>
<td>281.8 ± 36.0§</td>
</tr>
<tr>
<td>3MH/Creatinine, μmol/mmol</td>
<td>18.8 ± 2.5</td>
<td>22.1 ± 1.3</td>
</tr>
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</table>

1 Values are means ± SEM. Data were analyzed by two-factor ANOVA with main effects to be interpreted as the overall effect of age adjusted for gender and the overall effect of gender adjusted for age. Overall age effect: *P < 0.04, †P < 0.01. Overall gender effect for lower values in females: ‡P < 0.03, §P < 0.002. Abbreviations used: BMI, body mass index; 3MH, N-methyl histidine.
1.20 g/(kg BW \times d) and the average energy intake was 130 kJ/(kg BW \times d) (31 kcal/(kg BW \times d), which represented 148 to 182% of the RMR, as measured by indirect calorimetry. Each young subject received 27 g/(kg BW \times d) of protein with energy intake based on measured RMR multiplied by an activity factor of 1.7 (Table 3). The diet was divided into six small meals given every 3 h from 0800 to 2300. For breakfast, subjects received 30 g of whole-bran cereal (All Bran Cereal; Kellogg Canada, Etobicoke, ON) with 200 mL milk (2% fat). The rest of protein and energy intake was provided as a meal replacement product (Boost®, Mead Johnson, Montreal, QC) with additional energy sources as a combination of two-thirds glucose polymer (Polycolyte, Ross Laboratories, Montreal, QC) and one-third corn oil. The energy distribution was 15% as protein, 65% as carbohydrate and 20% as fat for the elderly and 13, 62 and 25%, respectively, for the young people. The elderly group received the diet for 9 d and the young group for 7 d, all at the CIU except for the first 2 d of the protocol for young subjects, who consumed it at home.

**Resting metabolic rate.** Following a training session and before admission, RMR was measured in the elderly and young subjects by continuous indirect calorimetry with a Deltatrac ventilated hood metabolic monitor (Sensor Medics, Yorba Linda, CA). All subjects were fasting for at least 10 h, arrived by car or by public transportation and were allowed to rest in a supine position in a thermally neutral and quiet room for at least 30 min before oxygen consumption and carbon dioxide production measurements were begun. Subjects breathed under the plastic canopy for 20 min, and the average of the last 15 min was used for calculation of the 24-h RMR based on the de Weir equation (de Weir 1949).

**Urinary nitrogen measurements.** Daily 24-h urine collections were made. Total urinary nitrogen (N) was analyzed by chemiluminescence (Anteck Pyro-Chemiluminescent Nitrogen System, Houston, TX) according to methods described by Ward et al. 1980. Standards were prepared from analytical grade urea (ICN Biomedicals, Aurora, OH) ranging from 1.0 to 10.0 g N/L. Standards and urine samples were diluted 1:100 or 1:200 with water and delivered in 5 mL aliquots in duplicate into the pyrolysis chamber by a quartz boat filled with quartz wool in a water-jacketed pyrolysis tube (Anteck Syringe Driver 735, Houston, TX).

**Protein turnover.** Protein kinetic studies were done using the 60-h oral [15N]glycine method during the last 3 d of the CIU stay. Details of the procedures are described by Gougeon et al. 1994. The method requires that within 60 h the enrichment of [15N] in urinary urea must reach a plateau. This determination, based on the mean plateau enrichment curves, is defined as the first plateau that extends for at least four points (12 h). Mean CV of plateau values was 4.2 and 3.5% for the elderly and young persons, respectively. The rate of entry of N (Q) into the metabolic N pool can be calculated from the mean plateau value, assuming that the fraction of the administered isotope (Q) into the metabolic N pool can be calculated from the mean enrichment curves, is defined as the first plateau that extends for at least 60 h. The rate of entry of N into the metabolic N pool is expressed as a percentage of whole-body protein breakdown and calculated in this manner from the whole-body breakdown from the stable isotope measurements (Moraes et al. 1997). Protein breakdown rates per unit of muscle and nonmuscle lean tissues were estimated by factoring the values for their individual volumes in L assessed by MRI. Urinary 3MH excretion was measured by reverse-phase HPLC (Hewlett-Packard 1090, Mississauga, ON) after derivatization with orthophthalaldehyde and mercaptopionic acid (Garrel et al. 1995). The sensitivity of the assay was 0.1 μmol/L. Interassay and intraassay variation was <5% (Garrel et al. 1995).

**Other analytical measurements.** Venous blood samples were drawn with minimal stasis in the overnight-fasted state on d 1 and 3 of the [15N]glycine study. The samples were drawn into serum separation tubes and into heparinized tubes containing one tenth of the volume of blood as aprotinin (Trasylol, 10,000 Kallikrein inhibitor kg/L, FBA, Pointe Claire, QC). The latter were cooled, centrifuged at 2000 000 g at 4°C for 15 min and stored in multiple aliquots at −20°C. Plasma was assayed for insulin by single-antibody charcoal precipitation radioimmunoassay with human standards and labeled hormone from Linco Research (St. Louis, MO) by methods described previously (Matliss et al. 1978). Immunoreactive glucagon was measured by double antibody method (Linco). Dole extracts of plasma by the radiochemical microtechnique (Fer et al. 1970). Plasma glucose was measured by the glucose oxidase method on a Beckman II glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma values were corrected for dilution by aprotinin, based on a concurrently measured hemocrit. Serum human growth hormone (hGH) was assayed using National Institutes of Health Standards (NIDDK-hGH-RP-1) and antibody (NIDDK-anti-hGH-2) and hGH obtained from NIH (product AFP-11019BNIDDK-NIDDK-hGH-I-3) that was labeled with 3H in the Royal Victoria Hospital’s polypeptide laboratory. Serum cortisol was measured at the Royal Victoria Hospital endocrinology laboratory by an automated immunoluminocenscence technique (CIBA Corning ACS 180, Cooperstown, NY). After thorough mixing, aliquots of the 24-h urine collections were analyzed daily for urea nitrogen, creatinine, and electrolytes (Na, K, Cl) and frozen at −20°C until assayed by the methods listed above. Daily urinary urea N and creatinine were obtained by the autoanalyzer method in the Clinical Biochemistry laboratory of the Hospital, the creatinine served as a means of assuring completeness of collection.

**Statistical methods.** Data are presented as means ± SEM. Outcomes were analyzed with two-factor ANOVA for age group and gender using the generalized linear model procedure. The main effects are to be interpreted as the overall effect of age adjusted for gender and the overall effect of gender adjusted for age. An interaction term for age group and gender was included in all models. Since no interaction term was found (P > 0.05), the models include only the main effects. Residual plots were used to examine model assumptions. Normality of the residuals was further tested using the Shapiro-Wilk statistic. When model assumptions were violated, analyses were redone after transforming the dependent variable; reported P-values are from the transformed model. Analyses were performed with the SAS (SAS Institute, Cary, NC) software programs. On the basis of an a priori standard deviation of 15% of the protein turnover results in our laboratory using the RIA (Linco method of Gougeon et al. 1994), and applying a power calculation formula for dietary studies (Hall 1983), we estimated that seven subjects per group would need to be studied for a 15% change in protein turnover to be detectable with a power of 90% (one-tailed α = 0.05, β = 0.10).

**RESULTS**

**Subject characteristics.** Groups were distinct with regard to age, as required by the design (Table 1). Elderly persons had higher (P < 0.04) BMI, and lower creatinine and 3MH excretions, compared with the young people. A gender effect independent of age was lower (P < 0.05) weight, height, creatinine and 3MH excretions was observed for females. The ratios of urine 3MH to creatinine were not significantly different between age groups.
MRI measurements. There were no significant differences between age groups for whole-body LT, muscle, or intraabdominal LT (Table 2). However, there was a trend for lower muscle volumes in elderly persons (19.7 vs. 25.0 L; \( P = 0.08 \)). There was an overall gender effect for females to have lower (\( P < 0.03 \)) volumes of LT. AT volumes as a whole, and at each site measured, were greater (\( P < 0.02 \)) in the elderly compared with young people. Percentage intraabdominal AT, and percentage abdominal subcutaneous AT (vs. total AT) and the ratio of intraabdominal/abdominal subcutaneous AT were also higher (\( P < 0.04 \)) in elderly compared with young people. This is in contrast with the percentage subcutaneous AT that was lower (\( P < 0.02 \)) in the aged group. As an overall gender effect, females also showed increased (\( P = 0.03 \)) volumes of intraabdominal AT. Their percentage subcutaneous and percentage intraabdominal AT (vs. total AT) were also higher than in males, as was their ratio of intraabdominal/abdominal subcutaneous AT (\( P < 0.004 \)).

Nutrient intakes. Elderly persons had lower (\( P < 0.03 \)) energy intake and RMR per day and per kg BW than young persons (Table 3). However, once these results were corrected for volumes of LT, no differences remained between groups. Protein intake was not significantly different between age groups irrespective of the denominator. As an overall gender effect, females also showed increased (\( P = 0.03 \)) volumes of intraabdominal AT. Their percentage subcutaneous and percentage intraabdominal AT (vs. total AT) were also higher than in males, as was their ratio of intraabdominal/abdominal subcutaneous AT (\( P < 0.004 \)).

Kinetics of protein metabolism. When rates were expressed in g N/(kg BW · d), the elderly group had a 16% lower mean Q that did not reach significance (\( P = 0.057 \)) and 20% lower (\( P < 0.04 \)) S and B (Fig. 1). However, when expressed per L of LT/d, differences were no longer significant (Fig. 2). No gender effect was observed for protein kinetics. Results of net endogenous protein balance (S-B; in mg N) showed no significant differences whether expressed per kg BW\(^{-1} \cdot d^{-1} \) or per L of LT/d.

**TABLE 2**

| Body composition assessed by magnetic resonance imaging of subjects participating in the study to determine the changes in the distribution of protein turnover with age \(^1\) |
|--------------------------|--------------------------|--------------------------|
|                          | Elderly                  | Young                    |
| **Lean tissue**          |                          |                          |
| Whole body, L            | 35.0 ± 2.2\( \ast \)     | 41.3 ± 3.3\( \ast \)     |
| Muscle, L                | 19.7 ± 1.5\( \ast \)     | 25.0 ± 2.4\( \ast \)     |
| Nonmuscle (whole body – muscle), L | 15.2 ± 0.7\( \ast \) | 16.3 ± 0.9\( \ast \)     |
| Intraabdominal, L        | 7.5 ± 0.3\( \ast \)      | 8.0 ± 0.5\( \ast \)      |
| **Adipose tissue**       |                          |                          |
| Whole body, L            | 25.7 ± 2.8\( \ast \)     | 14.5 ± 1.8               |
| Subcutaneous, L          | 21.4 ± 2.4\( \ast \)     | 13.0 ± 1.4               |
| Intraabdominal, L        | 2.6 ± 0.7\( \ast \)      | 0.7 ± 0.3\( \ast \)      |
| Abdominal subcutaneous, L| 5.2 ± 0.6\( \ast \)      | 2.0 ± 0.4                |
| Subcutaneous/whole body, %| 84.2 ± 3.7\( \ast \) | 91.2 ± 2.8\( \ast \)     |
| Intraabdominal/whole body, % | 9.6 ± 2.6\( \ast \) | 4.0 ± 1.4\( \ast \)      |
| Abdominal subcutaneous/whole body, % | 20.5 ± 1.5\( \ast \) | 13.7 ± 1.1               |
| Intraabdominal/abdominal subcutaneous | 0.53 ± 0.17\( \ast \) | 0.29 ± 0.11\( \ast \)    |

\(^1\) Values are means ± SEM. Data were analyzed by two-factor ANOVA with main effects to be interpreted as the overall effect of age adjusted for gender and the overall effect of gender adjusted for age. Overall age effect: \( P < 0.04 \), † \( P < 0.008 \). Overall gender effect with lower volumes of lean tissues and higher volumes of adipose tissue in females: \( \ast P < 0.03 \), § \( P < 0.004 \).
but did not change when results were expressed per unit lean body mass (Fugakawa et al. 1989, Morais et al. 1997, Robert et al. 1984, Uauy et al. 1978). Since these results represent the overall rate of individual lean tissues turning over at different rates, it could very well be that a decrease in one of them, e.g., muscle with slower protein turnover is concealed by another with a faster turnover, i.e., visceral tissue. As previously reported (Morais et al. 1997, Uauy et al. 1978), we found that the contribution of muscle to whole-body protein catabolism is significantly reduced in elderly persons (25.8 vs. 38.4%; \( P < 0.001 \)). This could be explained by a change in the amount of tissue participating, alterations in the intrinsic rates of turnover within tissues, i.e., their individual rates of protein turnover, or both. It is well known that muscle mass decreases with aging (Cohn et al. 1980, Forbes and Reina 1970). In our subjects, the muscle volume of elderly subjects was not significantly different from that of the young group, perhaps due to the number of subjects studied, as a trend for lower values was present. However, our findings indicate that there is a significant age-related decrease in protein breakdown within muscle of about 30%, reducing further the contribution of muscle to whole-body protein catabolism.

In support of our results of a lower rate of muscle protein breakdown per unit of tissue using 3MH and muscle volumes assessed by MRI are published data using \[^{13}C\]leucine incorporation into muscle in the postabsorptive state, showing that older subjects had lower fractional muscle protein synthesis than young subjects. These results suggest an age-related decline of muscle protein turnover (Welle et al. 1993, Yarasheski et al. 1993) in addition to the loss of total mass. Because muscle mass is relatively stable over long periods even in older persons, we can infer that the rates of skeletal myofibrillar protein breakdown would also reflect the decremental effect of aging. No reduction in rates of myofibrillar protein breakdown with aging was inferred by Uauy et al., using the ratio of 3MH to creatinine excretion (Uauy et al. 1978). Our 3MH/creatinine data also showed no significant differences, though both 3MH and creatinine excretion were substantially lower in the elderly subjects. The most plausible explanation for this lack of age-related change using 3MH to creatinine is that creatinine is not as good a surrogate for muscle mass as imaging.

The measurement of urinary 3MH is a reproducible and reliable method to quantify the turnover of skeletal myofibrillar protein (Young and Munro 1978). However, there are skin and endogenous gut sources contributing to the total urinary 3MH excretion (Afting et al. 1981, Harris 1981). Since aging affects primarily muscle mass (Cohn et al. 1980, Forbes and Reina 1970), elderly individuals are expected to have a higher contribution of nonskeletal muscle urinary 3MH to the total output than young subjects. Although there is uncertainty as
FIGURE 5 Percentage contribution by lean tissue to whole-body protein catabolism in humans. Data were analyzed by two-factor ANOVA with main effects to be interpreted as the overall effect of age adjusted for gender and the overall effect of gender adjusted for age. Overall effect of age was significant: \( P < 0.001 \). Overall gender effect for lower contribution by muscle and higher contribution by nonmuscle lean tissue in females was significant: \( \ddagger P < 0.002 \).

to the precise amount of 3MH produced by the skeletal muscle, these considerations serve to further strengthen the argument for our finding of lower skeletal muscle protein turnover with aging.

The finding of lower relative rates of myofibrillar protein catabolism in women than men is of interest. This gender effect was observed independent of age. In all but one study (Welle et al. 1995) comparing old with young individuals, the statistical analyses performed were not appropriate to demonstrate gender differences. Welle and coauthors did not report gender differences, but they may not have been sought in the analyses. In any case, this question needs to be confirmed by more direct measurements of muscle protein metabolism.

A novel finding of the present study is that the rate of nonmuscle protein breakdown tissue mass appears to remain unchanged with aging regardless of the denominator used. Our data indicate that volumes of nonmuscle LT and of intraabdominal LT were not affected by age, which is in accordance with other studies of body composition suggesting maintenance of nonmuscle LT with aging (Cohn et al. 1980). The finding of unchanged rates of visceral protein turnover is not surprising as visceral organs tend to maintain their functions, in contrast to skeletal muscle that decreases, because of the decrease in physical activity with aging. Rates of nonmuscle LT protein breakdown were 6.8 \( \pm \) 0.4 vs. 6.5 \( \pm \) 0.4 g/(L \cdot d) (NS) for elderly and young persons, respectively. For young persons, these nonmuscle tissue rates are 2.5 times higher than those of muscle which is in agreement with the nearly three-fold higher splanchnic than leg muscle leucine uptake in one human study using \( [1^{15}C] \)leucine (Gelfand et al. 1988). As a consequence of the unchanged volumes and rates of protein breakdown of visceral tissue, the percentage contribution of nonmuscle LT to whole-body protein breakdown significantly increased in older persons (74 vs. 62%) making up for the decreased contribution of muscle.

To our knowledge, the present study is the first to have assessed the effects of aging on rates of protein turnover of visceral lean tissues in humans. However, two other publications in human subjects support the notion that no decrease in visceral protein metabolism is observed with aging (Boirie et al. 1997, Gersovitz et al. 1980). The rates of albumin synthesis using the continuous oral \([ 1^{15}N ]\)glycine method (and estimating the enrichment of the free arginine guanidine-N in the liver from the plateau \( 15^N \) in urinary urea) were found to be unchanged in older compared with young males (Gersovitz et al. 1980). Recently, Boirie et al. 1997, using a combination of intravenous \([ 1^{13}C ]\)leucine and oral \([H_3] \)leucine tracers, have found that there is a higher leucine extraction by the gut and liver in older males. Interestingly, they also found that the ratio of \( \alpha \)-ketoisocaproic acid to leucine tracer enrichment, an indirect way of estimating splanchnic leucine transamination, was higher in old than young males. These results suggest that both liver and gut maintain their activity of protein metabolism with aging in humans. In vivo animal data looking at the effects of aging on different visceral tissues have given conflicting results. Some have shown a decline with age in liver protein synthesis rates in rats (Ward and Richardson 1991), whereas others that have found unchanged rates of fractional liver protein synthesis and breakdown (Goldspink and Kelly 1984, Mosoni et al. 1993). Increased heart and lung protein turnover rates have also been found (Mays et al. 1991).

MRI was able to discriminate among the different lean tissues, giving volumes of muscle and nonmuscle LT, and defining volumes of AT and its distribution. We are thereby able to adduce direct evidence of the preservation of visceral organ mass with aging in a cross-sectional design. Previous studies have concluded this by interpreting the faster decline of potassium to nitrogen using \( 4^K \) and the prompt gamma-neutron-activation technique (Cohn et al. 1980). Although this was not primarily a study on body composition measurement, and conclusions must be circumspect due to the small number of subjects, we showed a trend toward a reduced muscle mass with aging, as well as preservation of visceral lean tissues and increased adipose tissue in the elderly. Similar conclusions can be derived from reports that used a diversity of techniques (Cohn et al. 1980, Durnin and Wormersley 1974, Flynn et al. 1989). However, MRI enabled us to quantify the tissues by region, including the amount and distribution of AT. The AT is increased not only in total but also at all the sites evaluated: intraabdominal, subcutaneous and abdominal subcutaneous. The notion that there is a central distribution of fat with aging (Durnin and Wormersley 1974) has also been confirmed since there was an aging effect responsible for the increase in the percentage intraabdominal AT to total AT and in the ratio of intraabdominal AT to abdominal subcutaneous AT, with an opposite effect on the percentage subcutaneous AT. Female sex contributed to increased intraabdominal AT but contrary to aging, it contributed also to increase the percentage subcutaneous AT/whole-body AT. However, be-

### TABLE 4

**Plasma substrates and hormones of subjects participating in the study to determine the changes in the distribution of protein turnover with age**

<table>
<thead>
<tr>
<th></th>
<th>Elderly</th>
<th>Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8 ( \pm ) 0.1*</td>
<td>4.5 ( \pm ) 0.1</td>
</tr>
<tr>
<td>Fatty acids, ( \mu )mol/L</td>
<td>642.4 ( \pm ) 47.3</td>
<td>585.3 ( \pm ) 58.0</td>
</tr>
<tr>
<td>Insulin, ( \mu )mol/L</td>
<td>84.9 ( \pm ) 6.9†</td>
<td>76.7 ( \pm ) 8.4‡</td>
</tr>
<tr>
<td>Glucagon, ( \mu )mol/L</td>
<td>23.8 ( \pm ) 2.0</td>
<td>26.4 ( \pm ) 1.4</td>
</tr>
<tr>
<td>Glucagon/Insulin</td>
<td>0.29 ( \pm ) 0.04</td>
<td>0.36 ( \pm ) 0.04</td>
</tr>
<tr>
<td>Cortisol, ( \mu )mol/L</td>
<td>553.5 ( \pm ) 37.3</td>
<td>556.6 ( \pm ) 42.9</td>
</tr>
<tr>
<td>Growth hormone, ( \mu )g/L</td>
<td>3.4 ( \pm ) 0.5</td>
<td>4.1 ( \pm ) 1.1</td>
</tr>
</tbody>
</table>

* Values are means \( \pm \) SEM. Data were analyzed by two-factor ANOVA with main effects to be interpreted as the overall effect of age adjusted for gender and the overall effect of gender adjusted for age. Overall age effect: \( * P < 0.05 \). Overall gender effect for lower values in females: \( \ddagger P < 0.03 \).
cause no increase in the abdominal subcutaneous AT was found for females compared with males, the resulting effect is an “internalization” of fat in females with aging, in the abdominal region (T9-T10 to L4-L5). Although this distribution of fat in females is not a universal finding and could be due to a selection bias, the higher total fat in females compared with males is a well-recognized fact (Flynn et al. 1989).

Our panel of energy substrate measurements showed a significantly elevated fasting plasma glucose with age (4.8 ± 0.1 vs. 4.5 ± 0.1 mmol/L), although its value was still within the normal range. The magnitude of this difference is compatible with other studies comparing healthy elderly with young subjects (Meneilly et al. 1997, Robert et al. 1984) and is considered a manifestation of insulin resistance of aging (Jackson 1989). Although hyperglycemia due to diabetes mellitus is known to accelerate protein turnover (Gougeon et al. 1994 and 1997), this magnitude of glucose elevation per se is unlikely to be associated with altered protein turnover rates in the elderly. Fasting plasma insulin levels showed a gender effect, with lower values in females. This is of interest because of the increased intraabdominal AT which has been linked with insulin resistance and elevated plasma levels (Despré et al. 1989). Other factors are known to influence plasma insulin levels, including the current levels of energy intake (Ivy et al. 1991) and physical activity (Kohrt et al. 1992, Rosenthal et al. 1983), which could possibly account for this discrepancy. Of note is also the lack of significant aging effect on growth hormone levels in our subjects. Growth hormone deficiency affects approximately half of the elderly population (Rudman 1985) and thus is not a universal accompaniment of aging. This result and the absence of any significant aging effect on the level of plasma insulin support the statement that our aged group was composed of healthy elderly persons.

In summary, we found that despite unchanged rates of protein turnover per liter of lean tissue with aging, there is a significant reduction in the contribution by muscle to whole-body catabolism due to loss of muscle mass and to a slower myofibrillar protein catabolism. This is in contrast with non-muscle lean tissue whose mass and rates of protein catabolism remain constant with aging, thus increasing its percentage contribution to the whole body. The implications of a lesser contribution of muscle to whole-body protein breakdown are not fully understood, but it is postulated (Young 1990) that this could diminish the capacity of the elderly to respond successfully to restricted dietary intakes or to stressful conditions that require mobilization of amino acids from the myocyte for protein synthesis in vital organs, including cells of the immune system. Furthermore, the significance of the changing pattern of whole-body protein metabolism with aging on protein requirements remains to be defined. Debate prevails on whether the present FAO/WHO/UNU protein recommendations should remain the same (Millward et al. 1997) or be increased (Campbell et al. 1994) in older persons. Since the elderly are more prone to a variety of illnesses that can affect nutrient intake, efficiency of nutrient assimilation and metabolism and/or increased demand for nutrients, they are further at risk. Studies are needed in frail elderly subjects to ascertain what their body composition and protein turnover are, as well as alterations in nutrient requirements such illnesses impose, as they are expected to differ from those of younger persons.

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