Basal Muscle Amino Acid Kinetics and Protein Synthesis in Healthy Young and Older Men

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Context Sarcopenia is associated with loss of strength and function, eventually leading to loss of independence. Some studies suggest that basal muscle protein turnover is reduced with aging, but other studies do not confirm this finding.

Objective To determine if aging per se affects basal muscle protein turnover in men.

Design and Setting Cross-sectional study conducted from June 1997 to July 2000 in a general US community.

Participants Twenty-six young (mean [SE] age, 28 [2] years) and 22 older (mean [SE] age, 70 [1] years) men, who were healthy and independent based on activities of daily living, physical examinations, and screening tests. Subjects were excluded if they had cardiac, pulmonary, liver, or kidney disease; any impairment in activities of daily living; or steroid use.

Main Outcome Measures We measured basal muscle protein and amino acid kinetics, based on stable isotope techniques with femoral arteriovenous catheterization and muscle biopsies. Three models (arteriovenous balance, three-pool, and fractional synthesis rate) were used to estimate the metabolic parameters.

Results Mean (SE) total leg volume was 9.60 (0.32) L in older men vs 10.83 (0.43) L in younger men, which suggests muscle loss in the older men. Net muscle protein balance was similar in both groups (older men, −19 [2] nmol/min per 100 mL of leg volume vs younger men, −21 [2] nmol/min per 100 mL of leg volume; $P=.51$). Small differences were found in mean (SE) muscle protein synthesis in comparisons of older vs younger men: arteriovenous balance, 48 (5) nmol/min per 100 mL of leg volume vs 32 (3) nmol/min per 100 mL of leg volume; $P=.004$; three-pool, 58 (5) nmol/min per 100 mL of leg volume vs 43 (4) nmol/min per 100 mL of leg volume; $P=.04$; and fractional synthesis rate, 0.0601 (0.0046) %/h vs 0.0578 (0.0047) %/h; $P=.73$. Small differences were also found in mean (SE) muscle protein breakdown: arteriovenous balance, 66 (5) nmol/min per 100 mL of leg volume in older vs 53 (4) nmol/min per 100 mL of leg volume in younger men, $P=.045$; and three-pool, 76 (6) nmol/min per 100 mL of leg volume vs 64 (5) nmol/min per 100 mL of leg volume, $P=.14$.

Conclusion Differences in basal muscle protein turnover between older and younger men do not appear to explain muscle loss that occurs with age.

JAMA. 2001;286:1206-1212 www.jama.com

SARCOPENIA, THE INVOLUNTARY decrease in muscle mass with aging, is associated with loss of strength and function, eventually leading to loss of independence.[1-4] The reduction in lean body mass also may contribute to the development of metabolic alterations, such as diabetes mellitus and osteoporosis.[1,5]

The mechanisms leading to sarcopenia are still unclear. Numerous hypotheses have been suggested, including DNA damage,[6] reduced protein synthesis,[7] fiber type changes,[2] inactivity,[8] inadequate nutrition,[9,10] and hormonal changes.[11-14] A combination of several factors likely is responsible for the age-related changes in muscle mass and function.[5] Overall, any of the primary factors responsible for sarcopenia should, at some point, affect muscle protein turnover by creating an imbalance between muscle protein synthesis and breakdown, thus inducing skeletal muscle loss. Therefore, a better understanding of the metabolic alterations characterizing sarcopenia may help to clarify the etiological characteristics of this condition and to determine the efficacy of potential treatments.

Initial studies on muscle protein metabolism have suggested that the metabolic alteration responsible for sarcopenia is a reduction in basal muscle protein synthesis rate in older people as compared with younger controls.[15-19] However, more recent data did not confirm those results.[20-22] A careful review of these studies allows the exclusion of methodological problems as a reason for these discrepancies, since the methods used were similar and already validated in a variety of conditions.

Thus, 3 other possible explanations need to be considered. First, protein syn-

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thesis alone does not provide enough information about muscle loss or gain because protein breakdown influences net muscle protein balance as well. Muscle protein breakdown was directly measured only in the 2 studies from our group,[20,22] whereas the other studies did not measure breakdown[16] or used indirect measures of breakdown (3-methylhistidine or creatinine urinary excretion),[15,17-19,21] which are less sensitive and less specific than the methods used to measure protein synthesis, and they do not allow the estimation of muscle net protein balance. Second, the older population is more heterogeneous than the younger population, because the distinction between health and disease, activity and inactivity, or appropriate and inappropriate nutrition becomes less clear, thus increasing variability. Therefore, different investigators may have obtained contrasting results because they studied slightly different segments of the older population. Third, differences in the study design and insufficient power may have contributed to the discrepancies between different studies.

To assess if age is associated with a reduction in basal net muscle protein synthesis, we measured basal muscle protein turnover and amino acid kinetics in healthy young and older men, and we applied 3 different models to estimate muscle protein turnover to reduce a possible methodological bias. The volunteers were carefully selected to exclude any detectable confounders (eg, diseases, medications, exercise training) that might have affected measures of muscle protein turnover, and they were encouraged to maintain their usual lifestyle prior to the study to avoid the potential bias introduced by acute changes in diet, physical activity level, or both.

METHODS

Subjects

Forty-eight young and older men were recruited from June 1997 to July 2000 through the Sealy Center on Aging of the University of Texas Medical Branch (UTMB), Galveston, Tex (TABLE 1). There were 26 men in the young group with a mean (SE) age of 28 (2) years, and 22 men in the older group with a mean age of 70 (1) years. Women were not included because of difficulties in recruiting an adequate number representative of the population makeup (~50% per sex). After explaining the purpose and risks of the study, which was approved by the institutional review board of UTMB, written consent was obtained from each volunteer. Four young and 4 older subjects had participated in a previous study.[22] Volunteers were considered to be eligible if they were found to be healthy on the basis of clinical history, including a standard questionnaire on the activities of daily living (ADLs) in use at UTMB, physical examination, and screening tests, including complete blood count, blood chemistry workup, hepatitis panel, human immunodeficiency virus test, urinalysis, blood pressure, oral glucose tolerance test if necessary,[23] and electrocardiogram.

Exclusion criteria were the following: cardiac, pulmonary, liver, or kidney disease; peripheral vascular disease; cerbrovascular disease; recurrent deep venous thrombosis; acute, subacute, or chronic infection; active cancer; autoimmune disease; metabolic disease; diabetes mellitus and glucose intolerance as defined by the American Diabetes Association[23]; other endocrinopathies; anemia; hypertension treated with calcium channel blockers, angiotensin-converting enzyme inhibitors, β-blockers, or angiotensin receptor blockers; drug or alcohol abuse; coagulation defects; strength or aerobic training; impairment in the ADLs; history of falls; and anabolic steroid, corticosteroid, or antiandrogen therapy. Subjects with a history of anabolic steroid use or long-term corticosteroid therapy were excluded. Mild osteoarthritis was not an exclusion criterion, provided that it did not impair the ADLs. However, volunteers who could not discontinue analgesic or anti-inflammatory therapy for the time necessary for a complete washout (eg, 5 days for aspirin) were excluded. A careful clinical assessment of the amount of daily physical activity was performed to exclude any subjects engaged in regular aerobic or resistance exercise training, subjects whose jobs included daily heavy weight lifting or long walks, subjects with a history of falls (≥2/y), or subjects with any impairment in ADLs. We did not use a standardized questionnaire to measure the level of physical activity because several studies have shown that the individual variability is high, and the validity of these questionnaires for the estimation of the daily total energy expenditure is limited.[24,25]

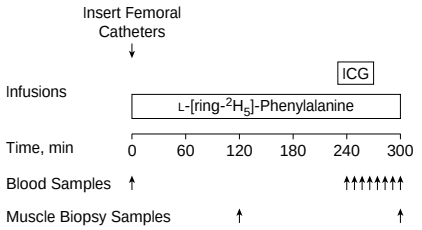
The volunteers performed their regular activities and maintained their usual diet during the week preceding the study. This routine was preferred to an early admission to the General Clinical Research Center (GCRC) and to the administration of a standardized diet for the 3 to 5 days preceding the study, as previously reported,[15-19,21] because these manipulations might influence basal muscle protein turnover.

Table 1. Characteristics of the Subjects*

	Young Men (n = 26)	Older Men (n = 22)	*P* Value	Power (β)	Least Significant Number†
Age, y	28 (2)	70 (1)	<.001	1.00	4
Height, cm	176 (1)	175 (2)	.79	.06	5187
Weight, kg	76 (2)	80 (3)	.22	.23	249
Body mass index, kg/m^2	24 (1)	26 (1)	.07	.44	114
Total leg volume, L	10.83 (0.43)	9.60 (0.32)	.03	.58	79
Leg blood flow, mL/min per 100 mL of leg volume	3.37 (0.25)	2.79 (0.20)	.08	.41	123
Total testosterone, nmol/L‡	21.26 (1.62)	19.66 (2.68)	.62	0.08	1262

*Values are the mean (SE).
†Total number of subjects (sum of both groups) that would produce an $\alpha = .05$ with $\beta = .80$, and δ and σ equal to those of the data.
‡Measured in 19 young and 21 older men.

Figure 1. Infusion Protocol



After the insertion of the femoral artery and venous catheters, L-[ring-$^2H_5$]-phenylalanine was infused for 5 hours. Indocyanine green (ICG) was infused from 230 to 270 minutes to measure rate of blood flow. At the time indicated by the arrows, muscle biopsy samples were taken and blood samples from femoral artery, femoral vein, and wrist vein were collected.

Study Protocol

The night before the study each subject was admitted to the GCRC. After 10 PM the subject was allowed only water ad libitum. At 6 AM on the morning of the study, a catheter was inserted in a forearm vein for phenylalanine tracer infusion and another catheter was inserted in the opposite forearm for blood sampling (FIGURE 1). Catheters were inserted in the femoral artery and femoral vein of one leg for blood sampling. The arterial catheter also was used for indocyanine green (ICG) (IC-Green, Akorn Inc, Buffalo Grove, Ill) infusion. Leg volume was measured using an anthropometric method.[26] After the insertion of the femoral arteriovenous (AV) catheters and the collection of a blood sample for background phenylalanine enrichment and ICG concentration, a primed-continuous infusion of L-[ring-$^2H_5$]-phenylalanine (priming dose: 2 µmol/kg; infusion rate: 0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was started at 6:30 AM (0 minutes) and continued for 5 hours. The background sample also was used to measure total testosterone concentration in 19 young and 21 older men.

At 120 minutes, a first muscle biopsy sample was taken from the lateral aspect of the vastus lateralis of the leg with the femoral catheters, using a 5-mm Bergström needle. The muscle tissue sample (50-150 mg) was quickly blotted and frozen in liquid nitrogen and kept at –80°C until analysis. At 230 minutes, the continuous infusion of ICG was started in the femoral artery (0.5 mg/min) and carried out until 270 minutes. To measure ICG concentration, 4 blood samples were taken every 10 minutes from the femoral vein and the forearm vein during the infusion. After stopping the ICG infusion, to measure phenylalanine concentration and enrichment, 4 blood samples were taken every 10 minutes from the femoral artery and vein until 300 minutes. At 300 minutes, before stopping the tracer infusion, a second muscle biopsy sample was taken as described above.

Approximately 1 week after the study, leg muscle volume was measured using magnetic resonance imaging (MRI) (GE Signa 1.5 T whole body imager, General Electric, Milwaukee, Wis)[20] in the first 10 older and 7 young subjects participating in the study. The remaining subjects could not undergo the procedure because the instrumentation was unavailable.

Analytical Methods

Muscle volume was calculated from the MRI scans as previously described.[20] Indocyanine green concentration in infusate and serum samples was measured spectrophotometrically at $\lambda = 805$ nm. Total testosterone was measured using a commercial radioimmunoassay (Diagnostic Products Corporation, Los Angeles, Calif). Phenylalanine concentrations and enrichments in blood samples were measured using gas chromatography mass spectrometry (GCMS; Hewlett Packard, Palo Alto, Calif).[27]

Muscle tissue from biopsy samples were processed as previously described.[27] Free phenylalanine enrichment and concentration were measured after extraction from the muscle tissue samples using GCMS.[27] Protein-bound phenylalanine enrichment was analyzed with GCMS after protein hydrolysis and amino acid extraction,[27] using the external standard curve approach for very low enrichments.[28,29]

Calculations

Muscle phenylalanine kinetic parameters were calculated using 2 different approaches: the AV balance method[30] and the three-pool model recently described and validated.[31] The AV balance method relies on the measurement of phenylalanine enrichments and concentrations in the femoral artery and vein to estimate muscle protein synthesis, breakdown, and net balance. These parameters are based on the extraction of labeled phenylalanine (tracer) from the femoral artery, the rate of appearance (Ra) of unlabeled phenylalanine (tracee) from the muscle in the femoral vein, and the net AV difference in phenylalanine concentrations, respectively.[30] The three-pool model is an expansion of the AV method and relies not only on the measurement of phenylalanine enrichments and concentrations in the femoral artery and vein but also on the direct measurement of free phenylalanine enrichment in the tissue water. This method allows for the direct calculation of phenylalanine intracellular utilization for protein synthesis and release from protein breakdown as well as the calculation of the rates of phenylalanine transport from the artery into the tissue and from the tissue into the venous blood.[31]

Phenylalanine is used with both methods because it is an essential amino acid, and it is not oxidized in the muscle tissue. Therefore, its utilization in the muscle is an index of muscle protein synthesis, and its release from the muscle is a measure of muscle protein breakdown.

The following 3 parameters are common to both the AV balance and the three-pool model:

(1) Delivery to the Leg = Influx = C_A BF

(2) Release from the Leg = Efflux = C_V BF

(3) Net Balance = NB = (C_A - C_V) BF

C_A and C_V indicate plasma phenylalanine concentrations in the femoral artery and vein, respectively; and BF, leg blood flow. Data are presented per 100 mL of leg volume.[30]

The other kinetic parameters of the AV method were calculated as follows:

(4) Rate of Appearance = Ra = ($C_A \cdot E_A / E_V$) BF

(5) Release from the Muscle = P = Ra – Influx = BF $\cdot$ C_A [(E_A/E_V) – 1]

(6) Rate of Disappearance = Rd = P + NB = BF [$C_A \cdot (E_A/E_V) - C_V$]

E_A and E_V indicate phenylalanine enrichments (tracer/tracee ratio) in the femoral artery and vein, respectively. Data are presented per 100 mL of leg volume.[30] In these calculations it is assumed that the phenylalanine enrichment in the vein is the closest to that of the intracellular pool.

From the AV balance data, it is also possible to calculate the fractional uptake of labeled phenylalanine (%), which is another indirect index of muscle protein synthesis:

(7) Phenylalanine Fractional Uptake = $(E_AC_A - E_VC_V)/(E_AC_A)$

The parameters specific to the three-pool model for labeled phenylalanine were calculated as follows:

(8) Transport Into Muscle = $F_{M,A} = \{[(E_M - E_V)/(E_A - E_M)\ C_V] + C_A\}$ BF

(9) Transport From Muscle = $F_{V,M} = \{[(E_M - E_V)/(E_A - E_M)\ C_V] + C_V\}$ BF

(10) Arteriovenous Shunting = $F_{V,A}$ = Influx − $F_{M,A}$

(11) Release From Proteolysis = $F_{M,0} = F_{M,A}\ [(E_A/E_M) - 1]$

(12) Utilization for Protein Synthesis = $F_{0,M} = F_{M,0} + NB$

E_M indicates the phenylalanine enrichment (tracer/tracee ratio) in the muscle. Data are presented per 100 mL of leg volume.[31]

Intracellular amino acid availability is given by the sum of transport into the muscle ($F_{M,A}$) and the muscle protein breakdown rate ($F_{M,0}$). Thus, it is possible to calculate protein synthesis efficiency as follows:

(13) Protein Synthesis Efficiency = $F_{0,M}/(F_{M,A} + F_{M,0})$

Leg plasma flow was calculated at dye dilution steady state, as previously described.[32,33] Leg blood flow was calculated by correcting the plasma flow by the hematocrit.

We also determined the fractional synthesis rate (FSR) of mixed muscle proteins by measuring the incorporation rate of L-[ring-$^2H_5$]-phenylalanine into the proteins and using the precursor-product model[34] to calculate the synthesis rate as follows:

(14) Fractional Synthesis Rate = $FSR = \{(\Delta E_P/t)/[(E_{M(1)} + E_{M(2)})/2]\}\ 60 \cdot 100$

where ΔE_P is the increment of protein-bound phenylalanine enrichment between 2 sequential biopsy samples, t is the time interval between the 2 sequential biopsy samples, and $E_{M(1)}$ and $E_{M(2)}$ are the phenylalanine enrichments (tracer/tracee ratio) in the free muscle pool in the 2 subsequent biopsy samples. The results are presented as percentage per hour.

We also calculated whole body phenylalanine Ra, an index of whole body protein breakdown, using the single-pool model[35]:

(15) Whole Body Phenylalanine Ra = i/E_A

where i is the infusion rate of L-[ring-$^2H_5$]-phenylalanine.

Statistical Analysis

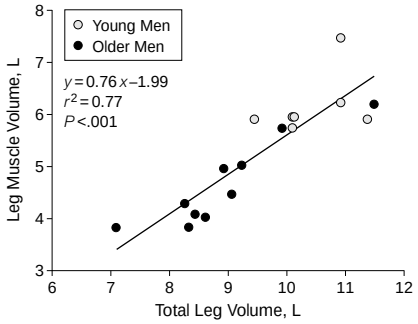
Differences between young and older men were analyzed for each variable using the 2-tailed 2-sample *t* test. Since the prior studies reported contrasting results with regards to muscle protein synthesis, some observing a reduction with age[15-19] and others finding no age-related differences,[20-22] we could not calculate a priori the least significant number of subjects to be studied to reach a set significance with a set power. Thus, we opted for a large-scale study, relative to the technology used. On the basis of the results obtained, we calculated for each measured parameter the power and the least significant number of observations, which we defined as the total number of observations that would produce an $\alpha = .05$ with a $\beta = .80$, with δ and σ equal to those of the data, to provide additional information regarding sample size for future investigations (JMP statistical software, version 4.0.2; SAS Institute Inc, Cary, NC). The relationships between total leg volume and leg muscle volume and between FSR and total testosterone concentration were assessed with the Pearson correlation coefficient. Significance was set at $P < .05$.

RESULTS

Subjects' Physical Features

Height, weight, and body mass index were similar in the older and in the young men (Table 1). However, the total leg volume was significantly lower in the older than in the young men, suggesting a reduced leg muscle mass (Table 1). Mean (SE) leg muscle volume measured by MRI in 10 older and 7 young men was lower in the older than in the young men (young men, 6.16 [0.23] L and older men, 4.64 [0.26] L; $P = .001$). There was a significant correlation between total leg volume and leg muscle volume ($r^2 = 0.77$; $P < .001$) in the 17 subjects who underwent MRI measurement of leg muscle volume (FIGURE 2). Leg blood flow was not different between groups (Table 1).

Figure 2. Relationship Between Total Leg Volume and Leg Muscle Volume



Muscle volume measured by magnetic resonance imaging in 7 young (gray circles) and 10 older (black circles) subjects was significantly correlated to the leg volume of the same subjects measured using an anthropometric method.[26]

Basal total testosterone concentration was measured in 19 young and 21 older subjects (Table 1). Total testosterone was slightly lower in the older than in the young men.

Phenylalanine Concentrations and Enrichments

Phenylalanine concentrations in the femoral artery and vein were significantly higher in the older than in the young men ($P < .001$) (TABLE 2). However, no difference in muscle free phenylalanine concentration was found between groups.

Phenylalanine enrichments were slightly but significantly higher in the femoral artery ($P = .04$) and in the muscle tissue water in the older men ($P = .03$). Phenylalanine enrichment in the femoral vein was not different in the

2 groups. The enrichments were at steady state during the sampling period (data available from authors).

Phenylalanine Kinetics

Phenylalanine delivery to the leg, release from the leg, and NB: across the leg were similar in older and young men (TABLE 3). Using the AV balance method, phenylalanine Ra was not different in both groups. Muscle proteolysis was slightly but significantly higher in the older men ($P=.045$) (an N=91 would have been needed to achieve a power of $\beta=.80$). Phenylalanine Rd, an index of protein synthesis, was significantly higher in the older than in the young men ($P=.004$).

Using the three-pool model, phenylalanine transport into the muscle ($F_{M,A}$) was slightly but not significantly higher in the older men ($P=.054$). Power analysis indicated that an additional 51 subjects should have been studied to achieve an $\alpha=.05$ and $\beta=.80$. Phenylalanine AV shunting ($F_{V,A}$) was slightly but significantly higher in the older men ($P=.03$), whereas the transport from the muscle ($F_{V,M}$), and the release from proteolysis ($F_{M,O}$) did not differ in the 2 groups. Phenylalanine utilization for protein synthesis was significantly higher in the older group ($P=.04$), confirming the AV model data. Also, the fractional uptake of phenylalanine, another index of protein synthesis, was significantly higher in the older group ($P<.001$). Protein synthesis efficiency was similar in young and older men.

Table 2. Phenylalanine Concentrations and Enrichments in Femoral Artery, Femoral Vein, and Muscle Tissue*

Phenylalanine	Young Men (n = 26)	Older Men (n = 22)	*P* Value	Power (β)	Least Significant No.†
Concentration, μmol/L					
Artery	53 (2)	64 (2)	<.001	.97	26
Vein	60 (2)	71 (2)	.001	.92	34
Muscle	115 (7)	125 (7)	.30	.18	348
Enrichment, tracer/tracee					
Artery	0.073 (0.002)	0.080 (0.003)	.04	.57	82
Vein	0.056 (0.001)	0.058 (0.002)	.38	.14	478
Muscle	0.042 (0.002)	0.048 (0.002)	.03	.58	80

*Values are the mean (SE).
†Total number of subjects (sum of both groups) that would produce an $\alpha=.05$ with $\beta=.80$, and δ and equal σ to those of the data.

Table 3. Leg and Muscle Phenylalanine Kinetics*

	Young Men (n = 26)	Older Men (n = 22)	*P* Value	Power (β)	Least Significant No.†
Common parameters, nmol/min per 100 mL of leg volume					
Delivery to the leg	182 (16)	175 (12)	.76	.06	4108
Release from the leg	202 (17)	194 (13)	.70	.07	2673
Net balance	−21 (2)	−19 (2)	.51	.10	890
AV balance model, nmol/min per 100 mL of leg volume					
Rate of appearance	234 (19)	241 (16)	.78	.06	4630
Endogenous proteolysis	53 (4)	66 (5)	.05	.52	91
Rate of disappearance	32 (3)	48 (5)	.004	.84	43
Three-pool model, nmol/min per 100 mL of leg volume					
Transport to the muscle	90 (8)	115 (9)	.054	.49	99
Transport from the muscle	111 (9)	134 (10)	.10	.36	141
AV shunting	91 (10)	60 (9)	.03	.58	80
Release from proteolysis	64 (5)	76 (6)	.14	.31	168
Utilization for protein synthesis	43 (4)	58 (5)	.04	.55	85
Mixed muscle protein FSR, %/h	0.0578 (0.0047)	0.0601 (0.0046)	.73	.06	3208
Other indexes of protein synthesis, %					
Phenylalanine fractional uptake	0.077 (0.006)	0.132 (0.015)	<.001	.94	32
Protein synthesis efficiency	28 (2)	30 (2)	.37	.14	471

*AV indicates femoral arteriovenous. Values are the mean (SE).
†Total number of subjects (sum of both groups) that would produce an $\alpha=.05$ with $\beta=.80$, and δ and σ equal to those of the data.

Mixed Muscle FSR

Mean (SE) mixed muscle FSR was not different in the older and in the young groups with a power of $\beta=.06$ (Table 3 and FIGURE 3). The FSR was 3.5% higher in the older group. We estimated that we should have studied at least 3208 subjects to achieve an $\alpha=.05$, with $\beta=.80$ and σ equal to that of the present data. We calculated that 8 subjects per group would be required to observe a 30% difference in FSR between young and older men. No relationship was found between total testosterone concentration and FSR (FIGURE 4).

Whole Body Phenylalanine Ra

Mean (SE) whole body phenylalanine Ra, an index of whole body proteolysis, was not different ($P=.07$) in the older group (0.64 [0.02] $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and in the young group (0.70 [0.02] $mol \cdot kg^{-1} \cdot min^{-1}$), with a power of $\beta=.47$. To achieve an $\alpha=.05$ and a $\beta=.80$, 105 subjects should have been studied.

COMMENT

Contrary to the general notion that muscle protein synthesis is reduced with age, we found that the kinetic indicators of muscle protein synthesis (phenylalanine Rd, fractional uptake, utilization for protein synthesis, and mixed protein FSR) all tended toward the opposite direction: a higher protein synthesis rate in older men. Nevertheless, phenylalanine net balance

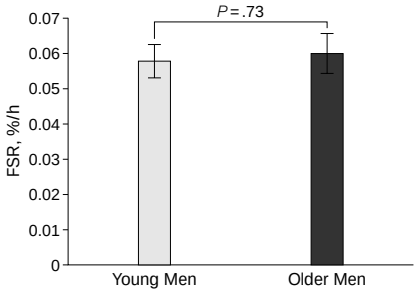
across the leg was similar in both age groups, due to a slightly higher breakdown rate in the older subjects, indicating that the degree of net protein catabolism was similar in the basal state in both the young and older men. In addition, the power analysis performed in this study provides evidence that when the study design is not longitudinal very large numbers of subjects are needed to observe significant age-related differences in basal muscle protein turnover with sufficient power.

The contrast between the present data and previous reports[15-19] that showed a difference in muscle myofibrillar protein synthesis, mixed protein synthesis, or both may be explained by a combination of factors. First, this investigation is the first large study to date to report not only basal muscle protein synthesis values but also direct measures of muscle protein breakdown and net muscle protein balance in older men. In the absence of quantification of breakdown, it is not possible to draw conclusions on the mechanisms leading to muscle loss. A lower protein synthesis rate would not induce muscle loss if the breakdown was reduced as well. The results of direct (present study and earlier studies[20,22]) and indirect[15,17-19,21] measurement of muscle protein breakdown have consistently indicated that breakdown does not change with age. If true, the 30% to 40% reduction in basal myofibrillar and mixed muscle protein synthesis with aging found in previous studies[15-19] would have resulted in a net loss of approximately 60% of the muscle mass within 1 year, assuming that an individual is in the basal state for approximately 16 h/d and that the response to feeding and other stimuli is preserved with age. This reduction is inconsistent with the natural history of sarcopenia that develops over decades and suggests that the measurement of that large of a decrease in basal myofibrillar and mixed muscle protein synthesis may reflect an acute response to the experimental design.

We purposely avoided any dietary manipulations prior to the study, whereas in some of the previous reports volunteers were given a standardized diet for several days prior to the study.[15-19,21] Recent data suggest that the recommended protein dietary allowance for people older than 55 years might be higher than that reported for adults younger than 55 years,[36] which is the cohort in which dietary allowance was assessed. Thus, standardized diets may not be adequate for healthy older men who may be compensating in their everyday life with increased ingestion of protein. Also, we cannot exclude that the older muscle is more sensitive to inactivity, so that an early admission to the GCRC (3 days before the study) as described in previous studies[15-18] might have contributed to a reduction in physical activity and, consequently, muscle protein turnover. This hypothesis is indirectly confirmed by the fact that 1 group, who found a slower muscle protein synthesis in older subjects after a 3-day admission to the GCRC,[18] did not confirm those findings when studying the subjects the day after admission.[21]

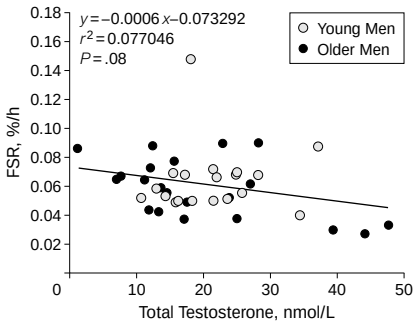
To assess the influence of age alone on muscle protein kinetics, we intentionally selected healthy and active, although not participating in exercise training, older subjects and compared them with healthy younger men. In addition, the older volunteers of our study had total testosterone concentrations within the mid- to-normal range, and we found no difference in total testosterone concentrations between the young and older men. Since other investigators have found age-related differences in testosterone concentrations,[11] the lack of a difference in testosterone concentration between the older and young men in our study might be explained by a high variability of total testosterone concentration in the older age group and by the fact that free testosterone was not measured. If our selection criteria allowed us to assess the effect of age per se on muscle protein turnover, our older volunteers may have not been representative of the general older population. Furthermore, it is possible that the subjects of other studies were not as healthy as the subjects in our study, although they were not clinically ill.

Figure 3. Mixed Muscle Protein Fractional Synthesis Rate (FSR)



Mixed muscle protein FSR was similar in 26 healthy young and 22 healthy older men, with a power of β=0.06. The least significant number of subjects to be studied to achieve an α=.05 with β=.80 should have been 3208 (sum of both groups). Error bars indicate SE.

Figure 4. Relationship Between Total Testosterone and Mixed Muscle Protein Fractional Synthesis Rate (FSR)



Basal total testosterone concentration was not significantly correlated with basal mixed muscle protein FSR in 19 young (gray circles) and 21 older (black circles) subjects.

One question that our study cannot answer is whether age affects the turnover rates and the net balance of specific muscle proteins. Although the synthesis rate of isolated muscle proteins, such as myofibrillar proteins or, more specifically, myosin heavy chain, can be measured,[15-17,19] at present no methods are available to determine their individual breakdown rates. However, myofibrillar proteins and myosin heavy chain represent the bulk of muscle proteins, and previous studies in which both mixed muscle proteins and muscle myosin heavy chain synthesis rates were measured together reported similar qualitative results.[16,19] Thus, we be-

lieve that our data closely reflect contractile muscle protein turnover.

In conclusion, since differences in the basal rate of muscle protein turnover are not apparently responsible for the loss of muscle with aging, it follows that research on sarcopenia should focus on the response of muscle to specific stimuli, such as feeding and physical activity. Recent data indicate that a blunted response to feeding may be in part responsible for the slow loss of muscle with aging[22] and that inactivity may be responsible as well, since exercise training results in increased muscle mass and muscle protein synthesis rate in both healthy and frail older individuals.[18,19,21,37] Furthermore, age-related changes in the hormonal pattern and the possible pharmacological correction of these imbalances should be considered. Specifically, the reduction in sex hormones and growth hormone/insulin-like growth factor I in some subjects are appealing targets for a replacement therapy, which could positively impact sarcopenia.[11,12,14] From a clinical perspective, it is encouraging that age-related reduction of basal muscle protein synthesis does not appear to explain sarcopenia, as it would be difficult to target such a fundamental response with an appropriate therapeutic intervention. On the other hand, it may be more feasible to treat alterations stemming from inactivity, altered response to nutrients, or hormonal imbalances.

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Obtained funding: Volpi, Wolfe.
Administrative, technical, or material support: Volpi, Sheffield-Moore, Wolfe.
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Funding/Support: This research was supported by grants from the National Institute on Aging (R01 AG15780 to Dr Wolfe, R01 AG18311 to Dr Volpi) and the University of Texas Medical Branch at Galveston, Claude Pepper Older Americans Independent Center (P60 AG17231 to Dr Goodwin). Conducted at GCRC of the University of Texas Medical Branch and funded by the National Center for Research Resources, NIH, USPHS (M01 RR00073). Dr Volpi is a Brookdale National Fellow.

Acknowledgment: We thank James S. Goodwin, MD, Director of the Sealy Center on Aging and Chief of the Division of Geriatric Medicine of the University of Texas Medical Branch at Galveston, for his continuous support and invaluable suggestions.

REFERENCES

1. Evans WJ. What is sarcopenia? *J Gerontol*. 1995; 50 Spec No:5-8.
2. Lexell J. Human aging, muscle mass, and fiber type composition. *J Gerontol*. 1995; 50 Spec No:11-16.
3. Wolfson L, Judge J, Whipple R, King M. Strength is a major factor in balance, gait, and the occurrence of falls. *J Gerontol*. 1995; 50 Spec No:64-67.
4. Tinetti ME, Williams CS. Falls, injuries due to falls, and the risk of admission to a nursing home. *N Engl J Med*. 1997;337:1279-1284.
5. Dutta C, Hadley EC. The significance of sarcopenia in old age. *J Gerontol*. 1995; 50 Spec No:1-4.
6. Barazzoni R, Short KR, Nair KS. Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart. *J Biol Chem*. 2000;275:3343-3347.
7. Proctor DN, Balagopal P, Nair KS. Age-related sarcopenia in humans is associated with reduced synthetic rates of specific muscle proteins. *J Nutr*. 1998; 128(suppl 1):351S-355S.
8. Evans WJ. Effects of exercise on body composition and functional capacity of the elderly. *J Gerontol*. 1995; 50 Spec No:147-150.
9. Roberts SB. Effects of aging on energy requirements and the control of food intake in men. *J Gerontol*. 1995; 50 Spec No:101-106.
10. Campbell WW, Evans WJ. Protein requirements of elderly people. *Eur J Clin Nutr*. 1996;50 (suppl 1): S180-S183; discussion S183-S185.
11. Bross R, Javanbakht M, Bhasin S. Anabolic interventions for aging-associated sarcopenia. *J Clin Endocrinol Metab*. 1999;84:3420-3430.
12. Dionne IJ, Kinaman KA, Poehlman ET. Sarcopenia and muscle function during menopause and hormone-replacement therapy. *J Nutr Health Aging*. 2000; 4:156-161.
13. Roubenoff R. Sarcopenia. *J Nutr Health Aging*. 2000;4:140-142.
14. Shetty KR, Duthie EH Jr. Anterior pituitary function and growth hormone use in the elderly. *Endocrinol Metab Clin North Am*. 1995;24:213-231.
15. Welle S, Thornton C, Jozefowicz R, Statt M. Myofibrillar protein synthesis in young and old men. *Am J Physiol Endocrinol Metab*. 1993;264:E693-E698.
16. Balagopal P, Rooyackers OE, Adey DB, et al. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol Endocrinol Metab*. 1997;273: E790-E800.
17. Welle S, Thornton C, Statt M. Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. *Am J Physiol Endocrinol Metab*. 1995;268:E422-E427.
18. Yarasheski KE, Zachwieja JJ, Bier DM. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol Endocrinol Metab*. 1993;265:E210-E214.
19. Hasten DL, Pak-Loduca J, Obert KA, Yarasheski KE. Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78-84 and 23-32 yr olds. *Am J Physiol Endocrinol Metab*. 2000; 278:E620-E626.
20. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first pass splanchnic extraction. *Am J Physiol Endocrinol Metab*. 1999;277:E513-E520.
21. Yarasheski KE, Pak-Loduca J, Hasten DL, et al. Resistance exercise training increases mixed muscle protein synthesis rate in frail women and men >/=76 yr old. *Am J Physiol Endocrinol Metab*. 1999;277:E118-E125.
22. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab*. 2001;85:4481-4490.
23. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes mellitus. *Diabetes Care*. 1999;22(suppl 1):S5-S19.
24. Jacobs DR, Ainsworth BE, Hartman TJ, Leon AS. A simultaneous evaluation of 10 commonly used physical activity questionnaires. *Med Sci Sports Exerc*. 1993; 25:81-91.
25. Bonnefoy M, Normand S, Pachiaudi C, et al. Simultaneous validation of ten physical activity questionnaires in older men. *J Am Geriatr Soc*. 2001;49:28-35.
26. Katch V, Weltman A. Predictability of body segment volumes in living subjects. *Hum Biol*. 1975;47: 203-218.
27. Wolfe RR. Appendix A: laboratory methods. In: Wolfe RR, ed. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, NY: Wiley-Liss; 1992:417-438.
28. Calder AG, Anderson SE, Grant I, McNurlan MA, Garlick PJ. The determination of low d5-phenylalanine enrichment (0.002-0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. *Rapid Commun Mass Spectrom*. 1992;6:421-424.
29. Patterson BW, Zhang XJ, Chen Y, Klein S, Wolfe RR. Measurement of very low stable isotope enrichments by gas chromatography/mass spectrometry: application to measurement of muscle protein synthesis. *Metabolism*. 1997;46:943-948.
30. Wolfe RR. Selection of tracer infusion and sampling sites. In: Wolfe RR, ed. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, NY: Wiley-Liss; 1992:167-188.
31. Biolo G, Fleming RY, Maggi SP, Wolfe RR. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol*. 1995; 268:E75-E84.
32. Jorfeldt L, Juhlin-Dannfelt A. The influence of ethanol on splanchnic and skeletal muscle metabolism in man. *Metabolism*. 1978;27:97-106.
33. Jorfeldt L, Wahren J. Leg blood flow during exercise in man. *Clin Sci*. 1971;41:459-473.
34. Chinkes DL, Rosenblatt J, Wolfe RR. Assessment of the mathematical issues involved in measuring the fractional synthesis rate of protein using the flooding dose technique. *Clin Sci*. 1993;84:177-183.
35. Wolfe RR. Calculation of substrate kinetics: single pool model. In: Wolfe RR, ed. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, NY: Wiley-Liss; 1992:119-144.
36. Pannemans DL, Wagenmakers AJ, Westerterp KR, Schaafsma G, Halliday D. Effect of protein source and quantity on protein metabolism in elderly women. *Am J Clin Nutr*. 1998;68:1228-1235.
37. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med*. 1994; 330:1769-1775.

normalities of any of those components can interfere with complete bladder emptying. We report the case of a patient with symptomatic abdominal distension, which was found to be due to urinary retention with bilateral hydroureter. We believe that this was induced by chronic alcoholism.

Report of a Case. A 45-year-old man presented with progressive abdominal distension for 6 months as well as dysuria and urinary frequency for 2 months. His medical history was significant for chronic alcohol abuse of 20 years (10 beers per day). He denied dizziness, dyspnea, abdominal pain, nausea, vomiting, or tenesmus.

Physical examination revealed normal vital signs without orthostatic changes. The sclerae were anicteric. The abdomen was extensively distended but soft and nontender without shifting dullness. Bowel sounds could not be heard. The liver was 5 cm in the right middle clavicular line. There was no splenomegaly. The prostate was small and smooth, and the stool was heme negative. The patient had no paresthesia, weakness, or other neurological deficits. Laboratory data revealed normal electrolytes and blood cell counts except for an elevated mean corpuscular volume of 105.8 fL. Liver function tests, including prothrombin time, were normal except for aspartate transaminase of 129 U/L. Serum ethanol level was 244 mg/dL.

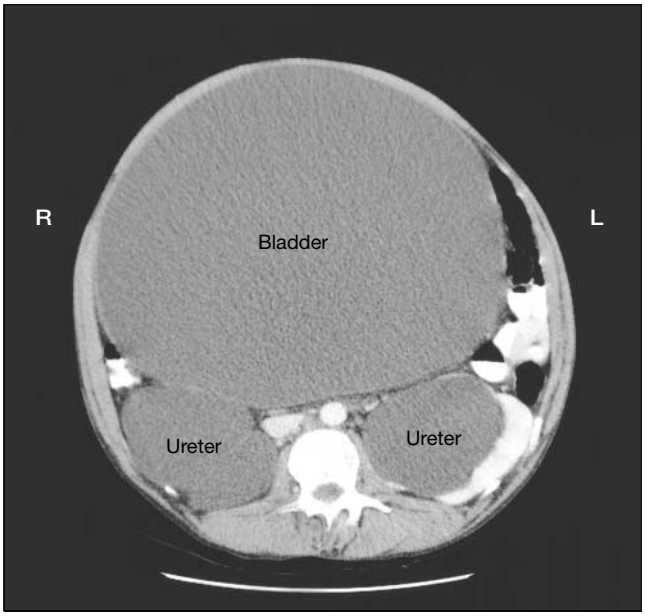
The results of an abdominal ultrasound suggested ascites and bilateral severe hydroureter. An abdominal computed tomography scan (FIGURE) showed bilateral hydroureteronephrosis with cortical thinning and marked distension of the urinary bladder, extending to the upper abdomen with compression of both ureters in the upper pelvis. No pelvic mass, adenopathy, or ascites was identified. The prostate and seminal vesicles were not enlarged.

A Foley catheter was inserted and 18650 mL of urine was drained over a 12-hour period. The patient reported complete relief from his abdominal distension. Cystoscopy confirmed bladder distension, but did not show any urethral obstruction. A suprapubic bladder tube was placed for recovery of bladder contraction. The patient was discharged and lost to follow-up.

Comment. Abdominal distension in the presence of alcoholism usually suggests ascites from portal hypertension. This patient's abdominal distension, however, was caused by urinary retention possibly due to alcohol-induced neuropathy.

Chronic alcoholism can cause both central and peripheral neuropathy. Two cases of alcohol-induced bladder dysfunction have been reported previously.[3,4] In 1 case, the etiology was thought to be thiamine deficiency,[3] and alcoholic myelopathy in the other.[4] Urinary retention as the presentation of alcoholism has only been reported once,[4] but alcohol-induced urinary distension with bilateral hydroureter, as described here, has not been reported to our knowledge. Several pieces of evidence suggest that this patient's urinary retention was due to alcoholic neuropathy. First, he had no symptoms or signs of either thiamine deficiency or alcoholic myelopathy. Second, neither physical examination, cystoscopy, nor imaging studies showed evidence of obstruction. Third, he had no medical conditions and was taking no medications or other drugs that would predispose him to urinary retention. Thus, we believe that this patient's abdominal distension was caused by urinary retention due to neurogenic bladder with autonomic dysfunction, which in turn was secondary to chronic alcohol abuse.

Figure. Abdominal Computed Tomography Scan of a Patient With Alcoholism



Abdominal computed tomography scan at the L2-L3 level shows marked distension of the bladder and severe bilateral hydroureter.

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1. Morrison JF. The physiological mechanisms involved in bladder emptying. *Scand J Urol Nephrol Suppl*. 1997;31:15-18.
2. Wein A. Pathophysiology and categorization of voiding dysfunction. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ, eds. *Campbell's Urology*. 7th ed. St Louis, Mo: WB Saunders; 1998:917-926.
3. Tjandra BS, Janknegt RA. Neurogenic impotence and lower urinary tract symptoms due to Vitamin B 1 deficiency in chronic alcoholism. *J Urol*. 1997;157:954-955.
4. Sheremata WA, Sherwin I. Alcoholic myelopathy with spastic urinary bladder. *Dis Nerv Syst*. 1972;33:136-139.

CORRECTION

Incorrect Regression Equation: In the Clinical Investigation titled "Basal Muscle Amino Acid Kinetics and Protein Synthesis in Healthy Young and Older Men" published in the September 12, 2001, issue of THE JOURNAL (2001;286:1206-1212), the regression equation in Figure 4 is incorrect. The intercept value should be 0.073292 (positive not negative value). The equation should thus be $y=-0.0006x+0.073292$.