Protein Feeding Pattern Does Not Affect Protein Retention in Young Women[1]

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ABSTRACT This study was undertaken to determine whether a pulse protein feeding pattern was more efficient than a spread pattern to improve protein anabolism in young women as was already shown in elderly women. After a 15-d adaptive period [1.2 g protein/(kg fat-free mass · d)], 16 young women (age 26 ± 1 y) were given a 14-d diet providing 1.7 g protein/(kg fat-free mass · d), using either a pulse pattern (protein consumed mainly in one meal, $n = 8$), or a spread pattern (spreading daily protein intake over four meals, $n = 8$). Nitrogen balance was determined at the end of both the 15-d adaptive and the 14-d experimental periods. Whole-body protein turnover was determined at the end of the 14-d experimental period using [^{15}N]glycine as an oral tracer. Nitrogen balance was 17 ± 5 mg N/(kg fat-free mass · d) during the adaptive period. It was higher during the experimental period, but not significantly different in the women fed the spread or the pulse patterns [59 ± 12 and 36 ± 8 mg N/(kg fat-free mass · d) respectively]. No significant effects of the protein feeding pattern were detected on either whole-body protein turnover [5.5 ± 0.2 vs. 6.1 ± 0.3 g protein/(kg fat-free mass · d) for spread and pulse pattern, respectively] or whole-body protein synthesis and protein breakdown. Thus, in young women, these protein feeding patterns did not have significantly different effects on protein retention. J. Nutr. 130: 1700–1704, 2000.

KEY WORDS: • *aging* • *protein feeding pattern* • *nitrogen balance* • *protein turnover* • *women*

Some of the nutritional factors regulating protein retention in humans have been studied extensively, in particular the effect of protein quantity or quality. A change in the repartitioning of daily protein feeding could be another way in which to modify nitrogen retention in humans. In young adults, it was shown that spreading protein and energy intake in multiple small meals over the day leads to a lower leucine balance than using three isoenergetic and isonitrogenous meals (El-Khoury et al. 1995). In young women, spreading daily protein intake from two (lunch and dinner) to three meals (breakfast, lunch and dinner) increased nitrogen retention (Leverton and Gram 1949), but not in young men fed a low protein diet (Taylor et al. 1973). Thus, from these studies, it seems that in young adults fed at an adequate protein level, spreading daily protein intake over three meals is the most appropriate protein feeding pattern.

However, we showed previously in elderly women that spreading protein intake over the daily meals (the spread pattern) improved nitrogen balance less than a pulse protein feeding pattern in which daily protein intake was consumed mainly (80%) in the midday meal (Arnal et al. 1999). We anticipated that this pulse protein pattern would become effective in elderly women because of better postprandial anabolism. Indeed, postprandial protein synthesis stimulation, which is less sensitive to meal feeding in elderly people than in younger adults (Mosoni et al. 1995, Volpi et al. 1998a), is restored when blood free amino acid levels are increased markedly (Mosoni et al. 1993, Volpi et al. 1998b), as was expected to occur after the protein pulse meal.

Such repartitioning of protein intake over the daily meals (the spread vs. the pulse pattern) has not been studied before in young adults. The literature quoted above suggests that the spread pattern would be more appropriate for young adults than the pulse pattern to improve protein retention. Thus, the positive effect of the pulse pattern observed previously in elderly women (Arnal et al. 1999) would be specific to their age.

To test this hypothesis, the experiment initially performed with elderly women (Arnal et al. 1999) was repeated in young women, and comparisons were made between the two age groups and the two protein feeding patterns.

SUBJECTS AND METHODS

Subjects. Young women ($n = 16$; 26 ± 1 y old) participated in this study. All subjects were certified to be in good health by the

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TABLE 1

Physical characteristics of young subjects[1]

Age, *y*	26 ± 1
Weight, *kg*	55.7 ± 2.0
Fat-free mass,[2] *kg*	40.8 ± 4.7
Fat mass, *g/100 g body*	26.1 ± 1.6
Body mass index, *kg/m*^{2}	20.8 ± 0.5
Resting energy expenditure,[3] *MJ/d*	4.9 ± 0.2

[1] Values are means ± SEM; $n = 16$.

[2] Fat-free mass was measured using an ^{18}O-enriched water dilution technique (Vaché et al. 1995).

[3] Resting energy expenditure was determined by indirect calorimetry.

medical staff of the Human Nutrition Research Center. Mean age, weight, fat-free mass (FFM; as measured with ^{18}O water dilution) (Vaché et al. 1995), body mass index and resting energy expenditure (REE as determined by open-circuit indirect calorimetry, Deltatrac, Datex, Geneva) are listed in **Table 1**. The volunteers were asked to maintain their usual physical activity immediately before and during the study.

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 (France).

Diets. The experimental design and the composition of the diets were similar to those previously used in elderly women (Arnal et al. 1999). All young subjects were given a controlled diet for 29 d. The diet was composed of usual food products selected from the following list: bread, meat, sugar, jam, milk, butter, gravy, fish, potatoes, vegetables, noodles, yogurt, cheese, juices, cake and fruit, and were provided by the experimental kitchen of the Human Nutrition Research Center. Energy intake was based on REE (measured before the study, Table 1) multiplied by an activity factor of 1.7 (National Research Council 1989). First, during a 15-d adaptive period, the women were fed a diet providing 1.13 ± 0.02 g protein/(kg FFM · d) [or 0.83 ± 0.02 g protein/(kg · d)], which was supposed to meet protein requirements in young adults (National Research Council 1989)]. During this adaptive period, the protein feeding pattern was 10, 60 and 30% in the morning, noon and evening meals, respectively, which was similar to the subjects' usual feeding pattern. During the subsequent 14-d experimental period, the protein intake was increased to 1.67 ± 0.03 g protein/(kg FFM · d) [or 1.24 ± 0.02 g protein/(kg · d)] to enable an increase in protein retention (Campbell et al. 1994). It was compensated for by a decrease in carbohydrates (from 55 to 51% of energy intake), making the adaptive and experimental periods isoenergetic (8.23 ± 0.30 and 8.33 ± 0.29 MJ/d, respectively); the proportion of fat was 35.5% and did not differ between the two periods. The 16 women were divided randomly into two groups to test the effects of protein feeding patterns. In one group ($n = 8$), dietary protein (79% of daily protein intake) was consumed mainly in one meal (1200 h) to create a pulse protein intake; the remaining 21% was distributed in meals fed at 0800 h (7%) and 2000 h (14%). This diet is referred to as the pulse diet. In the other group ($n = 8$), the women were fed the spread diet composed of four meals to spread protein intake over the 12-h feeding period of the day (22, 31, 19 and 28% of the daily protein intake given at 0800, 1200, 1600 and 2000 h, respectively). The protein intake at the midday meal in the pulse pattern (79% of daily intake) was significantly higher than the combined protein intake of meals fed at 1200 and 1600 h in the spread pattern (50%). This was not the case for energy intake because the two daily energy patterns were 21, 49 (34 plus 15%) and 30 for the 0800, 1200 plus 1600, and 2000 h meals in the spread pattern, and 17, 51 and 32% for the 0800, 1200 and 2000 h meals in the pulse pattern. There was no difference in the protein sources between the diets (70% animal, 30% vegetable).

Nitrogen balance and whole-body protein turnover. Urine and feces were collected for the last 5 d of each period (adaptive and experimental) to determine nitrogen balance, as described previously (Arnal et al. 1999). Duplicate meals were prepared and leftovers were collected during the same periods. Representative aliquots were stored at −20°C until further analysis. The nitrogen content of aliquots of urine, feces, duplicate meals and leftovers was measured by an automated Kjeldahl method using a single-channel autoanalyzer (Kjeltec Auto 1030 Analyser, Tecator, Paris, France). Daily miscellaneous nitrogen losses were assumed to be 8 mg protein/(kg · d) (National Research Council 1989). Nitrogen balance was calculated by subtracting the daily nitrogen losses (urine, fecal and miscellaneous) from the daily nitrogen intake. Urinary creatinine excretion was measured using the Jaffé reaction on an autoanalyzer (Cobas Mira, Roche Diagnostic Systems, Neuilly sur Seine, France) to ensure similar urine collection. Urinary creatinine excretion was stable throughout the experiment and not different between the two groups (for the spread group: 0.76 ± 0.07 and 0.73 ± 0.05 g/d at the end of the adaptive and experimental periods, respectively, and for the pulse group: 0.77 ± 0.04 and 0.75 ± 0.04 g/d, respectively, paired Student's *t* test, not significant), suggesting similar urinary recoveries in all groups.

At the end of the experimental period (d 27), whole-body protein turnover was measured using a single oral dose (200 mg) of [^{15}N]glycine (99 atom %, Mass Trace, Woburn, MA) as described earlier (Arnal et al. 1999). In brief, whole-body nitrogen flux was calculated from the urinary excretion of ^{15}N in urea during the subsequent 3 d, according to Waterlow et al. (1978) and using the following equation: $Q = E \times d/e$ where d is the dose of isotopic nitrogen (g ^{15}N), E is the excretion of urea over 3 d (g N/24 h), and e is the amount of isotope excreted in the urine as urea in 3 d (g ^{15}N). The rate of protein synthesis and breakdown in the whole body were calculated indirectly 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 supplied by whole-body protein breakdown. A factor of 6.25 was used to convert nitrogen into protein.

Statistics. Data are presented as means ± SEM. Nitrogen balance data were analyzed using repeated-measures ANOVA with diet as a between-subjects factor and period as a within-subjects factor. The effects of diet on whole-body protein turnover were compared at the end of the experimental period by an unpaired Student 's *t* test. The effects of age and diet during the experimental period on nitrogen balance and protein turnover were tested by a two-way ANOVA, including data from the previously published experiment with elderly women (Arnal et al. 1999) because they had been performed under the same conditions. An interaction term for age and diet was included in all models; when the interaction was significant, independent *t* tests were performed to establish the between-group differences.

RESULTS

Young women. Body composition was not significantly different in women fed the spread pattern diet and in women fed the pulse pattern diet. No variation in FFM was detected between the adaptive and experimental periods with either the spread pattern diet (39.9 ± 0.99 vs. 39.8 ± 1.5 kg) or the pulse pattern diet (41.9 ± 2.3 vs. 41.8 ± 2.2 kg).

During the adaptive period, mean nitrogen intake was 181.3 mg N/(kg FFM · d), and mean nitrogen balance was slightly positive (+17 ± 5 mg N/(kg FFM · d), i.e., + 0.74 ± 0.22 g N/d). During the experimental period when nitrogen intake was increased, urinary nitrogen excretion was higher ($P < 0.05$) than during the adaptive period, but was not significantly different in women fed the spread or the pulse patterns (**Table 2**). Fecal nitrogen excretion was not different in women consuming the spread or the pulse pattern diets during both the adaptive and experimental periods. Nitrogen balance was significantly higher when the two diets were consumed during the experimental period than during the adaptive period, but it was not significantly different in women fed the

TABLE 2

Nitrogen balance measurements in young women fed the spread pattern (protein intake over 4 meals) or the pulse pattern (protein consumed mainly in one meal) diets for 14 d after a 15-d adaptive period[1]

	Spread		Pulse		*P*[2]		
	Adaptive	Experimental	Adaptive	Experimental	Diet	Period	Diet × Period
	mg N/(kg FFM · d)						
Intake	181 ± 5	264 ± 6	181 ± 3	258 ± 5	0.68	0.0001	0.39
Urine	124 ± 12	161 ± 12	125 ± 8	183 ± 11	0.39	0.0001	0.25
Feces	28 ± 2	31 ± 2	29 ± 1	33 ± 3	0.60	0.12	0.81
Balance	17 ± 9	59 ± 12	17 ± 6	36 ± 8	0.24	0.001	0.16

[1] Values are means ± SEM, $n = 8$. 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 weight · d) were made (National Research Council 1989).
[2] By using a repeated-measures ANOVA.

spread pattern and in women fed the pulse pattern (Table 2). Whole-body protein turnover or protein synthesis was not significantly different in the two groups (**Table 3**), whereas protein breakdown tended ($P = 0.06$) to be higher in young women fed the pulse pattern than in young women fed the spread pattern.

Age-related effect of the protein feeding pattern on protein retention and metabolism. To compare the effects of protein feeding pattern at two different ages, the present data were compared with those obtained previously in elderly women fed the same diets (Arnal et al. 1999) using a two-way ANOVA. At the end of the experimental period, nitrogen balance was significantly lower in elderly women than in young women when they were fed the spread pattern, but was not different in young and elderly women when they were fed the pulse pattern (**Fig. 1**). Nitrogen balance was higher in elderly women fed the pulse pattern than in elderly women fed the spread pattern, but not in young women (Fig. 1). Whole-body protein flux was lower in elderly than in young women. It was higher in women fed the pulse pattern than in women fed the spread pattern (**Table 4**). This resulted from a different effect of those diets on protein breakdown and synthesis. Protein breakdown was lower in elderly than in young women regardless of the diet pattern. In contrast, protein synthesis was lower in elderly than in young women only when they were fed the spread pattern diet (Table 4).

TABLE 3

Whole-body protein turnover in young women fed the spread pattern (protein intake over 4 meals) or the pulse pattern (protein consumed mainly in one meal) diets for 15 d after a 14-d adaptive period[1]

	Spread	Pulse
	g protein/(kg FFM · d)	
Q[2]	5.47 ± 0.23	6.12 ± 0.26
Protein synthesis, *Z*[2]	4.47 ± 0.21	4.97 ± 0.23
Protein breakdown, *B*[2]	3.83 ± 0.23	4.51 ± 0.25

[1] Values are means ± SEM, $n = 8$.
[2] *Q*, nitrogen flux was determined using the ^{15}N end products method. The rate of protein synthesis and breakdown in the whole body were calculated indirectly 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 supplied by whole-body protein breakdown. Protein breakdown tended ($P = 0.06$, unpaired Student's *t* tests) to be higher in young women fed the pulse diet than in young women fed the spread diet.

DISCUSSION

This study was undertaken to determine whether the protein feeding pattern can affect protein retention and metabolism in young women, as it did in elderly women (Arnal et al. 1999). We showed that in young women, the protein feeding pulse pattern did not improve protein retention.

Such a result depends on the reliability and the precision of the nitrogen balance method. Overestimation is a general problem of nitrogen balance measurements. Even if it occurred in this study, however, it would have been minimized because much care was taken to avoid it, i.e., all meals were prepared by the cooking staff, leftovers were collected, duplicate meals were prepared and nitrogen contents were measured; fecal nitrogen excretion was measured and urinary creatinine excretion was used as an index of urinary recovery. The absolute

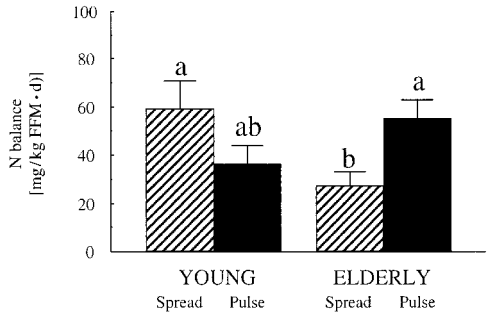

FIGURE 1 Nitrogen balance in young and elderly women fed the spread or the pulse diet, during the experimental period. Values are means ± SEM, $n = 7$ or 8. The effects of the protein feeding patterns in elderly subjects were published previously (Arnal et al. 1999). Data were analyzed by two-way ANOVA with age and diet as factors. Because age-by-diet interaction was significant ($P = 0.01$), an unpaired Student's *t* test was performed. Means allocated the same letter were not significantly different. Nitrogen intakes during the experimental period were 264 ± 6 or 258 ± 5 mg/[kg fat-free mass (FFM) · d] for the young women fed the spread or pulse diet, respectively, and 267 ± 4 or 274 ± 8 mg/(kg FFM · d), for the elderly women fed the spread or pulse diet, respectively. There were no significant differences between groups in nitrogen intake.

TABLE 4

Comparison of the responses of whole-body protein turnover to the (spread and pulse) feeding patterns in young and elderly women[1]

	Young		Elderly		*P*[2]		
	Spread	Pulse	Spread	Pulse	Age	Diet	Age × Diet
n	8	8	8	7			
	g protein/(kg FFM · d)						
Q	5.47 ± 0.23	6.12 ± 0.26	4.98 ± 0.17	5.58 ± 0.22	0.03	0.01	0.92
Z/*Q*[3]	0.82 ± 0.01	0.81 ± 0.01	0.75 ± 0.01[4]	0.80 ± 0.01[5]	0.001	0.03	0.02
B/*Q*[3]	0.69 ± 0.01	0.73 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.004	0.01	0.56

[1] Values are means ± SEM. The effects of the protein feeding patterns in elderly subjects were published previously (Arnal et al. 1999).
[2] By a two-factor ANOVA.
[3] Protein synthesis (*Z*) and breakdown (*B*) are expressed as the relative proportion of protein flux (*Q*) to detect the effects of the main components of protein turnover.
[4] Elderly vs. young within each diet group, unpaired Student's *t* tests, $P < 0.05$.
[5] Pulse vs. spread within each age group, unpaired Student's *t* tests, $P < 0.05$.

level of nitrogen balance obtained during the adaptive and experimental periods was similar to the level obtained in studies using similar protein intake (Campbell et al. 1994, Pannemans et al. 1995a and 1995b). Thus, it appears that nitrogen balances were not excessively overestimated. Furthermore, because all measurements were made under the same conditions, comparisons of the effects of protein feeding patterns should be valid.

Another issue that arises in comparing the effect of the protein feeding pattern in young women concerns the fact that in this study, the menstrual cycle was not taken into account. However, it appears that protein turnover and leucine oxidation increase during the luteal phase (Lariviere et al. 1994). The failure to account for this cycle could lead to an increase in intersubject variability (Calloway et Kurzer 1982). We cannot exclude that such a variability prevented us from detecting the diet effect on nitrogen balance because the difference between the spread and the pulse pattern groups was 23 mg N/(kg FFM · d), close to the significant difference found in elderly subjects [27 mg N/(kg FFM · d), Arnal et al. 1999]. It is very unlikely, however, that the pulse pattern induces a more marked improvement of protein retention than the spread pattern in young women.

These results in young women are in keeping with the lack of positive effect of concentrating protein feeding over two meals (Leverton and Gram 1949, Taylor et al. 1973). Indeed, when using three meals, restricting daily protein intake to the midday and evening meals did not modify protein retention in young men fed a diet providing 0.4–0.5 g protein/(kg body weight·d) (Taylor et al. 1973). Moreover, when the daily protein intake was 1 g protein/(kg body weight · d), nitrogen balance was lower in young women fed a two-protein meal diet than in young women fed a three-protein meal diet (Leverton and Gram 1949). The effect of the pulse or the spread pattern could also be compared with results obtained by Boirie et al. (1997a) who studied slow and fast proteins. Slow proteins (such as casein) are absorbed slowly by the gut and could mimic to a certain extent a spread protein pattern. By contrast, fast proteins (such as whey proteins) are absorbed rapidly by the gut, reflecting more a pulse protein pattern. In young men, consumption of slow proteins led to a higher postprandial protein accretion than fast proteins (Boirie et al. 1997a). Although no significant effect on nitrogen balance could be recorded in this study, ($P = 0.16$, Table 2), it appears that, in young women, nitrogen retention was 1.5 times higher with the spread pattern than with the pulse pattern. Thus, in young adults, spreading protein intake could be more efficient in improving protein retention than using a pulse pattern.

In contrast, in elderly women, the improvement in nitrogen retention was lower with the spread pattern than with the pulse pattern (Arnal et al. 1999). Consequently, nitrogen balance, which was lower in elderly women than in young women when using the spread pattern, became similar when using the pulse pattern (Fig. 1). Comparisons of nitrogen balance data in young and elderly subjects depend on the way nitrogen intake is normalized. Most studies use a weight basis. However, due to the loss of fat-free mass and the increase in fat mass during aging (Cohn et al. 1980), 1 kg of body weight does not represent the same proportion of metabolically active tissue in young and elderly women. This may explain why protein turnover, determined by nitrogen (Morais et al. 1997, Uauy et al. 1978) or leucine flux (Boirie et al. 1997b, Robert et al. 1984, Welle et al. 1994), is similar in adults and in elderly people only when expressed per FFM. Thus, we chose to adjust nitrogen intake to FFM, giving the same amount of protein per kilogram FFM to young [1.67 ± 0.03 g protein/(kg FFM · d)] and elderly women [1.69 ± 0.04 g protein/(kg FFM · d)]. This led us to feed the same daily amount of protein to young and elderly women (66.47 ± 2.10 and 64.24 ± 1.47 g protein/d, respectively, during the experimental period, $P = 0.43$). However, the amounts of protein per kilogram body weight fed to the young women [1.24 ± 0.02 g protein/(kg · d)] were higher than the amounts fed to the elderly women [1.05 ± 0.025 g protein/(kg · d), $P < 0.05$]. When protein intake per kilogram body weight was used as a covariate in variance analysis (not shown), it had no significant effect on nitrogen balances and protein metabolism, suggesting that our conclusions about the effects of the pulse and the spread patterns in young and elderly women are valid. Thus, under these conditions, we showed that the pulse pattern improved protein retention in elderly women but not in young women.

This specific age-related effect, which may also be interpreted as a default of the spread pattern to ensure whole-body maintenance in elderly women, results necessarily from modifications of whole-body protein metabolism. Nitrogen flux measurements (using a single dose of [^{15}N]glycine and urea as end product) revealed that the pulse pattern induced a similar 10% increase of whole-body protein turnover in both young

and elderly women. This resulted from a similar increase of protein breakdown in young and in elderly women. By contrast, the regulation of protein synthesis by the pulse pattern was different depending on age. Thus, protein synthesis was not different with the spread or the pulse pattern in young women, whereas in elderly women, protein synthesis was higher with the pulse pattern than with the spread pattern. This can explain the positive effect on nitrogen balance observed specifically in elderly women fed the pulse pattern. It was shown previously that an increase in the daily protein intake was not sufficient to normalize protein synthesis rates in elderly women (Pannemans et al. 1995b). We demonstrated in this study that if such an increase in protein intake was made following a pulse pattern, protein synthesis rates could be restored in elderly women.

Age-related alterations of whole-body protein synthesis should reflect in part some dysregulations at the muscle level. Indeed a lower stimulation of muscle protein synthesis through feeding was detected during aging in rats (Mosoni et al. 1995). In humans, this result was not observed in vastus lateralis (Welle et al. 1994) but was obtained by flux measurements at leg level (Volpi et al. 1998a). A marked increase in blood free amino acid levels was shown to stimulate muscle protein synthesis in both old rats (Mosoni et al. 1993) and elderly subjects (Volpi et al. 1998b). The positive effect of the pulse pattern on nitrogen balance and whole-body protein synthesis observed in elderly women likely results from a higher stimulation of muscle protein synthesis after consumption of the protein-rich meal. However, amino acid splanchnic extraction was shown to increase with age (Boirie et al. 1997b, Volpi et al. 1999), suggesting an age-related increase of protein utilization by liver and/or gastrointestinal tract. Thus, a stimulation of protein synthesis in those organs induced by the pulse pattern is likely to occur simultaneously.

In conclusion, this study demonstrates that the protein feeding pattern has a differential effect on protein retention in young and elderly women. The positive effect of the pulse pattern is specific to elderly women, whereas in young women, the protein feeding pattern did not significantly affect protein retention. This results from alterations of protein turnover regulation that occur during aging, which could be overcome, at least in part, by the use of the pulse pattern. Thus, this concept of a protein feeding pattern controlling protein metabolism in elderly subjects could be developed and used in other situations in which an improvement in protein anabolism is desired.

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