Short-term protein and energy supplementation activates nitrogen kinetics and accretion in poorly nourished elderly subjects[1–3]

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ABSTRACT

Background: An increase in protein intake exerts a stimulating effect on protein kinetics in children, young adults, and healthy elderly persons. However, there are few data on the response to such dietary changes in malnourished elderly subjects, despite important medical implications in this population.

Objective: The objective of this study was to determine the metabolic response to short-term nutritional supplementation in moderately malnourished elderly subjects.

Design: The influence of 10 d of supplementation (1.67 MJ/d and 30 g protein/d) on body composition, resting energy expenditure, and whole-body protein kinetics was studied in 17 malnourished elderly patients and 12 healthy young adults. A control group of 6 malnourished elderly patients received no supplementation.

Results: Supplemented elderly subjects had a significantly greater fat-free mass gain than did unsupplemented elderly subjects (1.3 and 0.1 kg, respectively; age effect, $P < 0.05$; diet effect, $P < 0.02$) and a significantly greater increase in fasting rate of protein synthesis than did young supplemented subjects (0.6 and 0.2 $g \cdot kg\ FFM^{-1} \cdot 11\ h^{-1}$; age effect, $P < 0.05$). The net protein balance in the supplemented elderly subjects in the fed state was positively correlated with protein intake ($r^2 = 0.46$) and in the fasted state was negatively correlated with protein intake ($r^2 = 0.27$). The sum of these regressions is a line with increasingly positive net diurnal protein balance produced by increasing protein intake.

Conclusion: These data provide evidence of a short-term anabolic response of protein metabolism to dietary supplementation in malnourished elderly patients that is likely to improve muscle strength and functional status. *Am J Clin Nutr* 2000;71:1129–37.

KEY WORDS Elderly subjects, short-term nutritional supplementation, protein-energy malnutrition, body composition, basal metabolic rate, protein turnover, protein accretion, nitrogen isotopes, France

INTRODUCTION

Protein-energy malnutrition (PEM) is a common disorder in the elderly (1–4). Malnutrition results from insufficient energy and protein intakes, a hypercatabolic state, or both. PEM accentuates the physiologic loss of fat-free mass (FFM) that occurs with advancing age, and that affects muscle mass in particular (5). Protein depletion leads to physical frailty and immune depression (6), exposing the elderly to falls, infections, and a decrease in functional ability. This deterioration of the overall condition has direct consequences for morbidity and mortality (7, 8).

The decline of lean body mass in the elderly was long thought to be paralleled by a decrease in metabolic functions, especially protein metabolism because of the lower contribution of muscle to whole-body protein turnover in the elderly (9). In studies in which whole-body protein turnover was compared between healthy elderly subjects and young adults, turnover was reported to be lower in elderly subjects when rates were expressed per kilogram of body weight, but to be not significantly different when the results were expressed per kilogram of FFM (10–13). Malnourished or ill elderly subjects have even higher protein turnover values per kilogram of body weight than do healthy elderly persons as a result of a hypercatabolic state (14, 15).

The influence of increasing protein intake on nitrogen metabolism was studied extensively in children and in young adults and increased protein intake was shown to stimulate protein kinetics (16–18). The findings were the same in healthy elderly subjects (19). However, there are few data on the response to such dietary changes in malnourished elderly persons, although nutritional replenishment of these patients is an important medical concern (15). Furthermore, rarely have the feeding and fasting components of whole-body protein metabolism in response to protein intake been studied under like conditions in the elderly: available data were obtained in the postabsorptive state (19), in the absorptive state (20), or during prolonged periods of both feeding and fasting (11). Therefore, the purpose of our study was to assess the effect of increased protein and energy intakes on protein metabolism, by using short-term oral supplementation,

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TABLE 1
Baseline characteristics of subjects

	Young healthy supplemented (n = 6 M, 6 F)	Elderly malnourished supplemented (n = 7 M, 10 F)	Elderly malnourished control (n = 3 M, 3 F)
Age (y)	29 ± 4 (22–37)[1]	80 ± 7 (69–90)	76 ± 6 (69–87)
BMI (kg/m^2)	22.4 ± 2.9 (19.6–25.0)	20.7 ± 3.9 (14.0–25.9)	20.2 ± 2.5 (17.5–24.9)
Weight loss (%)[2]	—	13 ± 9 (5–36)	12 ± 6 (6–25)
Albumin (g/L)	52.5 ± 4.5 (46.2–57.4)	35.2 ± 4.4 (28.6–44.0)	36.0 ± 5.2 (31.7–44.9)

[1] $\bar{x}$ ± SD; range in parentheses.
[2] Compared with usual body weight.

in malnourished, hospitalized elderly patients. The parallel changes in body composition and resting energy expenditure (REE) were also studied. A group of healthy young subjects was studied for comparison.

SUBJECTS AND METHODS

Subjects

The protocol was approved by the Ethical Committee of the St Germain-en-Laye Hospital. All subjects gave informed consent to participate in the study.

Elderly subjects

Twenty-three malnourished elderly volunteers (10 men and 13 women) participated in this study. Their characteristics at baseline are shown in **Table 1**. The subjects were patients recruited at Avicenne Hospital, Bobigny, France, and identified by clinical assessment, ie, a weight loss > 5% of their usual body weight (estimated from the patient's recall and confirmed by the patient's family whenever possible), a serum albumin concentration < 35 g/L, or both. There was no detailed assessment of the nutritional status of the patients at the time of admission. The medical conditions and dietary intakes of the patients were, however, consistent with a previous malnourished state that played an important role in their admission to the hospital. In most cases, weight loss was progressive and occurred over 6–12 mo. One patient in each experimental group had a high serum albumin concentration and, although the patients might have been dehydrated at the time of blood sampling, their 9% and 11% weight losses confirmed their malnourished states. All the other patients had serum albumin concentrations < 40 g/L. Five patients, despite having a body mass index (BMI; in kg/m^2) > 24, were categorized as being malnourished on the basis of weight loss and serum albumin concentration. The extent of malnutrition, evaluated by using the Buzby index, averaged 90.4 ± 8.1 (range: 75.8–105.3), placing the elderly subjects in a moderate class of malnutrition (21). Most of the subjects (21 of 23) lived at home before their hospitalization.

The medical conditions of the elderly patients on admission to the hospital were mobility impairment (n = 7) or acute events, including digestive bleeding (n = 3), diarrhea (n = 3), hemorrhoidal disease (n = 2), acute bronchopneumonia (n = 2), ophthalmic surgery (n = 1), asthma attack (n = 1), psoriatic arthritis (n = 1), iatrogenic bradycardia (n = 1), and need for bedsore care (n = 1). The acute conditions were fully treated at the time of enrollment in the study.

Elderly subjects entered the study 12 ± 1.5 d after hospital admission ($\bar{x}$ ± SEM; range: 1–27 d) and were divided into 2 groups: 17 subjects (7 men and 10 women) received oral nutritional support and 6 subjects (3 men and 3 women) received no supplementation (control group). The subjects were assigned sequentially to the supplemented or control groups (1 of every 4 patients). Data collection lasted for 12 mo. Apart from the 23 elderly subjects enrolled in the study, 12 other subjects who satisfied the inclusion criteria were screened; however, 6 of these could not enter the study because of falls (n = 2), intercurrent medical events (n = 2), or knee or hip prosthesis (n = 2). Six subjects entered the study but did not complete it because of refusal after beginning the study (n = 2), intercurrent medical events (n = 2), incomplete urine collection (n = 1), or discovery of a neoplastic disease (n = 1). Patients with significant cognitive impairment were excluded from the study, as were patients with neoplastic disease or pathologies related to food ingestion or nutrient absorption.

Young subjects

Twelve healthy young adults (6 men and 6 women) with stable body weights and no history of gastrointestinal disease were included in the supplementation protocol. The mean age of these subjects was 29 y (range: 22–37 y) and their mean BMI was 22.4. The young subjects were medical students or members of the hospital staff. For the duration of the study, the subjects ate their meals together in the hospital refectory. They were not hospitalized except for the 24 h after each dietary period, allowing for measurement of body composition, calorimetry, and whole-body protein kinetics. The subjects were asked to maintain a normal level of activity but to avoid participating in sports for the duration of the study.

Dietary supplementation and study design

The study lasted 17 d for each subject. During a baseline period of 7 d (period 1), the young healthy subjects ate a standard diet that provided 7.53 MJ/d (11.6% as protein, 33.2% as fat, and 55.2% as carbohydrate). The protein intake ranged from 0.52 to 1.12 $g \cdot kg^{-1} \cdot d^{-1}$ and the range of the ratios of energy intake to REE was 1.06–1.49 ($\bar{x}$ ± SEM: 1.29 ± 0.16) during the baseline period and 1.30–1.79 ($\bar{x}$ ± SEM: 1.54 ± 0.20) during the supplementation period. In the malnourished elderly subjects, individual dietary intakes were assessed by hospital dietitians and served as a basis for the dietary intakes proposed for the following days. Thereafter, for the duration of the study, actual dietary intakes of the elderly subjects were recorded by the medical staff. During the baseline period (5–7 d), the mean dietary intakes were 5.62 ± 1.37 MJ/d (12.3% as protein) and 5.94 ± 0.81 MJ/d (14.2% as protein) in the supplemented and control groups, respectively; the difference between groups was not significant. The mean protein intake ranged from

0.52 to 1.38 $g \cdot kg^{-1} \cdot d^{-1}$ and the range in the ratios of energy intake to REE was 0.68–1.92 ($\bar{x}$ ± SEM: 1.31 ± 0.36).

During the next 10-d period (period 2), 400 mL of an oral high-protein liquid formula (Nutrigil HP; Jacquemaire-Santé, BSA) was added to the diets of all young subjects and of 17 elderly subjects but not to the diets of the 6 elderly control subjects, who continued to receive the same amounts of food as during the baseline period. The daily supplementation provided 1.67 MJ, 30 g protein (calcium caseinates), 50 g carbohydrate, 9 g lipids, minerals (180 mg Na, 680 mg K, 460 mg Ca, 460 mg P, 48 mg Mg, 7.2 mg Fe, and 7.6 mg Zn), and vitamins (400 μg A, 2.4 μg D, 5 mg E, 30 mg C, 0.7 mg thiamin, 0.8 mg riboflavin, 9 mg niacin, 3 mg pantothenic acid, 1 mg B-6, 0.5 mg B-12, 100 μg folate, and 76 μg biotin). During period 2, the young healthy subjects consumed 9.20 MJ/d and 82 g protein/d (14.5%), whereas the supplemented and control groups of malnourished elderly subjects had a mean total energy intake of 7.43 ± 1.31 MJ/d (5.86 MJ from meals plus 1.57 MJ from dietary supplements) and 6.20 ± 0.78 MJ/d, respectively; mean total protein intakes were 71 g/d (19.4% of total energy intake) and 54 g/d (14.3% of total energy intake), respectively ($P < 0.02$). At the end of each dietary period, protein metabolism and body composition were measured to assess the effect of supplementation. Data for the elderly malnourished control subjects were used to control for the changes over time in dietary intake and body composition without supplementation.

Body composition and resting energy expenditure measurements

Whole-body composition was measured by using dual-energy X-ray absorptiometry (QDR 2000; Hologic, Walthman, MA) in all but 1 young subject and 3 supplemented elderly subjects. This measurement allowed for the calculation of FFM, fat mass, and appendicular skeletal muscle by summing the limbs' FFM according to the method described by Heymsfield et al (22). The scanner was calibrated during each measurement against a step phantom supplied by the manufacturer. Total body water (TBW) was assessed by single-frequency (50 kHz) bioelectrical impedance analysis (BIA 101 S; Akern RJL, Eugedia, Chambly, France) in a subsample of the supplemented groups (9 young and 10 elderly subjects) for validating the use of the equation of Watson et al (23). TBW was calculated from resistance by using the prediction equations of Kushner and Schoeller (24). REE was measured by using indirect calorimetry (Deltatrac II MBM-200; Datex, Helsinki) at the end of each dietary period in young ($n = 6$) and elderly ($n = 11$) supplemented subjects. Rates of oxygen consumption and carbon dioxide production were measured and averaged during 2 consecutive 30-min periods under thermoneutral conditions in the morning after an overnight fast. Glucose, lipid, and protein oxidation rates and energy expenditure were determined according to the method of Ferrannini (25). The protein oxidation rate was estimated from 24-h urinary nitrogen excretion, as measured by an elemental analyzer (NA 1500, series 2; Fisons Instruments, Manchester, United Kingdom).

Protein metabolism and turnover

The protein turnover study was conducted by using the end products method described by Picou and Taylor-Roberts (26) and modified by Fern et al (27). Whole-body protein turnover was measured on the last day of each dietary period with a single oral dose of 200 mg [^{15}N]glycine (99% atom [^{15}N]; Eurisotop, Saint-Aubin, France). Turnover, synthesis, and breakdown rates were estimated in either the fasted state (12 young and 10 supplemented elderly subjects) or the fed state (7 supplemented elderly subjects) from the urinary excretion of ^{15}N in ammonia and urea for 11 h. The elderly subjects were randomly assigned to the fed or fasted protocol. Under the fasted protocol, subjects neither drank nor ate from 1800 on day 1 to 0900 on day 2. They ingested the [^{15}N]glycine at 2200 on day 1. Urine was collected from 2000 to 2200 on day 1; from 2200 on day 1 to 0900 on day 2, corresponding to the 11-h period; and from 0900 to 2200 on day 2. Blood samples were taken at 2200 on day 1 and at 0900 on day 2. Under the fed protocol, the first meal was taken at 0830 after an overnight fast and basal blood sampling, urine collection, and [^{15}N]glycine administration were conducted at 0930 on day 1. Patients were then given 6 light meals, 1 every 2 h during the study day. Urine was collected from 0730 to 0930 on day 1; from 0930 to 2030 on day 1, corresponding to the 11-h period; and from 2030 on day 1 to 0930 on day 2. The second blood sample was taken at 2030 on day 1. The total energy and protein contents of the 6 meals for each subject corresponded to the subject's actual daily intake of food, established from ingesta of the previous days.

Analytic methods and calculations

Urine was collected in containers that included thymol crystal and liquid paraffin as preservatives. After the volume of urine was measured, the ammonia concentration was assayed by using an enzymatic method on a Kone automate (Kone, Evry, France). The urea concentration of both plasma and urine was determined by using an enzymatic method on a dimension automate (Dupont de Nemours, Les Ulis, France). Urea and ammonia were isolated by using the method described by Preston and McMillan (28), using a sodium-potassium form of the cation exchange resin (BioRad Dowex AG-50X8, 100–200 mesh; Interchim, Montluçon, France). The preparation of the sodium-potassium form of the resin was described previously (29). For plasma urea extraction, 2 mL plasma was added to 100 mg solid 5-sulfosalicylic acid, mixed, and kept for 1 h at 4 °C. After centrifugation at 2400 × *g* for 20 min at 4 °C, the supernate was collected. From the urine, ammonia was extracted first by using the prepared sodium-potassium form of the cation exchange resin. Excess fluid was collected for urea extraction. The urea was extracted from both plasma supernate and ammonia-free urine extract by hydrolysis with urease for 2 h at 30 °C on the cation exchange resin. The resins were washed 3 times with distilled water and stored at 4 °C. Before isotopic determination, ammonia and urea-derived ammonia were eluted from the resins by the addition of 2.5 mmol $KHSO_4$/L.

The total nitrogen content of the urine was measured by using an elemental nitrogen analyzer (NA 1500 series 2; Fisons Instruments). The [^{15}N]:[^{14}N] isotope ratios of urinary ammonia, urea, and plasma urea were determined by isotope ratio mass spectrometry: an aliquot of the liquid plasma or urine sample was burned at 1020 °C in an elementary analyzer coupled with an isotope ratio mass spectrometer (Optima; Fisons Instruments). The isotope ratio was measured in reference to a calibrated [^{15}N]:[^{14}N] nitrogen tank.

We assumed that at a steady state the following equilibrium was reached for the free amino acid pool:

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

TABLE 2
Dietary intakes and body composition of supplemented young, supplemented elderly, and control elderly subjects[1]

	Young healthy supplemented (n = 12)		Elderly malnourished supplemented (n = 17)		Elderly malnourished control (n = 6)	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Dietary intake						
Protein intake						
g·kg body wt^{-1}·d^{-1}	0.8 ± 0.2	1.3 ± 0.3[2]	0.9 ± 0.3	1.4 ± 0.3[2]	1.0 ± 0.2	1.1 ± 0.3[3]
g·kg FFM^{-1}·d^{-1}	1.1 ± 0.3	1.8 ± 0.4[2]	1.3 ± 0.3	2.0 ± 0.3[2]	1.4 ± 0.4	1.5 ± 0.3[3]
Energy intake						
kJ·kg body wt^{-1}·d^{-1}	118 ± 28	144 ± 33[2]	116 ± 35	146 ± 33[2]	119 ± 12	123 ± 10[4]
kJ·kg FFM^{-1}·d^{-1}	166 ± 40	200 ± 46[2]	168 ± 43	209 ± 38[2]	170 ± 16	177 ± 22[4]
Body composition[5]						
Body weight (kg)	64.5 ± 14.5	64.5 ± 13.7	56.1 ± 9.4	56.9 ± 9.1[6]	53.4 ± 9.7	53.6 ± 9.7[7]
Fat-free mass (kg)	46.3 ± 10.9	46.9 ± 10.6	36.7 ± 5.4	37.9 ± 5.4[8,9]	38.3 ± 9.7	38.3 ± 9.6[10]
ASM (kg)	19.5 ± 5.6	19.5 ± 5.3	13.9 ± 2.6	14.5 ± 2.6	14.1 ± 4.3	14.2 ± 4.5
TBW (L)	34.2 ± 7.6	34.6 ± 7.4	28.6 ± 4.9	29.1 ± 3.9	—	—
Fat mass (kg)	15.6 ± 6.1	15.1 ± 5.5	17.8 ± 6.5	17.4 ± 6.3	13.3 ± 4.8	13.5 ± 4.2
Percentage fat (%)	24.0 ± 7.3	23.3 ± 6.7	31.1 ± 8.1	29.9 ± 7.9[7,9]	25.4 ± 9.4	25.7 ± 8.6[11]

[1] $\bar{x}$ ± SD. Period 1, 7-d baseline period; period 2, 10-d supplementation period (control subjects not supplemented).
[2] Significantly different from period 1, $P < 0.0001$ (paired Student's t test).
[3,4,10,11] Significantly different from elderly malnourished supplemented subjects (one-way ANOVA): [3] $P < 0.0001$, [4] $P < 0.002$, [10] $P < 0.02$, [11] $P < 0.05$.
[5] Assessed on the last day of period 1 (day 7) and the last day of period 2 (day 17). Body weight, fat-free mass, appendicular skeletal muscle (ASM), fat mass, and percentage fat were measured by dual-energy X-ray absorptiometry in 11 young healthy subjects, 14 elderly malnourished supplemented subjects, and 6 elderly malnourished control subjects. Total body water (TBW) was measured by bioelectrical impedance analysis in 9 young healthy subjects and 10 elderly malnourished supplemented subjects.
[6,8] Significantly different from young healthy supplemented subjects (one-way ANOVA): [6] $P < 0.001$, [8] $P < 0.05$.
[7] Marginal means significantly different between day 7 and day 17 (both groups combined), $P < 0.005$ (paired Student's t test).
[9] Significantly different from day 7, $P < 0.001$ (paired Student's t test).

intake, B is the rate of protein breakdown, S is the rate of protein synthesis, and E is the rate of nitrogen excretion. When turnover was measured in the fasted state ($I = 0$), $Q = B$. The protein turnover rate (Q, g N/11 h) was calculated for each end product, ie, ammonia and urea, as follows:

$$Q_x = E_x \times d/e_x \quad (2)$$

where E_x is nitrogen excretion in the form of ammonia or urea, d is the amount of isotope ingested as [^{15}N]glycine, and e_x is the amount of [^{15}N] excreted as ammonia in 11 h or, for urea, the amount of [^{15}N] excreted as urea in 11 h corrected for the amount retained in the body urea pool. This pool was estimated from the plasma urea concentration and its volume of repartition was considered to be the TBW, either measured by bioelectrical impedance anlaysis or calculated by using the equation of Watson et al (23). The calculation of whole-body protein turnover was based on the arithmetic average of Q_{urea} and $Q_{ammonia}$. For each 11-h protocol, the net protein balance was calculated as the difference between synthesis and breakdown rates. In the supplemented elderly subjects, the net diurnal protein balance was derived from the sum of the net protein balances for the fasted and fed states.

Statistics

One-way analysis of variance (ANOVA) was used to compare changes in dietary intake and body composition between the supplemented and unsupplemented elderly subjects; the change from period 1 to period 2 was the dependent variable. The effect of age on changes in dietary intake, body composition, REE, and fasted protein turnover was analyzed between supplemented young and elderly subjects by using one-way ANOVA. The effect of feeding compared with fasting in the supplemented elderly subjects was analyzed by using one-way ANOVA. If the result of the ANOVA was significant, the effect of oral supplementation was tested within each group by a paired Student's t test because all subjects acted as their own controls. If the ANOVA result was not significant, only the marginal means (based on all of the data from both groups) were compared. Comparisons between groups were made by using an unpaired Student's t test. The relations among protein synthesis, protein breakdown, net protein balance, and protein intake were assessed by simple linear regression analyses. Data are given as means ± SDs. Analyses were performed by using STATVIEW 4.5 (Abacus Concepts Inc, Berkeley, CA).

RESULTS

Dietary intake, body composition, and resting energy expenditure

The effect on body composition of increased protein and energy intakes was examined (**Table 2**). During period 1, protein and energy intakes expressed per kilogram of body weight and kilogram of FFM did not differ significantly between groups. Oral supplementation (period 2) led to a significant increase in protein and energy intakes in young healthy subjects and elderly malnourished subjects; the increase did not differ significantly between the young and the elderly subjects (ie, 0.5 g protein and 26–30 kJ·kg^{-1}·d^{-1}). In the elderly malnourished control group, protein and energy intakes did not increase significantly during period 2 and were significantly lower than in supplemented subjects.

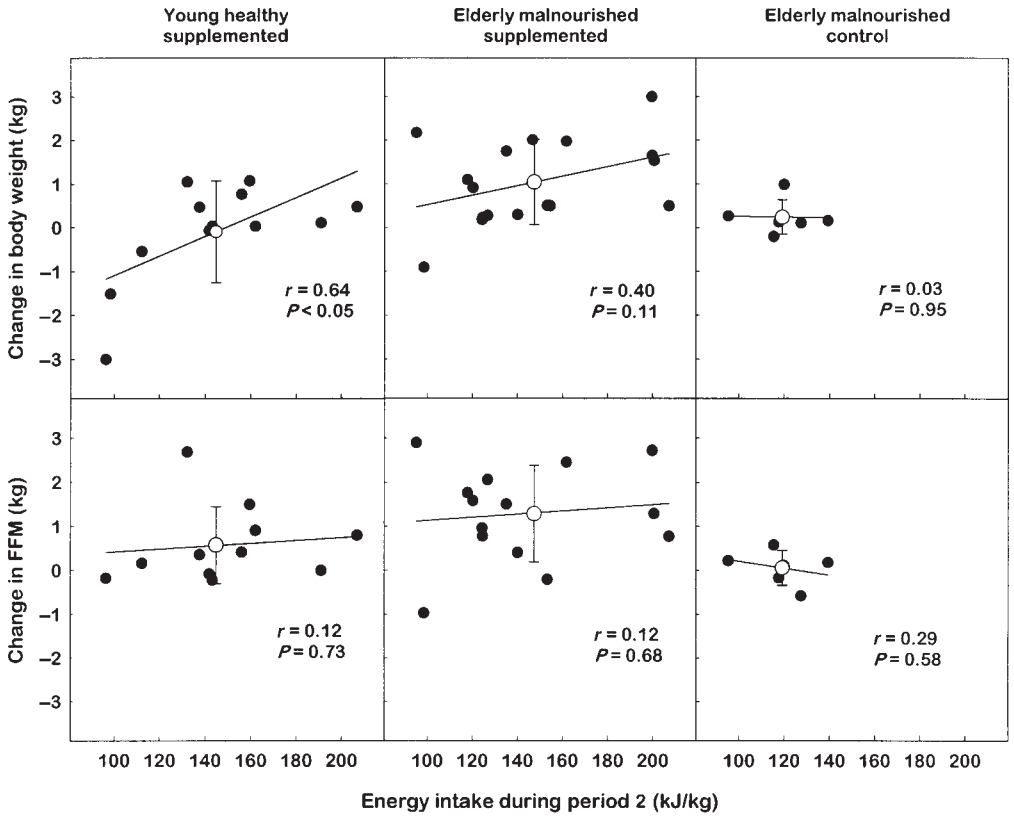

FIGURE 1. Changes in body weight and fat-free mass (FFM) between the last day of period 2 (a 10-d supplementation period; day 17) and the last day of period 1 (a 7-d baseline period; day 7) as a function of energy intake per unit of body mass during period 2 in young healthy subjects, elderly malnourished subjects, and elderly malnourished control subjects. Shown are simple regression lines and means ± SDs (○).

There were significant diet effects (ANOVA) on FFM and percentage of fat when the elderly supplemented and control groups were compared (Table 2). There were significant age effects on body weight and FFM when supplemented young and elderly subjects were compared. In young healthy subjects, supplementation had no significant effect on weight or on any body compartment on average, but the individual changes in body weight correlated positively with the energy intake per kilogram of body weight in this group (**Figure 1**). In contrast, in elderly malnourished subjects, supplementation resulted in a significant mean increase in FFM (3.7%), whereas there was no significant correlation of energy intake with the individual changes in body weight or FFM (Figure 1). In elderly malnourished control subjects, body composition did not vary significantly during the study.

There were no significant age effects of supplementation on the changes in REE expressed as kilojoules per day when young and elderly subjects were compared (ANOVA). REE expressed in kilojoules per day was significantly lower in malnourished elderly subjects than in healthy young subjects on days 7 and 17 ($P < 0.01$) (**Figure 2**). The changes in REE between day 7 and day 17 did not correlate with the changes in FFM. When expressed on an FFM basis ($kJ \cdot kg\ FFM^{-1} \cdot d^{-1}$), no significant difference was observed in REE between the young healthy subjects and the elderly malnourished subjects or between dietary periods. No changes in the percentages of oxidation of protein, fat, or carbohydrates were observed between groups or dietary periods.

Nitrogen kinetics and accretion

There was a significant age effect of supplementation on changes in ammonia flux and protein synthesis in the fasted subjects, on the basis of both body weight and FFM (**Table 3**). There was a significant feeding effect of supplementation on change in net protein balance when fed and fasted elderly subjects were compared. In the elderly subjects, supplementation induced an increase in ammonia flux and in protein synthesis and a decrease in net protein balance both per kilogram of body weight and per kilogram of FFM in the fasted state and an increase in net protein balance in the fed state. These changes resulted in a significant increase in net diurnal protein balance (sum of the fed and fasted net protein balance) in the elderly subjects after supplementation.

Protein kinetics and protein intake in individual subjects

Synthesis, breakdown, and net protein balance rates were plotted as a function of protein intake in individual subjects to analyze precisely the effect of protein intake on protein kinetics (**Figure 3**). In the fasted state, there was a significant positive correlation between protein intake and synthesis in elderly malnourished subjects and a similar trend in young healthy subjects. There was also a significant negative correlation between protein intake and breakdown fluxes in both groups. Net protein balance was also negatively correlated with protein intake in young and elderly fasted subjects. Increasing protein intake had a more pronounced effect on fasting protein synthesis in elderly malnourished subjects (slope: 0.97) than in young healthy subjects

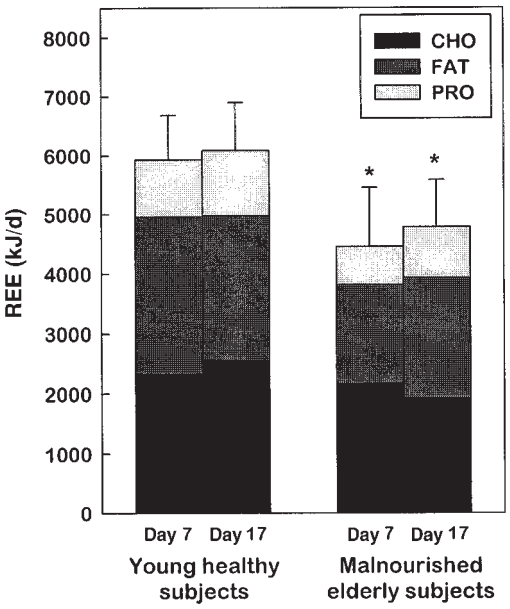

FIGURE 2. Mean (±SD) resting energy expenditure (REE) in young (n = 6) and malnourished elderly (n = 11) supplemented subjects before (day 7) and after (day 17) supplementation, with the repartition of oxidation of carbohydrates (CHO), lipids (FAT), and proteins (PRO). There was no age effect on the changes in REE; the marginal means differed significantly between day 7 and day 17 ($P < 0.05$). Mean REE expressed in kilojoules per day was significantly lower in the elderly subjects than in the young subjects at days 7 and 17 ($^{*}P < 0.01$) but not when expressed in kilojoules per kilogram of FFM per day.

(slope: 0.33). When measurements were carried out in the fed state in elderly subjects, there was no correlation between dietary protein and synthesis or breakdown rates. However, net protein balance was positively correlated with protein intake. Thus, the resulting plot of the net diurnal protein balance (ie, sum of the fed and fasted net protein balances) was

$$y = 0.319x - 7.3 \times 10^{-3} \quad (3)$$

The slope of this combined line was significantly different from zero ($P < 0.0001$). When expressed per FFM, the resulting line was similar ($y = 0.242x + 0.113$).

DISCUSSION

This study was designed to assess the response of body composition and protein metabolism to short-term oral supplementation in elderly malnourished patients. The results show that this supplementation significantly increased protein and energy intakes during the 10-d supplementation period and was associated with an increase in FFM and a stimulation of nitrogen kinetics in malnourished elderly patients.

An important finding was the variation in protein fluxes after energy and protein intakes were increased in supplemented elderly subjects. Supplementation produced 3 main effects in the diet. First, it resulted in a change in the mean protein intake from an intake near the recommended dietary allowance (30) to a moderately high intake. Second, it allowed for an increase in total energy intake. Third, it induced a rise in the ratio of protein to energy. Of these changes, the most influential in terms of protein kinetics was probably the increase in protein intake because protein intake is the main dietary determinant of whole-body protein turnover (31) and the energy provision was probably not limiting.

In most of the studies in which the relation between protein intakes and whole-body protein turnover flux was examined, increasing protein intake induced an increase in nitrogen excretion and in whole-body protein turnover, protein synthesis, and protein degradation in healthy young and elderly subjects (17, 18, 32). Consistent with these findings, we showed a significant correlation between protein intake and net protein balance, ie, synthesis minus breakdown rates in young and malnourished elderly subjects. To date, the only study in which the effect of refeeding on protein metabolism in malnourished elderly subjects was assessed did not show a significant increase in protein kinetics or in net protein balance, although a trend toward that was observed (15).

In young healthy volunteers, the change in dietary conditions led to a slight increase in all fluxes but the differences were not significant. In this context, the relatively low energy intake might have attenuated the effect of an additional 30 g dietary protein/d. In elderly subjects, we observed a significant stimulation of protein kinetics after protein and energy supplementation. The comparison between fasted and fed fluxes clearly indicated the influence of dietary change on the diurnal cycling of protein losses and deposition. As reported for young adults with increasing protein intakes (32), there was a parallel increase in nitrogen loss during the overnight fasting periods and in protein gain during feeding. In agreement with the results of previous studies in young adults, the losses in the fasting state were linked to the increasing breakdown rate that resulted from higher protein intake, whereas the increase in the fed state was the result of a slight inhibition of the breakdown rate associated with a slight stimulation of the protein synthesis rate (33). These variations in the amplitude of diurnal cycling resulted in a net protein deposition, ie, a higher absorptive protein gain than the postabsorptive protein losses, which was positively correlated with dietary protein intake. Furthermore, in the fed state, elderly malnourished subjects showed a trend toward a decrease in the ratio of Q_u to Q_a after supplementation (the relative increase in Q_a was 2-fold that measured for Q_u), suggesting a possible increase in muscle mass because there is a positive correlation between Q_u:Q_a and the ratio of nonmuscle mass to muscle mass in the fed state (34). These results are consistent with the measured increase in FFM and, more specifically, in appendicular skeletal muscle. Although the modulation of protein metabolism by protein intake in young adults is well known, this is the first report of such findings in elderly malnourished patients. These effects, together with their associated effects on body composition, are likely to be of clinical interest in this population.

We could not provide absolute protein turnover values in young subjects or malnourished elderly subjects because the results depend on the methods used (end products method or precursor method), even though several methods were shown to give comparable results in young adults (33). Pannemans et al (35) underlined a tracer-dependent assessment of whole-body protein turnover variations in response to an increase in protein intakes in elderly women. In that study, protein kinetics measured with either [^{15}N]glycine or [1-^{13}C]leucine were compared in the postabsorptive state after 2 different protein intake adaptations. Measurements based on [^{15}N]glycine allowed for the detection of a variation in protein turnover, whereas a variation was not detected when the [1-^{13}C]leucine tracer was used. Thus, the authors

TABLE 3
Protein kinetics in young and elderly supplemented subjects before (day 7) and after (day 17) supplementation[1]

	Young fasted (n = 12)		Elderly fasted (n = 10)		Elderly fed (n = 7)	
	Day 7	Day 17	Day 7	Day 17	Day 7	Day 17
Ammonia (Q_a)						
g P·kg body wt^{-1}·11 h^{-1}	1.6 ± 0.3	1.8 ± 0.4	2.1 ± 1.0	2.7 ± 1.2[2,3]	1.9 ± 0.7	2.5 ± 0.7[4]
g P·kg FFM^{-1}·11 h^{-1}	2.2 ± 0.4	2.5 ± 0.4	2.9 ± 1.2	3.7 ± 1.3[2,3]	2.9 ± 0.9	3.8 ± 1.1[4]
Urea (Q_u)						
g P·kg body wt^{-1}·11 h^{-1}	1.2 ± 0.6	1.5 ± 0.3	1.0 ± 0.6	1.5 ± 0.5[4]	2.5 ± 0.3	2.9 ± 0.2[4]
g P·kg FFM^{-1}·11 h^{-1}	1.7 ± 0.9	2.0 ± 0.4	1.5 ± 0.6	2.2 ± 0.5[4]	3.8 ± 0.6	4.4 ± 0.6[4]
Q_u:Q_a	0.75 ± 0.28	0.82 ± 0.19	0.54 ± 0.30	0.62 ± 0.22	1.37 ± 0.40	1.24 ± 0.37
Nitrogen excretion						
mg N·kg body wt^{-1}·11 h^{-1}	38.6 ± 19.2	53.0 ± 13.6	30.7 ± 30.2	50.3 ± 30.0[4]	88.6 ± 42.0	101.0 ± 38.2[4]
mg N·kg FFM^{-1}·11 h^{-1}	54.5 ± 28.4	73.4 ± 20.3	42.5 ± 36.5	68.4 ± 34.9[4]	135.3 ± 63.2	149.6 ± 46.8[4]
Protein turnover[5]						
g·kg body wt^{-1}·11 h^{-1}	1.4 ± 0.4	1.6 ± 0.3	1.6 ± 0.8	2.1 ± 0.8[4]	2.2 ± 0.5	2.7 ± 0.4[4]
g·kg FFM^{-1}·11 h^{-1}	2.0 ± 0.6	2.3 ± 0.3	2.2 ± 0.9	2.9 ± 0.9[4]	3.4 ± 0.6	4.1 ± 0.9[4]
Protein synthesis[5]						
g·kg body wt^{-1}·11 h^{-1}	1.2 ± 0.3	1.3 ± 0.2	1.4 ± 0.6	1.8 ± 0.7[2,3]	1.6 ± 0.4	2.1 ± 0.4[4]
g·kg FFM^{-1}·11 h^{-1}	1.6 ± 0.4	1.8 ± 0.2	1.9 ± 0.7	2.5 ± 0.7[2,3]	2.5 ± 0.6	3.2 ± 0.8[4]
Protein breakdown[5]						
g·kg body wt^{-1}·11 h^{-1}	1.4 ± 0.4	1.6 ± 0.3	1.6 ± 0.8	2.1 ± 0.8[4]	1.2 ± 0.4	1.3 ± 0.6[4]
g·kg FFM^{-1}·11 h^{-1}	2.0 ± 0.6	2.3 ± 0.3	2.2 ± 0.9	2.9 ± 0.8[4]	1.9 ± 0.7	2.1 ± 1.0[4]
Net protein balance[6]						
g·kg body wt^{-1}·11 h^{-1}	−0.24 ± 0.12	−0.33 ± 0.09	−0.19 ± 0.19	−0.32 ± 0.19[4]	0.41 ± 0.24	0.73 ± 0.27[2,7]
g·kg FFM^{-1}·11 h^{-1}	−0.34 ± 0.18	−0.46 ± 0.13	−0.27 ± 0.22	−0.43 ± 0.21[4]	0.64 ± 0.39	1.08 ± 0.30[2,8]

[1] $\bar{x}$ ± SD. Measurements based on the end products method with oral [^{15}N]glycine.
[2] Significantly different from day 7, $P < 0.005$ (paired Student's t test).
[3] Significantly different from young fasted subjects, $P < 0.05$ (one-way ANOVA).
[4] Marginal means significantly different between day 7 and day 17 (both groups combined), $P < 0.02$ (paired Student's t test).
[5] Calculated as the arithmetic average of urea and ammonia rates.
[6] Net protein balance = synthesis − breakdown.
[7,8] Significantly different from elderly fasted subjects (ANOVA): [7] $P < 0.01$, [8] $P < 0.001$.

focused on the importance of the tracer choice. In particular, the physiologic validity of the use of [^{15}N]glycine may be criticized in terms of the ability of this amino acid to correctly reflect the amino nitrogen precursor pool for protein synthesis. However, in the present study one important result was that supplementation led to an increase in protein kinetics in sick elderly persons that was strongly corroborated with body-composition data, ie, an increase in FFM and, especially, in appendicular skeletal muscle, along with a slight increase in REE. Furthermore, we estimated the resulting body protein gain in elderly subjects after 10 d of supplementation from the combination of the net protein balances obtained in the fed and the fasted state, because they lasted for 11 h and were suitable to figure the 2 components of the diurnal cycle. The net protein gain was 0.45 g·kg body wt^{-1}·d^{-1}. Over a 10-d period, considering that the deposition of 1 g N corresponds to an increase of 30 g hydrated lean tissue (36) and using a factor of 6.25 to convert nitrogen to protein, we found a mean theoretical gain of 1.2 kg, which is consistent with our body-composition results. Thus, we have convincing reasons to consider that the protein turnover variations and the resulting net protein deposition we measured in the elderly subjects were in fact not derived from any artifact linked to the measurement method used. In the same manner, Castaneda et al (37) reported complementary metabolic responses to a 9-wk low-protein diet in elderly women, ie, a loss of body cell mass and the occurrence of physiologic impairments associated with a fall in both leucine flux and oxidation. However, there was no influence of marginal protein intake on protein synthesis and breakdown rates.

The question as to whether protein turnover is modified with aging has been studied extensively and it now appears clear that there is no decrease in whole-body protein flux expressed per kilogram of FFM (11–13, 38–41). However, all of these studies were conducted in healthy elderly subjects and much less information is available concerning sick elderly people, in particular, during nutritional rehabilitation after illness. The protein kinetics values we measured in malnourished elderly subjects agree well with those described by Phillips (14) in sick and malnourished elderly subjects but were higher than those described in malnourished elderly subjects by Beaumont et al (15). In young adults, it was shown previously that chronic undernutrition leads to a lower Q_a and a lower protein synthesis rate in the fed state than that in healthy age-matched subjects (34). We could not compare our data from malnourished elderly subjects with data from healthy age-matched subjects, so this point would need further investigation. When fasted sick elderly subjects and healthy young subjects were compared, there was no significant difference between basal protein kinetics; the 2 main age effects of supplementation were a higher stimulation of Q_a and of the protein synthesis rate. After supplementation, Q_u:Q_a in the fasted state was significantly higher in young subjects, which is surprising because Q_u reflects urea labeling in the liver whereas Q_a is indicative of ammonia labeling from glutamine metabolism in the periphery (27), and

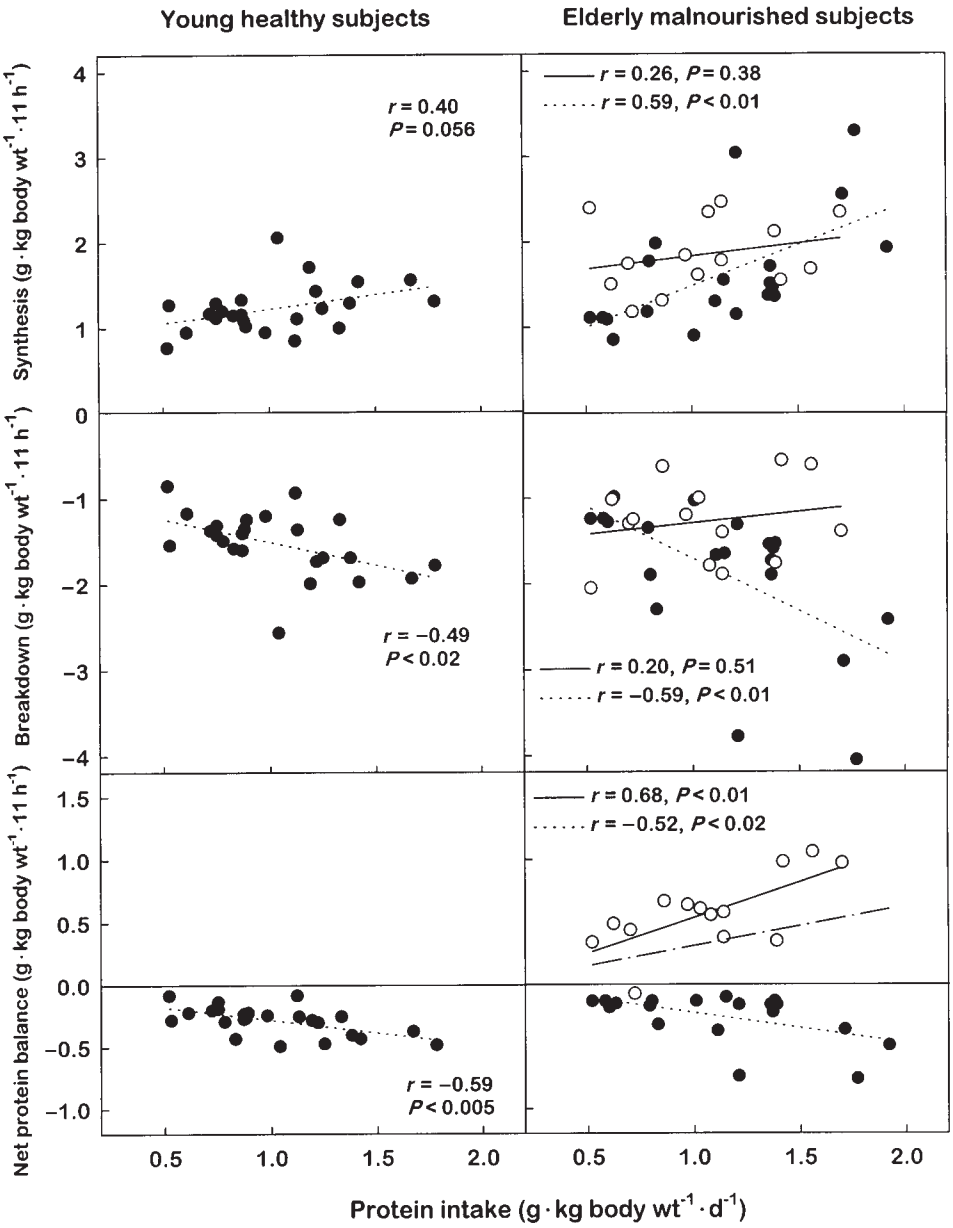

FIGURE 3. Protein synthesis, protein breakdown, and net protein balance as a function of protein intake in fasted young healthy subjects and fasted (●; ···) and fed (○; —) elderly malnourished subjects. All subjects at each of the 2 measurements with [^{15}N]glycine are included. In elderly subjects, the resulting equation (– ·) from postabsorptive and absorptive net protein balance was $y = 0.319x - 7.3 \times 10^{-3}$.

because the elderly subjects in our study were expected to have a fall in Q_a, probably because of both malnutrition and age-related sarcopenia. Finally, the differences between the protein kinetics of healthy young subjects and malnourished elderly subjects were of minor importance despite the discrepancy of metabolic states between these 2 populations, ie, aging and malnutrition.

In conclusion, we addressed the possibility of inducing an anabolic response of protein metabolism in moderately malnourished elderly subjects by short-term dietary supplementation. Increasing energy and protein intakes and the ratio of protein to energy in these subjects led to a positive net diurnal protein balance with a concomitant FFM gain. These changes are likely to produce beneficial functional effects that require further study. Measurement of muscle protein turnover would help in understanding the mechanisms that produce an anabolic response after refeeding in malnourished elderly persons.

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