Frailty amplifies the effects of aging on protein metabolism: role of protein intake[1–3]

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ABSTRACT

Background: We previously showed that muscle contributes less to whole-body protein breakdown with healthy aging.

Objective: We hypothesized that frailty further compromises protein metabolism and that short-term protein supplementation improves protein status.

Design: Protein metabolism was studied with the oral, 60-h [^{15}N]glycine and N^τ-methylhistidine methods in 8 frail and 13 healthy elderly women during a 9-d isoenergetic, isonitrogenous formula diet and then after increased protein intakes in the frail women, to match the intakes of healthy subjects, for 12 d.

Results: Compared with healthy women, frail women had higher rates of whole-body protein synthesis and breakdown per kg fat-free mass and lower rates of muscle protein breakdown when expressed as total amounts per day but higher rates when expressed per kg muscle. Because muscle mass was lower in frail women, the contribution of muscle to whole-body protein breakdown was lower and that of nonmuscle lean tissues was higher. The protein-enriched diet had no effect on these variables but resulted in an increase in net endogenous protein balance and a positive nitrogen balance at the end of the diet period.

Conclusions: Frailty exacerbates age-related changes in protein metabolism by inducing an increase in muscle protein catabolism and a decrease in muscle mass. At low protein intakes, the increase in muscle catabolism may be a form of protection for both nonmuscle lean tissue mass and function at the expense of muscle mass. Frail women maintained the capacity to retain nitrogen after increased protein intakes, which could convey health benefits if sustained over a long enough period to result in lean tissue accretion. *Am J Clin Nutr* 2003;78:422–9.

KEY WORDS Aging, frailty, protein metabolism, muscle breakdown, protein supplementation

INTRODUCTION

Frail elderly persons are those who experience a loss of autonomy with impairment in performing the essential activities of daily living because of functional and physical disabilities (1). Frailty also has nutritional implications (2). Frailty commonly afflicts the oldest segment of the geriatric population, in whom the functional decline is due to many interactive factors, including poor appetite, low food intake, involuntary weight loss, sarcopenia, mobility impairment, illnesses, polypharmacy, and depression. Many intervention strategies, namely resistance exercise training (1, 3–5) and dietary protein-energy supplementation (6, 7), have been successful in slowing this process.

We previously showed that the contribution of muscle protein to whole-body protein breakdown is lower in healthy elderly persons (8, 9), even though their rates of whole-body protein flux, synthesis, breakdown, and net balance were not different from those of young subjects, when expressed per fat-free mass (FFM). Others have suggested an age-dependent decline in whole-body protein turnover, but this was based on calculations expressed per kg body wt and did not account for the decline in FFM (10). Few protein kinetic studies have been undertaken in frail elderly persons (7, 10–13), mainly because of the physical limitations of frailty and the complexity of such studies. In one study that used [^{14}C]leucine, no differences in protein metabolism were found between malnourished and healthy elderly subjects, but again the results were not corrected for differences in FFM or muscle mass (12). These authors found no changes in protein kinetics with enteral protein-energy replenishment. Others have reported increased nitrogen flux, breakdown, and synthesis rates in ill elderly patients, which they attributed to tissue trauma and inflammation (13). Protein deposition and a gain in lean body mass were found in malnourished elderly subjects after a 10-d protein-energy supplementation (7). A greater increase in postabsorptive protein synthesis rates was reported in the malnourished elderly group than in a young group after supplementation, indicating that the capacity to respond was present.

We hypothesized that frailty imposes an additive effect to that found with healthy aging (8, 9). The study of muscle protein breakdown in frail elderly subjects is particularly relevant because of underlying sarcopenia and because no data on this aspect are yet available. Most studies of protein metabolism at the muscle tissue level in healthy elderly subjects have focused on protein synthesis (14–16); few studies have assessed protein breakdown (17, 18). In view of recent suggestions to revise the recommended dietary allowance of protein for elderly persons upward (19), our objective was to test whether an increase in protein but not in energy intake would improve protein status in frail elderly women.

TABLE 1
Characteristics of frail and healthy elderly women during an isoenergetic, isonitrogenous (ISO) diet[1]

	Frail ISO group (n = 8)	Healthy ISO group (n = 13)
Age (y)	84.0 ± 2.2[2]	71.2 ± 1.1
Height (m)	1.50 ± 0.04	1.57 ± 0.01
Weight (kg)	44.3 ± 4.0[2]	55.3 ± 2.1
BMI (kg/m²)	19.4 ± 1.5[2]	22.6 ± 0.8
Urinary creatinine (g/d)	0.45 ± 0.03[2]	0.80 ± 0.03
Serum albumin (g/L)	36.9 ± 1.2[2]	42.5 ± 1.1
Triceps skinfold thickness (mm)[3]	11.3 ± 1.8 (5–10)[2]	24.4 ± 2.3 (50–75)
Midarm muscle area (cm²)[4]	25.1 ± 5.3	34.7 ± 4.3

[1] $\overline{x}$ ± SEM.
[2] Significantly different from healthy ISO group, $P < 0.05$ (unpaired Student's t test).
[3] The percentile ranking according to reference 21 in parentheses.
[4] Corrected for bone area according to reference 22.

SUBJECTS AND METHODS

Subjects

Thirteen healthy elderly women aged 65–78 y were recruited from local newspaper advertisements. Eight frail elderly women aged 73–91 y were recruited from the McGill University Health Centre, Royal Victoria Hospital Acute Geriatric Unit at their discharge ($n = 4$), from the Geriatric Day Hospital rehabilitation program ($n = 2$), from the Outpatient Clinic ($n = 1$), or from home ($n = 1$). The subjects were screened for medical history and underwent a physical examination, an assessment of cognitive status with the Mini-Mental State Examination (20), and a laboratory investigation, which included a complete blood count, a fasting lipid profile, renal and liver function tests, a test for hepatitis B, and measurements of plasma glucose, serum electrolytes, total proteins and albumin, and thyroid hormones. In addition, the subjects received a chest X-ray and an electrocardiogram. The frail subjects completed a 5-d food diary, which was reviewed by a dietitian to estimate energy and protein intakes. Inclusion criteria were a clear understanding of the nature and requirements of the study, a stable weight in the preceding 4 mo, no recent smoking or alcohol abuse, minimal help needed in performing activities of daily living, urinary continence, and no use of estrogen replacement medication. Exclusion criteria were cognitive deficits identified by a Mini-Mental State Examination score < 24, mobility impairment requiring a wheelchair, infections, endocrine diseases (eg, diabetes or untreated thyroid dysfunction), anemia (hemoglobin < 115 g/L), a systolic blood pressure > 160 mm Hg or a diastolic blood pressure > 95 mm Hg, angina class III or IV (New York Heart Association), congestive heart failure, chronic obstructive lung disease, a serum creatinine concentration > 120 μmol/L, neoplasia other than of the skin during the preceding 5 y, and use of the following drugs: diuretics, β-blockers, theophylline preparations, nonsteroidal antiinflammatory drugs, antianginal agents, antiarrhythmics, and oral steroids.

All subjects were admitted to the Clinical Investigation Unit: the healthy subjects for 9 d and the frail elderly women for 21 d. Informed written consent was obtained from all subjects, and the research protocol was approved by the Human Ethics Review Committee of the Hospital. Subject characteristics are presented in **Table 1**.

TABLE 2
Nutrient intakes and resting energy expenditure of frail and healthy elderly women during an isoenergetic, isonitrogenous (ISO) diet and of frail elderly women during an isoenergetic, protein-enriched diet (PED)[1]

	Frail ISO group (n = 8)	Frail PED group (n = 8)	Healthy ISO group (n = 13)
Energy intake			
(MJ/d)	5.23 ± 0.21[2]	5.37 ± 0.31[2]	7.43 ± 0.23
(kJ·kg body wt⁻¹·d⁻¹)	123 ± 9	126 ± 9	136 ± 5
(kJ·kg FFM⁻¹·d⁻¹)	174 ± 6[2]	178 ± 8[2]	213 ± 8
REE			
(MJ/d)	3.91 ± 0.10[2]	3.90 ± 0.13[2]	4.75 ± 0.14
(kJ·FFM⁻¹·d⁻¹)	130 ± 5	130 ± 5	136 ± 5
Energy intake/REE	1.34 ± 0.04[2]	1.38 ± 0.06[2]	1.57 ± 0.03
Protein intake			
(g/d)	38.4 ± 3.4[2]	54.4 ± 5.0[3,4]	67.5 ± 2.5
(g·kg body wt⁻¹·d⁻¹)	0.87 ± 0.03[2]	1.23 ± 0.02[4]	1.25 ± 0.08
(g·FFM⁻¹·d⁻¹)	1.25 ± 0.09[2]	1.78 ± 0.12[4]	1.95 ± 0.10
(% of energy)	12.1 ± 0.8[2]	16.9 ± 1.3[4]	15.4 ± 0.8

[1] $\overline{x}$ ± SEM. REE, resting energy expenditure; FFM, fat-free mass.
[2,3] Significantly different from healthy ISO group (unpaired Student's t test): [2] $P < 0.005$, [3] $P < 0.05$.
[4] Significantly different from frail ISO group, $P < 0.001$ (paired Student's t test).

Diets

Individualized isoenergetic, isonitrogenous (ISO) diets were devised for the frail women (frail ISO group) on the basis of their 5-d food diaries, with Genesis R&D (ESHA Research Inc, Salem, OR). Energy and protein intakes are shown in **Table 2**. Protein accounted for 12.1 ± 0.8% of the total energy intake. The diet was composed mainly of a meal-replacement liquid formula (Ensure; Abbott Laboratory Ltd, St Laurent, Canada) that was divided into 6 equal meals per day and given at 3-h intervals in addition to a standard breakfast of 30-g bran cereal (All-Bran; Kellogg Canada Inc, Etobicoke, Canada) and 200 mL milk (2% fat). Additional energy sources were used as a combination of two-thirds glucose polymer (Polycose; Ross Laboratories, Montreal) and one-third corn oil. The frail women received this weight-maintaining diet for 9 d, after which the protein content of the diet was increased by an average of 42%, to match that of the healthy women, by eliminating the additional energy sources described above and replacing them with a protein-rich soup (Bariatrix International Inc, Lachine, Canada) or more formula. This protein-enriched diet (PED), which provided 16.9 ± 1.3% ($\overline{x}$ ± SEM) of energy as protein (or 1.2 g·kg⁻¹·d⁻¹), was fed for the subsequent 12 d (frail PED group).

The healthy elderly women received the same ISO diet (healthy ISO group), calculated to equal their resting energy expenditure (REE)—measured by indirect calorimetry as described earlier (8)—multiplied by an activity factor of 1.6, and modified according to a 24 h-food recall. Protein intake was maintained between 1.2 and 1.3 g·kg⁻¹·d⁻¹ for both the healthy and frail groups. No other foods, supplements, or beverages were permitted in either group. Adherence to the diets was ensured through supervision by a member of the Clinical Investigation Unit during consumption of 5 of the 6 meals per day.

Resting energy expenditure

After the subjects completed a training session, REE was measured by continuous indirect calorimetry with a Deltatrac

TABLE 3
Body composition of frail and healthy elderly women during an isoenergetic, isonitrogenous (ISO) diet[1]

	Frail ISO group (n = 8)	Healthy ISO group (n = 13)
From BIA[2]		
FFM		
(kg)	30.3 ± 1.3[3]	35.0 ± 0.8
(%)	71 ± 5	64 ± 1
Body fat		
(kg)	14.0 ± 3.1[4]	20.3 ± 1.5
(%)	29 ± 5	36 ± 1
From anthropometric measures[5]		
FFM		
(kg)	32.0 ± 2.1	34.7 ± 0.7
(%)	74 ± 2[3]	63 ± 1
Body fat		
(kg)	12.3 ± 2.0[3]	20.6 ± 1.5
(%)	26 ± 2[3]	37 ± 1
Muscle mass (kg)[6]	8.3 ± 0.7[7]	14.8 ± 0.6
Nonmuscle lean mass (kg)[8]	22.0 ± 1.0	20.2 ± 0.6

[1] $\bar{x}$ ± SEM. FFM, fat-free mass; BIA, bioelectrical impedance analysis.
[2] FFM was estimated by BIA with the use of Roubenoff's equation (26); body fat was obtained by subtracting FFM from body weight.
[3,4,7] Significantly different from healthy ISO group (unpaired Student's t test): [3] $P < 0.005$, [4] $P = 0.05$, [7] $P < 0.00001$.
[5] Body fat was estimated as the sum of triceps, biceps, suprailiac, and subscapular skinfold thicknesses and with the use of the equation of Durnin and Womersley (25); FFM was obtained by subtracting body fat from body weight.
[6] Estimated from urinary creatinine excretion (27).
[8] Calculated as FFM (from BIA) minus muscle mass (from urinary creatinine).

ventilated-hood metabolic monitor (Sensor Medics, Yorba Linda, CA). All subjects had fasted for ≥ 10 h and were allowed to rest in a supine position in a thermally neutral and quiet room for 30 min before the oxygen and carbon dioxide measurements were made. The subjects breathed under the plastic canopy for 20 min, and measurements obtained during the last 15 min were averaged and used to calculate 24-h REE (Table 2) according to the de Weir equation (23).

Body composition

Daily morning body weights were measured in the postabsorptive state, after the subjects had voided and while they were wearing light clothing, to the nearest 100 g on a Scale-Tronix digital scale (Ingram and Bell-Meditron; Le Groupe Inc, Don Mills, Canada). Standing body height was measured, while the subjects were shoeless, to the nearest 0.1 cm with a wall-mounted stadiometer. In 2 frail subjects with moderate kyphosis, height was estimated from knee height by using a caliper and equations provided by Ross Laboratories (Columbus, OH). Skinfold thicknesses were measured by the same observer with a Lange caliper at the biceps, triceps, subscapular, and suprailiac sites as described (24), and 2 measurements of < 1-mm difference were averaged. The sum of the 4 skinfold thicknesses was used to estimate body fat according to the Durnin and Womersley (25) regression equations. FFM was obtained by subtracting body fat from body weight (**Table 3**).

Body composition was also assessed by bioelectrical impedance analysis (BIA) with the use of the RJL-101A instrument (RJL Systems Inc, Detroit). After the subjects fasted overnight and voided in the morning, resistance and reactance were measured on the dominant side of the body after the subjects had rested in a supine position for 10 min with their arms and legs abducted. Electrodes were placed as described by Lukaski et al (28), and the average of 2 measurements was used to estimate FFM with the use of the age- and sex-specific equation of Roubenoff (26), which were validated in a healthy elderly population. The same equation was used for the frail women, because there are no specific BIA equations available for this group. Body fat was obtained by subtracting FFM from body weight. Muscle mass was estimated from urinary creatinine excretion (27) averaged from 3 consecutive 24-h urine samples and also with the use of a published BIA equation (29).

Nitrogen balance

Total daily urine was collected during the whole study period. Completeness of collections was assessed on the basis of day-to-day variability of urinary creatinine and nitrogen excretion. Nitrogen balance was calculated by subtracting total daily nitrogen losses (urinary, fecal, and miscellaneous) from daily nitrogen intake (as 16% of dietary protein). Urinary nitrogen was analyzed by chemi-luminescence (Pyro-Chemiluminescence Nitrogen System; Antek, Houston) as described earlier (8, 30). A factor of 0.07 g fecal N/g N intake, measured in subjects after similar formula-based diets in previous studies (8), was applied, and miscellaneous losses were set at 8 mg N/kg body wt (31). The mean nitrogen balance during the last 3 d of each dietary period was used for comparisons between groups.

Protein turnover

The kinetics of protein metabolism were determined by using the oral, 60-h [15N]glycine method with urea nitrogen as the end product. The method was described in detail previously (8, 32). Briefly, the method requires that a steady state of 15N isotopic enrichment in urinary urea be reached within 60 h. *Steady state* was defined as the first plateau (identified by a slope not different from zero) that extends for 4 points (12 h). The mean CVs of steady state values were 3.7% for the frail nonsupplemented, 2.4% for the frail supplemented, and 3.3% for the healthy women. Protein flux (Q) was estimated from the 15N enrichment of urinary urea, and protein synthesis (S) and breakdown (B) were calculated from the equations of Picou and Taylor-Roberts (33):

$$Q = I + B = S + E \qquad (1)$$

because nitrogen intake (I) and total urinary excretion (E) are known. Dietary and endogenous proteins were derived assuming 16% of protein as nitrogen. 15N enrichment of urinary urea was measured with a dual-inlet, triple collector isotope ratio mass spectrometer (Micromass 903D; Vacuum Generators, Winsford, United Kingdom) after correction for background values determined in a urine sample obtained immediately before the test.

Urinary N^τ-methylhistidine

Urinary N^τ-methylhistidine was used to estimate skeletal muscle protein breakdown as described by Young and Munro (34). The urinary concentration of N^τ-methylhistidine was measured by reversed-phase HPLC (Hewlett-Packard 1090; Mississauga, Canada) after derivatization with orthophthalaldehyde and mercaptopropionic acid (35). The average of three 24 h-urine collections taken on the same days as those for protein turnover studies was used as the mean excretion. Nonmuscle protein breakdown

was obtained by subtracting muscle protein breakdown estimated from urinary N^τ-methylhistidine excretion from whole-body breakdown estimated with the use of the [^{15}N]glycine method (8, 9), and their relative contributions to the whole body were calculated as percentages.

Other analytic measurements

Postabsorptive venous blood samples were collected as described previously (8). Immunoreactive insulin was measured in plasma by single-antibody charcoal precipitation radioimmunoassay with human standards and labeled hormone (Linco Research Inc, St Louis) by methods previously described (36). Values were corrected for dilution by aprotinin on the basis of a concurrently measured hematocrit value. Serum cortisol was measured at the McGill University Health Centre, Royal Victoria Hospital's endocrinology laboratory by an automated immunoluminescence technique (ACS 180; CIBA Corning, Cooperstown, NY). Serum insulin-like growth factor I (IGF-I or somatomedin C) was measured in serum by a 2-site immunoradiometric assay (IRMA) with the DSL-5600 ACTIVE IGF-I IRMA kit (Diagnostic Systems Laboratories Inc, Webster, TX). Insulin-like growth factor binding proteins 1 and 3 (IGFBP-1 and IGFBP-3) were assessed with the DSL-7800 and DSL-6600 ACTIVE IRMA kits, respectively, from the same company.

After being thoroughly mixed, 24-h urine samples were portioned and analyzed daily by the hospital's clinical biochemistry laboratory for urea nitrogen, creatinine, and electrolytes (sodium, potassium, and chloride) and frozen at −20 °C until assayed with the methods described above.

Statistical analysis

Results are presented as means ± SEMs, and data were analyzed with the use of SPSS 10.0 for WINDOWS (SPSS Inc, Chicago). The effect of the PED on all variables in the frail group was measured by paired Student's t test. Differences between the frail group, at both diet periods, and the healthy group were assessed by unpaired Student's t test. Because there was an age difference between the frail and healthy groups, differences between groups (found by unpaired Student's t test) were corrected by multivariate analysis of variance. Partial correlation was used to correlate variables (hormones and binding proteins) after control for age and FFM. A P value < 0.05 was considered significant.

RESULTS

Subject characteristics and nutrient intakes

The frail women were older than the healthy women (Table 1). The lower body weight, body mass index, urinary creatinine, triceps skinfold thickness, and serum albumin in the frail women all indicated frailty. Frail elderly women were taking a mean of 4 different prescribed medications, other than vitamin and mineral supplements and those included in the exclusion criteria; the healthy group was taking no medication at the time of the study. Of the coexisting illnesses present only in the frail subjects, osteoporosis was present in 6, osteoarthritis in 2, general deterioration with prior falls in 2, treated anemia in 3, and previous hip fractures, glaucoma, cataracts, varicose veins, treated hypothyroidism, and peptic ulcer were also present. All frail women required a walking aid for mobility. Of the measured variables shown in Table 1, only urinary creatinine remained significantly lower in the frail ISO

group when age was used as a covariate in the multivariate analysis of variance. Energy intake was lower in the frail than in the healthy ISO group when reported as the total intake per day or per kg FFM, but not when reported per kg body wt (Table 2). REE was not different between groups when expressed relative to FFM, but was lower when reported as the total amount per day, which resulted in a lower ratio of energy intake to REE. Both energy and protein intakes per FFM were lower in the frail group when age was entered as a covariate in the multivariate analysis of variance. Protein intake was lower in frail women during the ISO diet, independent of the denominator used. Protein intake was 0.87 g/kg body wt at baseline and increased by design by 42%, on average, to levels that were similar to those of healthy women (1.2 g/kg body wt). Because total energy intake was maintained constant by decreasing the carbohydrate and lipid contents of the diet, the percentage of energy provided by protein increased significantly and was not different from that of healthy women during the ISO diet.

Body composition

As shown in Table 3, body composition was assessed by anthropometric measures and BIA, but only FFM obtained by BIA was used as the denominator in the expression of the protein kinetic data. Although results from BIA and anthropometric measures tended to agree better within the healthy than within the frail group, body-composition values obtained with both methods were not significantly different when compared by Student's t test. Frail women had a lower FFM than did healthy women, significantly so when determined by BIA. The proportion of FFM (%) based on anthropometric measures was significantly higher in the frail than in the healthy women and, inversely, the percentage body fat was lower than in the healthy women.

Muscle mass, estimated on the basis of urinary creatinine, was significantly lower in the frail than in the healthy women. When the age difference between the 2 groups was accounted for by multivariate analysis, both FFM estimated by BIA ($P = 0.026$) and muscle mass ($P = 0.001$) remained significantly lower in the frail than in the healthy women. Body composition was not changed significantly by the protein-supplemented diet (data not shown).

Nitrogen balance

The mean CVs for urinary creatinine values from 3 consecutive days were 3.9%, 5.0%, and 4.1% for the frail ISO, frail PED, and healthy ISO groups, respectively. The CVs for urinary nitrogen excretion were 5.4%, 4.9%, and 3.2% for the frail ISO, frail PED, and healthy ISO groups, respectively. Healthy elderly women were in nitrogen equilibrium during the last 3 d of the ISO diet, during which time oral [^{15}N]glycine tests were performed (**Figure 1**). The nitrogen balance of the frail ISO group was not significantly different from zero. Positive nitrogen balance was observed at the onset of the PED and, despite a subsequent decrease, it remained positive.

Protein kinetics

During both the ISO diet and the PED, the frail women had higher synthesis and breakdown rates per kg body wt or FFM than did the healthy women, but net protein balance was not significantly different between the groups (**Table 4**). The significance of these data remained after the age difference between the groups was accounted for. Protein flux increased significantly with the PED in frail women, whether expressed as the total amount per day or per kg FFM. Relative to body weight and FFM, protein flux increased to higher levels than in the healthy women. Although
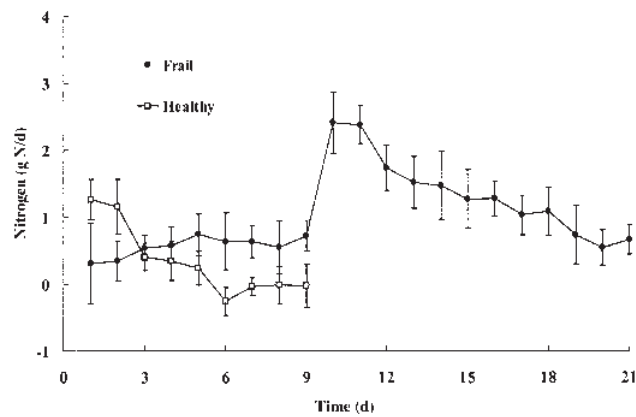
FIGURE 1. Mean (± SEM) average daily nitrogen balance in frail elderly ($n = 8$) and healthy elderly ($n = 13$) women during an isoenergetic, isonitrogenous (ISO) diet from days 1 to 9 and in frail elderly women ($n = 8$) during an isoenergetic, protein-enriched diet (PED) from days 10 to 21. Both the frail and the healthy women were in nitrogen equilibrium; the mean balance from days 6 to 9 of the ISO diet was not different from zero (one-tailed Student's t test). After 12 d of the PED, the nitrogen balance of the frail women remained positive; the mean from days 19 to 21 was different from zero ($P = 0.03$, one-tailed Student's t test).

protein synthesis and breakdown rates were not significantly modified by the diet, the net protein balance increased. These data agree with the nitrogen balance results.

Distribution of protein breakdown

The mean CVs for urinary N^τ-methylhistidine were 4.4%, 5.2%, and 4.9% for frail ISO, frail PED, and healthy ISO groups, respectively. Rates of muscle protein breakdown were lower in the frail than in the healthy women when expressed as the total amount per day or per FFM, were not different when expressed per kg body wt, and were actually higher in the frail group when

expressed per kg muscle mass (**Figure 2**). Rates of nonmuscle protein breakdown were not significantly different between groups when expressed as the total amount per day and per kg nonmuscle lean mass but were higher in the frail than in the healthy women when reported per kg body wt or per FFM (Figure 2). As a result, muscle protein breakdown contributed significantly less (13.9% compared with 20.1%) and, inversely, nonmuscle breakdown contributed more (86.1% compared with 79.9%) to whole-body breakdown in the frail than in the healthy women (**Figure 3**). Muscle and nonmuscle protein breakdown rates were not significantly changed by the PED (Figure 2). However, there was a significant positive correlation ($r = 0.58$, $P = 0.005$; controlled for age) between protein intake at baseline and the total muscle protein breakdown (data not shown).

Hormones

Fasting insulin and cortisol concentrations were not significantly different between the frail and healthy groups (**Table 5**). IGF-I concentrations tended to be lower ($P = 0.06$) in the frail than in the healthy women and were correlated with muscle mass ($r = 0.57$, $P = 0.01$, when controlled for age and FFM; data not shown). IGFBP-1 concentrations were significantly higher in the frail than in the healthy women, as was the ratio of IGFBP-1 to IGFBP-3, and were negatively correlated with IGF-I concentrations ($r = -0.44$, $P = 0.02$). As expected, plasma IGF-I and IGFBP-3 concentrations were strongly correlated ($r = 0.77$, $P < 0.001$; data not shown). There was no PED effect on the hormones and growth factors assessed.

DISCUSSION

This study provided evidence that *1*) frailty exacerbates the effects of aging on protein metabolism that we showed previously (8, 9) by a combined effect of reduced muscle mass and higher rates of muscle catabolism, and *2*) short-term protein supplementation is unable to counteract this effect. We previously reported

TABLE 4
Protein kinetics of frail and healthy elderly women during an isoenergetic, isonitrogenous (ISO) diet and of frail elderly women during an isoenergetic, protein-enriched diet (PED)[1]

	Frail ISO group ($n = 8$)	Frail PED group ($n = 8$)	Healthy ISO group ($n = 13$)
Flux			
(g protein/d)	156.25 ± 9.25[2]	187.76 ± 19.69[3]	186.68 ± 4.50
(g protein·kg body wt^{-1}·d^{-1})	3.68 ± 0.28	4.29 ± 0.33[2]	3.42 ± 0.15
(g protein·kg FFM^{-1}·d^{-1})	5.21 ± 0.21	6.18 ± 0.41[2,3]	5.35 ± 0.18
Synthesis			
(g protein/d)	127.95 ± 9.78	145.36 ± 18.48	128.62 ± 5.54
(g protein·kg body wt^{-1}·d^{-1})	3.02 ± 0.27[2]	3.31 ± 0.35[2]	2.35 ± 0.11
(g protein·kg FFM^{-1}·d^{-1})	4.26 ± 0.26[2]	4.76 ± 0.47[2]	3.67 ± 0.14
Breakdown			
(g protein/d)	117.86 ± 8.41	133.42 ± 16.84	119.18 ± 5.63
(g protein·kg body wt^{-1}·d^{-1})	2.81 ± 0.28[2]	3.06 ± 0.34[2]	2.17 ± 0.10
(g protein·kg FFM^{-1}·d^{-1})	3.94 ± 0.25[2]	4.38 ± 0.40[2]	3.40 ± 0.14
Balance[4]			
(g protein/d)	10.06 ± 2.31	11.94 ± 0.25[3]	9.44 ± 1.44
(g protein·kg body wt^{-1}·d^{-1})	0.21 ± 0.04	0.26 ± 0.04[3]	0.18 ± 0.03
(g protein·kg FFM^{-1}·d^{-1})	0.32 ± 0.01	0.38 ± 0.08[3]	0.28 ± 0.04

[1] $\overline{x} \pm$ SEM. Protein kinetics were calculated with the oral, 60-h [^{15}N]glycine method. FFM, fat-free mass.
[2] Significantly different from healthy ISO group, $P < 0.05$ (unpaired Student's t test).
[3] Significantly different from frail ISO group, $P < 0.05$ (paired Student's t test).
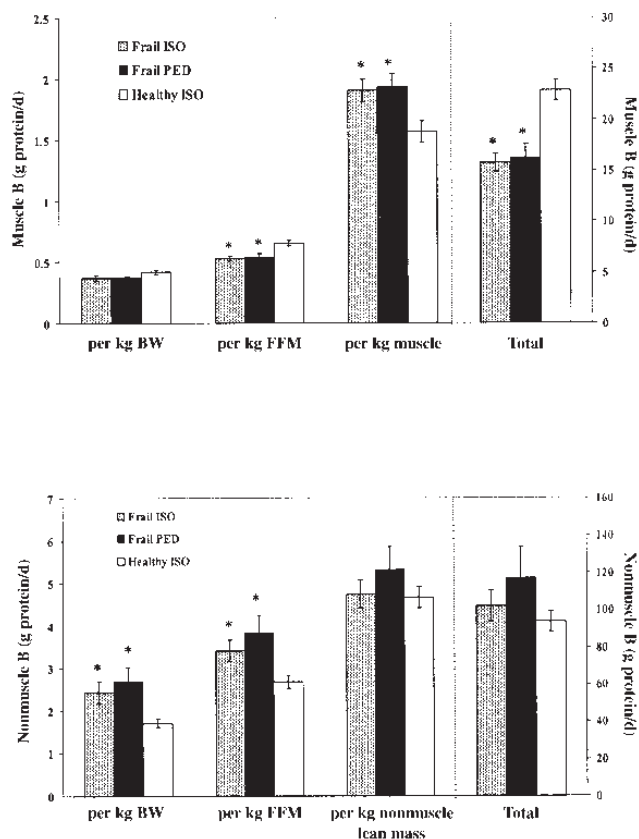[4] Calculated as protein synthesis minus protein breakdown.

FIGURE 2. Mean (± SEM) skeletal muscle and nonmuscle protein breakdown (B) rates estimated from urinary N^τ-methylhistidine excretion in frail elderly ($n = 8$) and healthy elderly ($n = 13$) women during an isoenergetic, isonitrogenous (ISO) diet and in frail elderly women ($n = 8$) during an isoenergetic, protein-enriched diet (PED). The data were obtained by subtracting the muscle protein breakdown rates shown in the top panel from the whole-body protein breakdown rates estimated with the use of the [^{15}N]glycine kinetic studies during the same time period. *Significantly different from healthy ISO group, $P < 0.05$ (unpaired Student's t test).

FIGURE 3. Mean (± SEM) contribution of muscle and nonmuscle to whole-body protein breakdown (B) in frail elderly ($n = 8$) and healthy elderly ($n = 13$) women during an isoenergetic, isonitrogenous (ISO) diet and in frail elderly women ($n = 8$) during an isoenergetic, protein-enriched diet (PED). *Significantly different from healthy ISO group, $P < 0.05$ (unpaired Student's t test).

(9) reduced skeletal muscle protein breakdown rates per unit of muscle mass in healthy elderly than in younger subjects. The present study showed that in frail elderly women, muscle protein breakdown rates were only lower when reported as the total amount per subject or per kg FFM. A major departure with frailty is that these rates were in fact higher than in healthy women when expressed per unit of muscle mass, which suggests increased catabolism of muscle. These increased rates of muscle protein breakdown would ordinarily have augmented the contribution of muscle to the whole body but did not because of a dramatic reduction in muscle mass in the frail women, which explains the very low contribution of muscle to the whole-body turnover.

The increased muscle protein breakdown rates observed theoretically reflect the presence of a catabolic state. However, the frail subjects did not manifest any clinically apparent catabolic diseases nor were they taking medications known to cause catabolism. Their serum cortisol concentrations, although reaching the upper limit of the reference range for our laboratory, were not significantly different from those of the healthy elderly women. However, a trend toward lower IGF-I concentrations ($P = 0.06$) compared with healthy women and higher concentrations of fasting IGFBP-1 in the
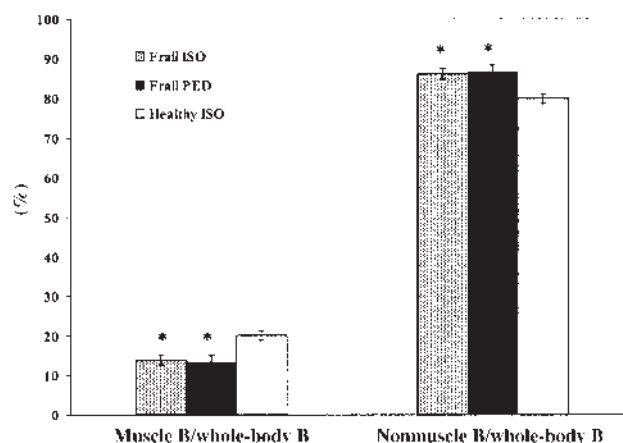
absence of differences in fasting insulin concentrations, which is known to suppress IGFBP-1, would support the presence of undernutrition (37–39). The frail elderly women appear to have accomodated to the low protein intakes in terms of energy and protein metabolism. The frail women were weight stable for ≥ 4 mo, were in nitrogen equilibrium even at their usual low protein intake, and had rates of whole-body protein breakdown that matched those of synthesis. Thus, although frail women appeared to be in protein balance at the whole-body level, they had higher muscle catabolism.

Such increased muscle protein breakdown found in frail women may play a role in sarcopenia, the gradual loss of muscle mass and strength that occurs with aging (40). For this situation to occur, muscle protein breakdown rates have to be greater than synthesis rates. To our knowledge, fractional synthesis rates of muscle protein have never been measured in such frail elderly and, because we did not perform tracer studies at the skeletal muscle level in our subjects, we can only hypothesize on their muscle synthesis rates. Our data suggest that the lower values for muscle mass in frail women may have been due to higher muscle protein breakdown than synthesis, occurring at such a slow pace that it did not translate to detectable body-composition changes during the course of the study (21 d). The fact that there were no nitrogen losses in the weight-stable frail elderly women suggests that amino acids coming from muscle breakdown must have been reutilized as substrates for nonmuscle protein synthesis, hence an explanation for apparent nitrogen conservation. In summary, rates of protein degradation and synthesis and exchanges in amino acids between muscle and nonmuscle compartments may differ in frailty without affecting whole-body protein balance. Because nonmuscle lean tissue has a more rapid protein turnover than does muscle, its higher proportion relative to FFM in frailty likely explains the higher rates of whole-body protein synthesis and breakdown we found.

The frailty of our subjects precluded our using invasive techniques to assess protein kinetics. The urinary N^τ-methylhistidine method was thus chosen, although it provides an estimate rather than a precise quantitative measurement of myofibrillar protein catabolism. This is because N^τ-methylhistidine is also produced

TABLE 5
Hormones, insulin-like growth factor I, and binding proteins in frail and healthy elderly women during an isoenergetic, isonitrogenous (ISO) diet and in frail elderly women during an isoenergetic, protein-enriched diet (PED)[1]

	Frail ISO group ($n = 8$)	Frail PED group ($n = 8$)	Healthy ISO group ($n = 12$)
Fasting insulin (pmol/L)	60.7 ± 4.7	62.2 ± 1.7	69.3 ± 3.3
Fasting cortisol (nmol/L)	587.7 ± 29.7	—	679.3 ± 62.6
IGF-I (nmol/L)	19.1 ± 2.2	21.6 ± 2.6	29.2 ± 4.0
IGFBP-1 (nmol/L)	48.3 ± 8.0^2	47.2 ± 6.7^2	24.8 ± 1.6
IGFBP-3 (nmol/L)	107.0 ± 5.6	112.2 ± 5.3	127.0 ± 8.9
IGFBP-1:IGFBP-3	0.47 ± 0.10^2	0.44 ± 0.07^2	0.23 ± 0.04

[1] $\bar{x} \pm$ SEM. IGF-I, insulin-like growth factor I; IGFBP-1 and -3, insulin-like growth factor binding proteins 1 and 3.

[2] Significantly different from healthy ISO group, $P < 0.05$ (unpaired Student's t test).

by other tissues that contain actin, although the contribution is by far dominated by that of skeletal muscle (34). It is true that when muscle mass is reduced relative to other protein-rich tissues, the proportion from smooth muscle to the total N^τ-methylhistidine pool increases. However, nonmuscle lean mass did not differ between the frail and healthy groups nor did their contribution to the N^τ-methylhistidine pool, which indicates that the lower concentrations of urinary N^τ-methylhistidine per day in frail women reflected both their lower muscle mass (41) and degradation of skeletal muscle in total amounts per day. Only when urinary N^τ-methylhistidine excretion was corrected for urinary creatinine, to account for the difference in muscle mass, was muscle protein breakdown higher than that of the reference group.

We estimated muscle mass from urinary creatinine excretion (42) because this method was validated previously (27, 43) and widely used. This method is acknowledged to be less precise than imaging techniques (44). However, we were able to validate muscle mass on the basis of urinary creatinine against that estimated by magnetic resonance imaging because both were available for 5 of our healthy elderly subjects and for 3 young women. The results of the 2 methods were strongly correlated ($r = 0.88$, $P = 0.004$), which added validity to our use of muscle mass data obtained from urinary creatinine excretion (JA Morais et al, unpublished observations, 2002). Although the use of urinary creatinine to estimate muscle mass has never been validated in frail elderly humans, none of the subjects in our study had any conditions known to interfere with this method (infection, fever, trauma, and renal dysfunction) (27).

The present study was designed to assess the possible effects of increased protein intakes in frail elderly persons to higher amounts than the current recommended dietary allowance, as suggested by many authors (19, 45). Despite the known effect of energy on nitrogen retention (46), energy intake was purposely not increased for reasons of potential clinical applicability. Our results did not show any effect of increasing protein intake alone on muscle and nonmuscle protein breakdown rates nor on their respective contributions to whole-body protein breakdown. A possible explanation for this is that frail women may have overreported their protein intakes from the ISO diet, therefore masking a more positive nitrogen balance during the following PED. Nonetheless, our frail subjects avidly retained more nitrogen at the onset of the PED and, most importantly, nitrogen balance was still slightly positive at the end of the 12-d study period. Equilibrium in nitrogen balance would probably have been reached had the study been prolonged (47). With the PED, the positive nitrogen balance agreed with an increase in net protein balance (assessed by [^{15}N]glycine kinetics) that was either too small or of too short a duration to be translated into increases in muscle or lean mass, as was shown with combined energy and pro-

tein supplementation (7). Assuming a gain of 30 g lean hydrated tissue per each 1 g N retained (48), the increase in net balance of 0.3 g N/d over 12 d would correspond to a theoretical gain of 108 g lean tissue, a change too small to be detected by our methods. However, extrapolation of these values would yield a theoretical gain of 3 kg lean tissue over 1 y if all other conditions were maintained, a change that could well be accompanied by health benefits.

Our data indicate that the frail women's mean usual low protein intake of 38 g/d, which matches the current recommended intake ($0.87 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), relative to their correspondingly low body weights, was nonetheless associated with less muscle mass and greater muscle catabolism. For this reason, the current dietary recommendation for protein may not be appropriate for frail elderly persons, whose energy intake is minimal, even if they are in nitrogen equilibrium. In fact, if protein requirements were calculated according to an ideal body weight at a body mass index (in kg/m^2) of 25, they would be higher for frail women.

In conclusion, muscle mass was lower and the rate of muscle catabolism was higher in the frail elderly women than in the healthy elderly women. The higher muscle catabolism may represent an accommodation mechanism through which muscle provides amino acids to maintain visceral mass and function, at the expense of muscle mass. The low usual protein intake of the frail women, despite their compliance with the current recommendations for protein intake, contributed significantly to this decremental state encountered with aging. Although short-term protein supplementation did not reverse this situation, frail women maintained the capacity to retain nitrogen when they consumed higher protein intakes, which could potentially convey health benefits in the long term. ❧

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