Protein pulse feeding improves protein retention in elderly women

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ABSTRACT

Background: Adequate protein nutrition could be used to limit gradual body protein loss and improve protein anabolism in the elderly.

Objective: We tested the hypothesis that an uneven protein feeding pattern was more efficient in improving protein anabolism than was an even pattern.

Design: After a controlled period, 15 elderly women (mean age: 68 y) were fed for 14 d either a pulse diet (n = 7), providing 80% of the daily protein intake at 1200, or a spread diet (n = 8), in which the same daily protein intake was spread over 4 meals. Both diets provided 1.7 g protein · kg FFM–1 · d–1. Protein accretion and daily protein turnover were determined by using the nitrogen balance method and the end product method (ammonia and urea) after an oral dose of [15N]glycine.

Results: Nitrogen balance was more positive with the pulse than with the spread diet (54 ± 7 compared with 27 ± 6 mg N · kg FFM–1 · d–1; P < 0.05). Protein turnover rates were also higher with the pulse than with the spread diet (5.58 ± 0.22 compared with 4.98 ± 0.17 g protein · kg FFM–1 · d–1; P < 0.05), mainly because of higher protein synthesis in the pulse group (4.48 ± 0.19 g protein · kg FFM–1 · d–1) than in the spread group (3.75 ± 0.19 g protein · kg FFM–1 · d–1) (P < 0.05).

Conclusion: A protein pulse-feeding pattern was more efficient than was a protein spread-feeding pattern in improving, after 14 d, whole-body protein retention in elderly women. Am J Clin Nutr 1999;69:1202–8.

KEY WORDS Aging, protein feeding pattern, fat-free mass, nitrogen balance, protein metabolism, elderly women, protein retention

INTRODUCTION

In elderly humans there are marked alterations in body composition, particularly a loss of fat-free mass (FFM), most of which is skeletal muscle protein (1, 2). This loss results in a decrease in muscular strength, reduced social independence, and because muscles are the major source of amino acids for other tissues, a decrease in the resistance to stress (disease or trauma) (3). More studies are necessary to determine whether improving the quantity or quality of protein nutrition in elderly people can reduce this age-related decrease in FFM and muscle protein.

A loss of protein necessarily involves an imbalance between protein synthesis and protein degradation. There is controversy as to whether whole-body protein synthesis and degradation, expressed per kg FFM, change with aging. In some experiments, no changes were found (4–7), whereas in others, protein turnover decreased with age (8). Furthermore, an alteration in muscle protein synthesis has been observed in the elderly compared with young adults (8–10). Such a decrease in protein synthesis could be involved in the age-related loss of muscle proteins.

Numerous experiments have shown that dietary protein intakes affect whole-body and muscle protein metabolism. High-protein diets in elderly people could increase nitrogen retention (11–14). However, because of the methodologic limitations of the nitrogen balance method, this positive effect is debatable (15). In other experiments (16, 17), such diets increased whole-body protein synthesis in the postabsorptive state without significant effect on nitrogen balance. Studies in mature and old rats suggest that an increase in plasma amino acids is required in old animals for stimulation of muscle protein synthesis (18, 19). This was confirmed recently in elderly humans (20). However, high protein intakes could have deleterious effects on renal function in the elderly (21). One way to maintain the advantageous effects yet reduce the deleterious effects of high-protein diets would be to maintain the total daily protein intake but modulate the pattern of protein feeding during the day by using a pulse-feeding pattern. Interestingly, modifying the pattern of protein feeding by using slow (diary amino acids are slowly absorbed) and fast (diary amino acids are rapidly absorbed) proteins (22) or by using different meal patterns (eg, 3 discrete meals compared with continuous feeding) (23) was shown to modulate protein anabolism in young adults. A protein feeding pattern that combines meals rich and low in protein during the day may improve protein anabolism in elderly people by allowing for the advantages of both diets, ie, higher protein anabolism during postprandial periods and lower

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protein catabolism during postabsorptive periods (24). We tested this hypothesis by comparing 2 protein feeding patterns: 1) a pulse diet providing 80% of the daily protein intake in the midday meal and the remaining 20% in the morning and evening meals and 2) a spread diet in which the daily protein intake was provided fairly evenly over 4 meals fed over a 12-h period.

SUBJECTS AND METHODS

Subjects

Fifteen elderly women with a mean (±SEM) age of 68 ± 1 y participated in this study. Each subject was determined to be healthy after a physical examination and had no history of renal, cardiovascular, or endocrine disease; no history of endocrine therapy; and no currently evolving disease. All subjects were asked to maintain their usual physical activity immediately before and during the study. The physical characteristics of the subjects are listed in Table 1. The purpose and the potential risks of the study were explained fully to the subjects and written, informed consent was obtained from each participant. The experimental protocol was approved by the Ethical Committee of Clermont-Ferrand.

Diets

Food intake was controlled rigorously in the 2 consecutive periods: a 15-d adaptive period and a 14-d experimental period. Energy intake was based on measured resting energy expenditure (REE) multiplied by an activity factor of 1.5 (26). REE was determined for each woman before the study by open-circuit indirect calorimetry (Deltatrac; Datex, Geneva).

Before the controlled dietary period, a dietitian met the subjects and customized a menu for each of them, taking care not to alter their dietary habits greatly (3 or 4 meals daily composed of usual food products). The diets were composed of ingredients selected from the following list: bread, sugar, jam, milk, butter, meat, gravy, fish, potatoes, vegetables, noodles, yogurt, cheese, juices, cakes, and fruit. All diets were provided by the experimental kitchen of the Human Nutrition Research Center. During the adaptive and experimental periods, all meals and drinks were served daily either at home or at the Human Nutrition Research Center and subjects were not allowed to eat or drink anything else except water, tea, or coffee (without sugar, milk, or any energy-containing substance). All subjects were motivated to participate in the study and were aware of the importance of compliance.

Because usual protein intake differed between the volunteers (0.8–1.6 g protein·kg body wt⁻¹·d⁻¹), we used a 15-d adaptive period to achieve a similar protein status for all subjects. During this period, subjects received a controlled diet providing 1.2 g protein·kg FFM⁻¹·d⁻¹ (ie, 0.74 g·kg body wt⁻¹·d⁻¹; Tables 2 and 3). The protein feeding pattern was similar to the subjects' usual feeding pattern, ie, the daily protein distribution was 10%, 60%, and 30% in the morning, noon, and evening meals, respectively. Energy intake was calculated by using dietary tables contained in the computer program GENI (Micro 6, Nancy, France) (Table 2). During the following 14-d experimental period, 2 protein feeding patterns were compared. In one group (n = 7), most of the daily protein intake was provided in 1 of the 3 meals to provide a pulse feeding pattern: 79% of daily protein in the noon meal and the remaining 21% in the meals fed at 0800 (7%) and 2000 (14%). This was the pulse diet. In the other group (n = 8), subjects were fed 4 meals daily at 0800, 1200, 1600, and 2000 in which the protein content was spread out more evenly. This was the spread diet (Table 3). The protein intake was greater during the experimental than during the adaptive period to promote an anabolic state. The increase in protein intake to 1.7 g protein·kg FFM⁻¹·d⁻¹ (ie, 1.05 g·kg body wt⁻¹·d⁻¹; Tables 2 and 3). The protein feeding pattern was similar to the subjects' usual feeding pattern, ie, the daily protein distribution was 10%, 60%, and 30% in the morning, noon, and evening meals, respectively. Energy intake was calculated by using dietary tables contained in the computer program GENI (Micro 6, Nancy, France) (Table 2). During the following 14-d experimental period, 2 protein feeding patterns were compared. In one group (n = 7), most of the daily protein intake was provided in 1 of the 3 meals to provide a pulse feeding pattern: 79% of daily protein in the noon meal and the remaining 21% in the meals fed at 0800 (7%) and 2000 (14%). This was the pulse diet. In the other group (n = 8), subjects were fed 4 meals daily at 0800, 1200, 1600, and 2000 in which the protein content was spread out more evenly. This was the spread diet (Table 3). The protein intake was greater during the experimental than during the adaptive period to promote an anabolic state. The increase in protein intake to 1.7 g protein·kg FFM⁻¹·d⁻¹ (ie, 1.05 g·kg body wt⁻¹·d⁻¹) was compensated for by a decrease in carbohydrates, making the adaptive and experimental periods isocaloric (Table 2). During the experimental period, 70% of the protein was from animal products (dairy products, meat, and fish) and 30% was from plant products (cereals and legumes); the protein source was similar for both the pulse and spread diet groups. The daily energy repartition was similar between the 2 groups: 50.9 ± 1.4% of energy provided by the meal fed at 1200 in the pulse group and 48.6 ± 1.5% provided by the meals fed at 1200 and 1600 in the spread group (Table 3).

Body composition

Subjects were weighed in underclothes before lunch (1200) once a week to the nearest 0.1 kg on a SECA scale (model 109; Les Mureaux, France). On days 14 and 31, FFM was measured by the H₂¹⁸O isotopic dilution method as described previously (25). Saliva samples were collected from fasted subjects before and 4, 5, and 6 h after they drank H₂¹⁸O-enriched water (0.44 g/kg

### Table 1

Physical characteristics of the subjects

<table>
<thead>
<tr>
<th>Value</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>68 ± 1 (60–73)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.4 ± 1.8 (50.3–76.0)</td>
</tr>
<tr>
<td>Fat-free mass (kg)²</td>
<td>38.3 ± 0.9 (31.7–45.0)</td>
</tr>
<tr>
<td>Fat mass (% of body wt)</td>
<td>38.2 ± 1.5 (29.3–47.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 0.8 (21.0–31.1)</td>
</tr>
<tr>
<td>REE (MJ/d)¹</td>
<td>5.1 ± 0.2 (4.3–6.6)</td>
</tr>
</tbody>
</table>

¹± SEM; range in parentheses. n = 15.
²Measured by using an ¹⁸O-enriched water dilution technique (25).
³Resting energy expenditure as determined by indirect calorimetry.

### Table 2

Energy and macronutrient intakes during the adaptive and experimental periods

<table>
<thead>
<tr>
<th></th>
<th>Spread diet</th>
<th></th>
<th>Pulse diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adaptive</td>
<td>Experimental</td>
<td>Adaptive</td>
<td>Experimental</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Energy (kJ · kg FFM⁻¹·d⁻¹)</td>
<td>194.7 ± 7.1</td>
<td>203.7 ± 7.7</td>
<td>196.1 ± 4.1</td>
<td>198.6 ± 3.9</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>10.2 ± 0.4</td>
<td>14.2 ± 0.6²</td>
<td>10.5 ± 0.3</td>
<td>14.5 ± 0.4²</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>35.7 ± 0.7</td>
<td>34.8 ± 0.7</td>
<td>35.4 ± 0.4</td>
<td>35.1 ± 0.5</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>54.1 ± 0.7</td>
<td>51.0 ± 0.9²</td>
<td>54.0 ± 0.8</td>
<td>50.4 ± 0.7²</td>
</tr>
</tbody>
</table>

¹± SEM.
²Significantly different from the adaptive period, P < 0.05 (paired Student’s t test).
Nitrogen losses were assumed to be 8 mg · kg⁻¹ body wt⁻¹ · d⁻¹ (26).

Automated Kjeldahl method with a single-channel autoanalyzer was used for the nitrogen analysis of urine, feces, and leftovers. Freeze-dried samples were homogenized, and representative aliquots were stored at -20°C until analyzed further.

Twenty-four–hour nitrogen flux was determined from the urinary excretion of [¹⁵N]ammonia during the first 12 h after [¹⁵N]glycine ingestion, which represents the rate of nitrogen flux in ammonia over 12 h in the fed state (g N/12 h).

Nitrogen balance was calculated by subtracting the daily nitrogen intake from the rate of nitrogen used for whole-body protein synthesis, I, and the rate of nitrogen used for whole-body protein breakdown, B.

Protein turnover

Whole-body protein turnover was measured according to Waterlow et al (28). Two hundred milligrams [¹⁵N]glycine (99 atom%; Mass Trace Inc, Woburn, MA) was given orally to each subject on day 27 at 0800. On days 27, 28, and 29, urine was collected over the last 5 d of the adaptive and experimental periods. After the total body water was measured by using the ¹⁸O dilution space method as described previously (25, 27), the ¹⁵N enrichment of urinary ammonia and urea was measured by using a sodium-potassium form of a cationic ion-exchange resin that specifically binds ammonia from neutral solutions (29).

Urea enrichment was determined with enzymatic methods (Ammonia kit 171-C; Sigma-Aldrich Chimie, St Quentin Fallavier, France). [¹⁵N]enrichment of urinary ammonia and urea was measured by using a mass spectrometer (IRMS VG 903 E5; Micromass, Manchester, United Kingdom).

Nitrogen flux was calculated as described previously (28):

\[ Q = E_{\text{urea}} \times d/e \]  

where \( d \) is the dose of isotopic nitrogen (g [¹⁵N]). For urea, \( Q \) is the rate of nitrogen flux in urea over 24 h (g N/24 h); \( E_{\text{urea}} \) is the excretion of urea (g N/24 h), assumed to be equal to the mean amount of urea nitrogen excreted in the urine during the 3-d collection period; and \( e \) is the amount of urea nitrogen excreted in the urine as urea in 3 d (g [¹⁵N]). For ammonia, \( Q \) is the rate of nitrogen flux in ammonia over 12 h in the fed state (g N/12 h); \( E_{\text{ammonia}} \) is the rate of ammonia nitrogen excretion (g N/12 h) in the urine during the first 12 h after [¹⁵N]glycine ingestion, and \( e \) is the amount of isotope excreted in the urine as ammonia in 12 h (g [¹⁵N]) (30).

The rates of protein synthesis and breakdown in the whole body were derived from the expression

\[ Q = E + Z = I + B \]  

where \( E \) is the rate of excretion of total nitrogen in urine, \( Z \) is the rate of nitrogen used for whole-body protein synthesis, \( I \) is the rate of nitrogen intake from the diet, and \( B \) is the rate of nitrogen used for whole-body protein breakdown. \( I \) and \( E \) were not corrected for nonabsorbed dietary nitrogen and metabolic fecal plus dermal nitrogen losses, respectively, because the corrections were minor and similar between the spread and pulse diets. Consequently, although \( Z \) was slightly overestimated and \( B \) slightly underestimated, comparative measurements between diets remained valid. A factor of 6.25 was used to convert grams of nitrogen into grams of protein.
mean daily values estimated over 3 d, whereas whole-body protein synthesis and degradation rates calculated from ammonia data represent fed state values.

Statistics

Results are given as means ± SEMs. When comparisons were made between results obtained during the adaptive period and those obtained during the experimental period, a paired Student’s t test was used because subjects acted as their own controls. Interactions between protein pattern (pulse or spread diet) and protein intake (adaptive or experimental period) were tested by two-way analysis of variance with repeated measures. The effect of diet on protein turnover (Z and B) was analyzed by using an unpaired Student’s t test. The Statview program (version 4.5; Abacus Concepts, Inc, Berkeley, CA) was used for the statistical analyses.

RESULTS

Body composition

There were no significant differences in mean body-composition indexes between the spread and pulse diet groups at the end of either the adaptive or the experimental period (Table 4). A slight but significant decrease in body weight was observed during the adaptive period (−0.66 ± 0.17 and −0.48 ± 0.25 kg in the spread and pulse diet groups, respectively) and during the experimental period. FFM, which was calculated from total-body water mass, assuming a constant coefficient of hydration, decreased significantly from baseline in the spread diet group, but was maintained in the pulse diet group.

Nitrogen balance

During the adaptive period, mean nitrogen intake was 190 ± 3 mg·kg FFM$^{-1}·d^{-1}$. Nitrogen balance was not significantly different between groups and was in equilibrium (0.4 ± 6.0 mg N·kg FFM$^{-1}·d^{-1}$), although 7 of the subjects were in slight negative nitrogen balance. During the experimental period, the increase in nitrogen intake to 267 ± 4 and 274 ± 7 mg·kg FFM$^{-1}·d^{-1}$ in the spread and the pulse diet groups, respectively, led to a significant increase in urinary nitrogen excretion in both groups (Table 5). In contrast, fecal nitrogen loss remained constant. Nitrogen balance also was significantly greater during the experimental period than during the adaptive period (Table 5). Nitrogen balance was significantly more positive with the pulse diet than with the spread diet. Thus, during the experimental period, the spread diet group had lower nitrogen gains than the pulse diet group and had a slight decrease in FFM, whereas the pulse diet group had virtually no change in FFM.

Protein turnover

When calculated from the urea data, whole-body protein turnover, synthesis, and breakdown rates were significantly greater with the pulse diet than with the spread diet: by 12%, 19%, and

| TABLE 4 | Body composition during the adaptive and experimental periods$^1$
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Spread diet</td>
<td>Pulse diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adaptive ($n = 8$)</td>
<td>Experimental ($n = 8$)</td>
<td>Absolute variation</td>
</tr>
<tr>
<td>Body weight</td>
<td>61.2 ± 2.7</td>
<td>60.8 ± 2.7</td>
<td>−0.46 ± 0.13$^3$</td>
</tr>
<tr>
<td>FFM</td>
<td>38.1 ± 0.7</td>
<td>37.8 ± 0.7$^4$</td>
<td>−0.33 ± 0.10$^3$</td>
</tr>
<tr>
<td>Fat mass</td>
<td>23.0 ± 2.2</td>
<td>22.9 ± 2.2</td>
<td>−0.13 ± 0.13</td>
</tr>
</tbody>
</table>

$^1$x ± SEM. Initial body weight was not significantly different between groups: 61.9 ± 2.5 and 64.2 ± 2.6 kg for the spread and pulse diet groups, respectively. Body-composition indexes were measured at the end of the adaptive and experimental periods. Fat-free mass (FFM) was determined by using an isotopic-dilution method; fat mass was calculated as body weight − FFM.

$^2$By two-way ANOVA with repeated measures.

$^3$Significantly different from baseline, $P < 0.05$ (Student’s $t$ tests).

$^4$Significantly different from the adaptive period, $P < 0.05$ (paired Student’s $t$ tests).

| TABLE 5 | Nitrogen balance during the adaptive and experimental periods$^1$
<table>
<thead>
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</thead>
<tbody>
<tr>
<td></td>
<td>Spread diet</td>
<td>Pulse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adaptive ($n = 8$)</td>
<td>Experimental ($n = 8$)</td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>192 ± 2</td>
<td>267 ± 4</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>147 ± 4</td>
<td>197 ± 4$^3$</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>30 ± 2</td>
<td>29 ± 2</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td>2 ± 6</td>
<td>27 ± 6$^4$</td>
<td></td>
</tr>
</tbody>
</table>

$^1$x ± SEM. Nitrogen balance was calculated by subtracting the daily nitrogen losses from the daily nitrogen intake. Corrections for miscellaneous losses of 8 mg N·kg body wt$^{-1}·d^{-1}$ were made (26).

$^2$By two-way ANOVA with repeated measures.

$^3$Significantly different from the adaptive period, $P < 0.05$ (paired Student’s $t$ tests).

$^4$Significantly different from the spread diet, $P < 0.05$ (unpaired Student’s $t$ tests).
TABLE 6
Whole-body protein turnover

<table>
<thead>
<tr>
<th></th>
<th>Spread diet (n = 8)</th>
<th>Pulse diet (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h protein turnover (g · kg FFM⁻¹ · d⁻¹)</td>
<td>4.98 ± 0.17</td>
<td>5.58 ± 0.22²</td>
</tr>
<tr>
<td>Q (urea data)</td>
<td>3.33 ± 0.16</td>
<td>3.87 ± 0.20²</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>3.75 ± 0.19</td>
<td>4.48 ± 0.19²</td>
</tr>
<tr>
<td>Fed state protein turnover (g · kg FFM⁻¹ · 12 h⁻¹)³</td>
<td>2.47 ± 0.11</td>
<td>2.61 ± 0.21</td>
</tr>
<tr>
<td>Protein intake</td>
<td>1.23 ± 0.02</td>
<td>1.50 ± 0.04²</td>
</tr>
<tr>
<td>N × 6.25 urinary excretion</td>
<td>0.63 ± 0.06</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Protein breakdown</td>
<td>1.24 ± 0.10</td>
<td>1.10 ± 0.19</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>1.84 ± 0.13</td>
<td>2.03 ± 0.21</td>
</tr>
</tbody>
</table>

² Significantly different from the spread diet, P < 0.05 (unpaired Student’s t tests).
³ During the 12-h period (0800–2000) of day 27, protein intake was equal to 86 ± 1% of total daily intake for the pulse diet group (0800 and 1200 meals) and 72 ± 1% for the spread diet group (0800, 1200, and 1600 meals).

The significantly higher protein gain over the day with the pulse diet (0.61 ± 0.14 g · kg FFM⁻¹ · d⁻¹) than with the spread diet (0.42 ± 0.11 g · kg FFM⁻¹ · d⁻¹) was due to higher protein synthesis. Furthermore, the percentage of [¹⁵N]glycine not recovered in urine 3 d after [¹⁵N]glycine ingestion was slightly higher in the pulse (83.7 ± 0.5%) than in the spread (81.3 ± 0.6%, P < 0.01) diet group. When calculated from the ammonia data, whole-body protein turnover in the fed state was not significantly different between the spread and pulse diat groups. Although both protein synthesis and breakdown were not significantly different between the 2 groups, the mean values for protein synthesis and breakdown were 10% higher and 11% lower, respectively, with the pulse than with the spread diet (Table 6). Consequently, protein gain in the fed state was higher with the pulse than with the spread diet (0.92 ± 0.03 compared with 0.60 ± 0.07 g · kg FFM⁻¹ · 12 h⁻¹, P < 0.001).

Therefore, during the pulse diet, FFM was maintained and nitrogen balance improved more efficiently than during the spread diet, when the protein intake was increased from 1.2 to 1.7 g · kg FFM⁻¹ · d⁻¹ because of the resulting higher protein synthesis rates.

DISCUSSION

The purpose of this study was to investigate whether the pattern of protein feeding can modulate protein metabolism in elderly women. We hypothesized that a pulse protein feeding pattern, without marked changes in dietary habits, could improve protein anabolism. To detect the effects of the protein feeding patterns, the protein intake was increased from 0.74 g · kg body wt⁻¹ · d⁻¹ during the adaptive period to 1.05 g · kg body wt⁻¹ · d⁻¹ during the experimental period; this increase improved nitrogen balance (Table 6), which is consistent with the results of previous studies in the elderly (11–14, 31).

In the anabolic state resulting from this increase in protein intake, the pulse protein pattern was more efficient at improving nitrogen balance than was the spread pattern. This was shown by 2 independent methods: FFM variations and nitrogen balance, with a significant, positive correlation between the 2 (r = 0.70, P < 0.005). This finding is consistent with the fact that the percentage of [¹⁵N]not recovered in urine 3 d after ingestion of [¹⁵N]glycine was higher with the pulse than with the spread diet.

Feeding frequency (number of daily meals) was shown to modulate body composition and nitrogen retention (32), but the effects of protein feeding patterns on protein metabolism remain poorly studied and have given conflicting results. In young women, nitrogen balance was higher when the daily protein intake was spread fairly evenly over 3 meals than when spread over 2 meals, with 1 meal containing no protein (33); in young men, the same conditions resulted in no significant changes in nitrogen balance (34). Several differences between these studies, such as sex and age of the subjects, daily protein intake, and methods used, might explain the discrepancies. The present study provides the first evidence that at least in the short term in this group of elderly women, protein retention was more efficiently improved by a protein pulse feeding pattern than a spread feeding pattern.

Small discrepancies between some indexes used in the present study to assess the anabolic effects of protein intakes would not affect this conclusion. First, some subjects, mainly in the spread diet group, showed a decrease in FFM but were in positive nitrogen balance. This may have been because nitrogen retention was overestimated, which typically occurs with the nitrogen balance method, or because variations in FFM were measured over the whole experimental period (14 d), whereas nitrogen balance was determined on only the last 5 d of the experimental period. Nevertheless, because all determinations were made under the same experimental conditions, it was valid to compare the changes during the experimental period.

Second, the stimulation of protein anabolism that occurred during the experimental period corresponded to slight body weight losses. This was also observed by others (35). In fact, body weight losses were higher (40%) during the adaptive period than during the experimental period, but were not significantly different between the 2 groups. Thus, the women were in a more anabolic state during the experimental period than during the adaptive period. These small body weight losses may have been due to a slight energy deficiency because the women were fed 1.5 × their REE in accordance with the recommended dietary allowances for the elderly (26). However, Campbell et al (36) suggest that energy intake should be equal to 1.82 × REE to maintain energy balance, although other studies indicate that 1.5 (31) or 1.6 (13) × REE is sufficient to maintain body weight. The discrepancies between these studies may be a result of differences in physical activities of elderly subjects in free-living conditions. Nevertheless, because the energy contents of both diets were similar and because the effects of energy intakes on body weight were not significantly different between the 2 groups during the adaptive period, we conclude that the differences in body composition and nitrogen balance during the experimental period resulted from differences in the protein feeding patterns.

To obtain insight into the mechanisms involved in the regulation of protein balance, protein turnover was determined by using the end product method (ammonia and urea). Harmonic and arithmetic averages were not calculated because urea data represent whole-body protein turnover over the whole day, whereas ammonia data represent whole-body protein turnover in the fed state. In the present study, the nitrogen flux values, calculated from ammonia or urea data, were similar to those obtained by others (6, 30).

The protein pulse feeding pattern increased 24-h whole-body protein turnover. This effect was analogous to results obtained...
with an increase in protein intake (35, 37, 38). However, elderly women seemed to be less responsive to an increase in protein intake (17). The present study showed that the higher stimulation of daily protein synthesis with the pulse diet (19%) accounted for most of the increase in daily nitrogen balance and suggests that this pulse feeding pattern can improve the protein anabolic effect of an increased protein intake in elderly women.

In the fed state, the higher postprandial protein gain with the pulse diet resulted from slight alterations in whole-body protein turnover, i.e., a small increase in protein synthesis (10%) and a small decrease in protein degradation (11%). We hypothesized that the protein feeding pattern might influence the diurnal protein cycle, which was described by Pacy et al (24). The increase in plasma amino acid and insulin concentrations that occurred after the midday pulse diet might have been responsible for the changes in protein synthesis and breakdown observed. Indeed, it is known that protein breakdown is inhibited by postprandial hyperinsulinemia (39) or a slight increase in amino acid concentrations (40) and that protein synthesis is stimulated by an even greater increase in amino acid concentrations (40, 41). Low-protein meals (e.g., the pulse diet meals fed at 0800 and 2000) are known to induce modifications in protein turnover, especially by limiting postabsorptive losses (24). However, whether this occurred in the present study could not be verified because 24-h protein breakdown (urea data) values were higher during the pulse diet than during the spread diet, whereas they were similar between diet groups postprandially (ammonia data). This implies that postabsorptive protein breakdown was higher in the pulse than in the spread diet group.

In the present study, protein metabolism was measured in the whole body. However, responses of individual tissues to different diet patterns must be considered because of age-associated modifications in tissue protein metabolism. Muscle protein synthesis has been shown to decrease with aging (8, 9), but is still responsive to dietary amino acid concentrations (40, 41). Low-protein meals (e.g., the pulse diet meals fed at 0800 and 2000) are known to induce modifications in protein turnover, especially by limiting postabsorptive losses (24). However, whether this occurred in the present study could not be verified because 24-h protein breakdown (urea data) values were higher during the pulse diet than during the spread diet, whereas they were similar between diet groups postprandially (ammonia data). This implies that postabsorptive protein breakdown was higher in the pulse than in the spread diet group.

In conclusion, the 14-d pulse diet had a positive effect on the body composition of elderly women, particularly on FFM. It also resulted in higher nitrogen retention than did the spread diet because of higher whole-body protein turnover rates, particularly protein synthesis, over the whole day in the pulse diet group. Insofar as such results will be confirmed in a larger group of subjects, modulation of the pattern of protein feeding with respect to dietary habits (i.e., the pulse diet in this study) may be a more attractive option than simply increasing the protein intake to improve protein turnover and protein retention in elderly women.

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