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M. A. ARNAL,[1] L. MOSONI,[1] Y. BOIRIE,[2] P. GACHON,[2] M. GENEST,[2] G. BAYLE,[1] J. GRIZARD,[1] M. ARNAL,[1] J. M. ANTOINE,[3] B. BEAUFRÈRE,[2] AND P. PATUREAU MIRAND[1]
[1]*Unité d'Etude du Métabolisme Azoté, Institut National de la Recherche Agronomique et Centre de Recherche en Nutrition Humaine, 63122 Clermont-Ferrand-Theix;* [2]*Laboratoire de Nutrition Humaine, Université d'Auvergne et Centre de Recherche en Nutrition Humaine, 63009 Clermont-Ferrand; and* [3]*Société Danone, 92350 Le Plessis-Robinson, France.*

Arnal, M. A., L. Mosoni, Y. Boirie, P. Gachon, M. Genest, G. Bayle, J. Grizard, M. Arnal, J. M. Antoine, B. Beaufrère, and P. Patureau Mirand. Protein turnover modifications induced by the protein feeding pattern still persist after the end of the diets. *Am J Physiol Endocrinol Metab* 278: E902–E909, 2000.—This study was undertaken to determine whether the protein feeding pattern could induce chronic adaptation of protein turnover. After a 15-day adaptive period, elderly (68 yr) and young (26 yr) women received, for 14 days, a diet providing 200 KJ·kg fat-free mass $(FFM)^{-1}\cdot day^{-1}$, where the daily protein intake (1.7 g protein·kg $FFM^{-1}\cdot day^{-1}$) was either spread over 4 meals in the spread pattern or mainly (80%) consumed at noon in the pulse pattern. One day after the end of the dietary treatment, whole body leucine kinetics were measured by use of a continuous [^{13}C]leucine infusion, both in the postabsorptive state and in the same fed state. The pulse pattern was able to induce, in young as in elderly women, a lower postabsorptive leucine oxidation and endogenous leucine flux than the spread pattern and improved the responsiveness of nonoxidative leucine disposal during 4-h oral feeding. Thus the pulse pattern was able to induce chronic regulation of protein metabolism in young as in elderly women.

women; postabsorptive state; postprandial state; aging

SEVERAL EXPERIMENTS HAVE SHOWN that protein retention can be improved in elderly people by adequate protein feeding. This may be useful in limiting the gradual body protein loss occurring during aging. Thus it is generally admitted that, in elderly people, an increase in the daily amount of protein intake improves, at least in the short term, protein balance (9, 21, 24). We previously demonstrated, in elderly women, that the increase of protein retention due to an increase of daily protein intake [from 1.2 to 1.7 g protein·kg fat-free mass $(FFM)^{-1}\cdot day^{-1}$] was modulated by the protein feeding pattern (1). Indeed, when 80% of the daily protein intake was consumed during the midday meal (as in the pulse pattern), the increase of nitrogen balance was higher than when protein intake was spread over 4 meals (as in the spread pattern). Similar protein retention was obtained in young and in elderly women fed the pulse but not the spread pattern (2). It was suggested that this effect was the consequence of a defect in the response of whole body protein anabolism to the spread pattern in elderly women. We analyze now what mechanisms are involved in the effects of the protein pulse feeding pattern.

Indeed, protein intake levels modulate protein deposition by acting on protein turnover regulation during the diurnal cycle of postabsorptive/postprandial phases (22). Low protein intake can reduce postabsorptive protein breakdown and, consequently, postabsorptive protein losses. A high protein intake improves postprandial protein gains by a higher stimulation of protein synthesis and a higher inhibition of proteolysis (20). The pulse pattern, which included both high- and low-protein meals, induced a higher protein synthesis in elderly women than the spread pattern (1). This might correspond to a specific recovery of protein synthesis stimulation by nutrients during the dietary treatment. However, the pulse pattern may also induce chronic metabolic adaptations of protein turnover, such as a better protein-sparing effect during the postabsorptive period and/or a higher responsiveness of protein metabolism to nutrients. In this case, these modifications of protein turnover had to persist after the dietary treatments had stopped.

In the present study, we tested the hypothesis that the positive effect of the pulse pattern on protein anabolism in elderly women involves chronic adaptations of protein turnover. Thus we measured, in young and in elderly women, leucine kinetics on the day after the end of the dietary pulse or spread diets both in the basal state (i.e., postabsorptive) and during the fed state (i.e., small repeated meals). This allowed us to detect whether or not protein metabolism responses to postabsorptive and fed states remained different in women previously fed the spread or the pulse diet, according to their age.

METHODS

Subjects. Thirty-one healthy women who had previously participated in the nitrogen balance and flux determination studies (1, 2) were included in the present study; they were 16

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young [26 ± 1 (SE) yr] and 15 elderly adults (68 ± 1 yr). Each subject had a normal physical examination without any medical history of renal, cardiovascular, endocrine, or currently evolving disease. All subjects were asked to maintain their usual physical activity before and during the study. Their physical characteristics were recorded. Mean body weights were 55.7 ± 2.0 and 62.4 ± 1.8 kg (in young and elderly women, respectively, $P < 0.05$), FFM values (as measured with ^{18}O water dilution) were 73.9 ± 1.6 and 61.7 ± 1.4% of body weight ($P < 0.05$), and body mass index values were 20.8 ± 0.5 and 25.3 ± 0.8 kg/m^2 ($P < 0.05$) in young and elderly women, respectively.

The purpose and the potential risks of the study were fully explained to the subjects, and written informed consent was obtained from each participant. The experimental protocol was approved by the Ethical Committee of Clermont-Ferrand.

Materials. L-[1-^{13}C]leucine [99 mole percent excess (MPE)], L-[5,5,5-$^2H_3$]leucine (97 MPE), and [^{13}C]bicarbonate (99 MPE) were obtained from MassTrace (Woburn, MA). The isotopic and chemical purity of the leucine compounds was checked by gas chromatography-mass spectrometry (GC-MS). Solutions of tracers were tested for sterility and pyrogenicity before use and prepared in sterile nonpyrogenic saline. During each experiment, the tracers were filtered through 0.22-μm filters.

Experimental design. The food intake was entirely controlled for 29 days. The experimental design and the composition of the diets are described in detail elsewhere (1, 2). Briefly, during the first 14 days, each subject received a controlled diet adjusted to 1.2 g protein·kg FFM^{-1}·day^{-1} and 200 KJ·kg FFM^{-1}·day^{-1}. This adaptive period was achieved to obtain a similar protein status for all of the subjects. Between *days 15* and *29*, two isonitrogenous (1.7 g protein·kg FFM^{-1}·day^{-1}) and isoenergetic (200 KJ·kg FFM^{-1}·day^{-1}) diets were fed to test the effects of two protein feeding patterns. The daily protein and energy repartition of these diets is shown in Table 1. The first diet was composed of four meals, spreading daily protein intake over 12 h (0800, 1200, 1600, and 2000): it is referred to as the spread pattern. In the second diet, 80% of daily protein intake was concentrated at midday, and the remaining 20% was provided at breakfast (0800) and dinner (2000): it is referred to as the pulse pattern. The protein intake from the midday meal in the pulse pattern was significantly higher than the combined protein intake from meals fed at 1200 and 1600 in the spread pattern. This was not the case for energy intakes (Table 1).

Table 1. *Daily protein and energy intake during the 14-day experimental period*

Time of Meal	Spread Diet ($n = 16$)	Pulse Diet ($n = 15$)
	Protein (% of intake)	
0800	21.4 ± 0.3	6.8 ± 0.3*
1200	31.2 ± 0.3	79.6 ± 0.4*
1600	19.3 ± 0.4	—
2000	28.1 ± 0.3	13.6 ± 0.4*
1200 + 1600	50.5 ± 0.3	79.6 ± 0.4*
	Energy (% of intake)	
0800	20.6 ± 1.1	17.0 ± 1.4
1200	33.8 ± 0.9	50.9 ± 0.8*
1600	15.6 ± 0.6	—
2000	29.9 ± 0.6	32.1 ± 0.9
1200 + 1600	49.4 ± 0.9	50.9 ± 0.8

Values are means ± SE of young and elderly women; *n*, no./group. Because protein and energy feeding patterns were not different between young and elderly women, values were pooled, and effects of diet were analyzed using an unpaired Student's *t*-test. *$P < 0.05$.

On the 30th day, the day after the end of the dietary treatment, L-[1-^{13}C]leucine infusion was performed as described by Boirie et al. (7) to measure leucine kinetics. Subjects were admitted to Research Center facilities at 0700 in the postabsorptive state. At 0730, a catheter was inserted retrogradely into a dorsal vein of the hand for arterialized blood sampling after introduction of the hand into a 60°C-heated, ventilated box. A second catheter was inserted into a vein of the contralateral arm for tracer infusion. At 0800, after a prime dose of [^{13}C]bicarbonate (6 mg), a primed (6 μmol/kg), continuous (0.10 μmol·kg^{-1}·min^{-1}) infusion of L-[1-^{13}C]leucine was begun and continued for 8 h. After 210 min, a semiliquid diet was given orally for the 270 remaining minutes (from 210 to 480 min). The diet provided 3.7 MJ/kg, 13.3% as protein (in the form of whey protein concentrate, containing 10% leucine by weight), 52.3% as carbohydrates (dextrin maltose hydrolyzed from potato starch of low natural ^{13}C abundance), and 35% as fat (in the form of vegetable oil). The liquid meal was prepared on the day of protocol, just before the beginning of the study, and was ingested in small (50-ml) aliquots given every 20 min. [$^2H_3$]leucine was added to the meals to obtain an oral administration rate of 0.07 μmol·kg^{-1}·min^{-1}.

Blood and breath samples were taken before any infusion and at 20-min intervals during the last hour of each plateau in the postabsorptive state (i.e., from 150 to 210 min) and in the fed state (from 420 to 480 min). The plasma supernatant was separated, an internal standard was added, and the sample was stored at −20°C until further analysis. Breath samples were kept in 10-ml evacuated containers (Vacutainer, Becton-Dickinson, Grenoble, France). Total carbon dioxide production rates were measured at isotopic plateau during the last hour of the two postabsorptive and fed states by an open-circuit indirect calorimeter (Deltatrac, Datex, Geneva, Switzerland).

During this infusion protocol, two elderly women (one in the pulse and one in the spread group) did not endure the continuous feeding mode with the semi-liquid diet. Consequently the fed state plateau could not be achieved (not shown), and these two subjects were excluded from this study.

Analytical methods. Plasma [^{13}C]- and [$^2H_3$]leucine and ketoisocaproate (KIC) enrichments were measured by selected ion monitoring electron impact GC-MS (model 5971A, Hewlett-Packard, Palo Alto, CA) with *t*-butyldimethylsilyl derivatives, and corrections for ^{13}C and $^2H_3$ enrichments were applied according to Biolo et al. (4). As shown in Fig. 1, enrichment plateaus were achieved during both postabsorptive and postprandial states, and there was no significant difference in [^{13}C]- and [$^2H_3$]leucine or KIC enrichments between groups. $^{13}CO_2$ enrichments were measured on a GC combustion isotope ratio mass spectrometer (μGas system; Fisons Instruments, VG Isotech, Middlewich, UK). Plasma amino acid concentrations, after deproteinization with 0.6 M TCA, were measured on an autoanalyzer (Biotronic LC 3000, Roucaire, Vélizy, France, with BTC 2410 resin). Measurements of leucine content of the liquid meal were performed by GC (model 5971A, Hewlett-Packard) after a 24-h hydrolysis at 110°C with HCl 6N and with norleucine as internal standard. Plasma insulin concentrations were determined by RIA (CIS, Gif-sur-Yvette, France). Plasma glucose, total proteins, and urea concentrations were measured using enzymatic reactions on an autoanalyzer (Cobas Mira, Roche Diagnostic Systems, Neuilly sur Seine, France).

Calculations. As described previously (3), leucine kinetics were calculated as follows

$$\text{Leu } R_a = F[^{13}C]\text{Leu}/([^{13}C]\text{Leu MPE} \times 0.01) \quad (1)$$

where Leu R_a (μmol·kg $FFM^{-1}\cdot min^{-1}$) is total leucine systemic flux, F[^{13}C]Leu is the [^{13}C]leucine infusion rate (μmol·kg $FFM^{-1}\cdot min^{-1}$) corrected for isotopic purity, and [^{13}C]Leu MPE is the plasma [^{13}C]leucine enrichment. This flux includes the tracer infusions.

Leucine splanchnic extraction (%) was calculated as follows

$$\text{Leu Sp Ext} = [1 - (R_a[^{13}C]\text{Leu}/R_a[^2H_3]\text{Leu})] \times 100 \quad (2)$$

where R_a [^{13}C]Leu and R_a [$^2H_3$]Leu are total leucine fluxes calculated according to *Eq. 1* with either the intravenous (^{13}C) or oral ($^2H_3$) tracer. Splanchnic extraction represents the fraction of ingested leucine taken up by the gut and/or liver during its first pass.

Leucine oxidation (Leu Ox, μmol·kg $FFM^{-1}\cdot min^{-1}$) was calculated as follows

$$\text{Leu Ox} = {}^{13}CO_2 \text{ excretion}/([^{13}C]\text{KIC MPE} \times 0.01) \quad (3)$$

where $^{13}CO_2$ excretion (μmol·kg $FFM^{-1}\cdot min^{-1}$) is the product of carbon dioxide production and $^{13}CO_2$ atom percent excess (APE), corrected for incomplete recovery by a factor of 0.70 in the postabsorptive state and 0.82 in the fed state, according to Hoerr et al. (16).

Nonoxidative leucine disposal (NOLD, μmol·kg $FFM^{-1}\cdot min^{-1}$), an index of whole body protein synthesis, is the difference between total leucine flux (Leu R_a) and leucine oxidation (Leu Ox). Endogenous leucine production (μmol·kg $FFM^{-1}\cdot min^{-1}$) represents whole body protein breakdown and is the difference between total leucine flux (minus the intravenous tracer infusion rate) and either leucine intake (Endo Leu R_a) or leucine intake corrected for splanchnic extraction (corrected Endo Leu R_a) calculated as follows

$$\text{corrected Leu intake} = \text{Leu intake} \times [1 - (\text{Leu Sp Ext} \times 0.01)] \quad (4)$$

Finally, net leucine balance is the difference between total leucine intake (including the tracers) and leucine oxidation.

Statistical analysis. Results are means and residual SD (RSD) or SE. Leucine kinetics and plasma parameters were compared by a three-way ANOVA [age (A), protein feeding pattern (P), and nutritional state (N) as factors] with repeated measures for nutritional state. The analyses were performed with the Superanova software (Abacus Concepts, Berkeley, CA). The error term subject was used to test the effects of A, P, and their interactions; it was the N × subjects interaction for factors including nutritional state. Then, to analyze the effects of age and diet in the postabsorptive state or in response to feeding (%postabsorptive), a two-way ANOVA was performed to discriminate between the effects of age and protein feeding pattern.

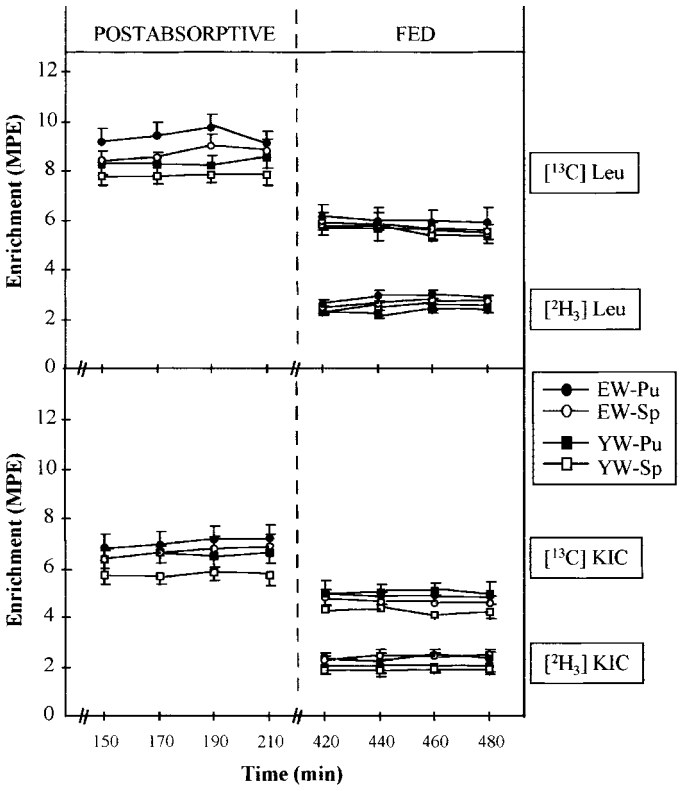

Fig. 1. Leucine and ketoisocaproate (KIC) enrichments during postabsorptive and fed states in young (YW) and elderly women (EW). Values are means ± SE. MPE, mole percent excess; Pu, pulse; Sp, spread.

RESULTS

Plasma substrate and hormone concentrations. In the postabsorptive state, no significant effects of age were detected, either on plasma glucose or on insulin concentrations. Feeding induced an increase of both glucose and insulin concentrations (Table 2). In the fed state, glycemia and insulinemia were higher in the elderly women than in the young women (+30 and +68%, for glucose and insulin concentrations, respectively). Plasma urea concentrations were higher in the elderly than in the young women and were increased during feeding only in the elderly women (Table 3). Feeding induced an increase in plasma free amino acid concentrations (Table 3). A significant ($P < 0.05$) effect of age was detected for His, Leu, Tyr, and Val, and especially in the fed state for Ile, Leu, and Val, for which concentrations were, respectively, 22, 17, and 15% higher in the elderly subjects than in the young subjects. Concentrations of His, Lys, and Phe were lower in the pulse than in the spread groups (−13, −11, and −13%, respectively).

Whole body leucine kinetics. A complete analysis of results regarding leucine kinetics is given in Table 4. Feeding induced a significant stimulation of Leu R_a and Leu Ox and an inhibition of Endo Leu R_a. The stimulation of NOLD in the fed state was dependent on the protein feeding pattern (N × P interaction): it was significantly different from zero in the pulse but not in the spread groups. Because the protein feeding pattern had no significant effect according to age (A × P interaction not significant for each leucine flux), the effects of age were analyzed by pooling the data of both protein feeding patterns, and the effects of protein feeding pattern were analyzed by pooling the data of both age groups.

Effect of age. In the postabsorptive state, Leu R_a was not significantly different in the young and the elderly women (Fig. 2). Leu Ox was 20% lower in the elderly

Table 2. *Plasma glucose and insulin concentrations during [^{13}C]leucine infusion*

	Young				Elderly						
	Spread		Pulse		Spread		Pulse				Variance Analysis Significant Effects
n	PA 8	Fed 8	PA 8	Fed 8	PA 7	Fed 6	PA 7	Fed 6	RSD_1	RSD_2	
Glucose, mmol/ml	4.58	6.18	4.76	6.83	4.79	8.50	4.70	8.28	0.9	0.9	A, N, A×N
Insulin, μU/ml	5.83	44.9	5.58	58.7	5.88	75.6	5.28	102.2	31.9	31.3	A, N, A×N

Values are means and residual SD (RSD); *n*, no./group. Data were analyzed using a 3-way ANOVA [age (A), protein feeding pattern (P), and nutritional state (N) as factors] with repeated measures for nutritional state. PA, postabsorptive; RSD_1, term that was used to test A, P, and A × P effects; RSD_2, subject × N interaction, which was used to test effects including the repeated factor N. All effects reported are significant ($P < 0.05$).

women than in the young women. Neither NOLD nor Endo Leu R_a differed between the two age groups. However, postabsorptive leucine balance was less negative in the elderly than in the young women (−0.23 ± 0.01 and −0.33 ± 0.03 μmol·kg $FFM^{-1}\cdot min^{-1}$, respectively). The increase of Leu R_a and Leu Ox induced by feeding was higher in the elderly women than in the young women (Fig. 2). The significant NOLD changes were not dependent on the age of the subjects. In the fed state, Endo Leu R_a inhibition tended ($P = 0.10$) to be lower in the elderly women (65 ± 6%) than in the young women (75 ± 4%). When it was corrected for splanchnic extraction, this inhibition was significantly lower in the elderly women than in the young women (Fig. 2). Leucine balance became positive, reflecting fed state protein gains, but no significant effect of age was detected (+1.12 ± 0.07 and +1.08 ± 0.08 μmol·kg $FFM^{-1}\cdot min^{-1}$ for young and elderly women, respectively).

Effect of diet. Postabsorptive Leu R_a, corrected Endo Leu R_a, and Leu Ox were, respectively, 9, 8, and 26% lower in the pulse than in the spread groups (Fig. 3). No diet effect was detected on NOLD in the postabsorptive state. Postabsorptive Leu balance was higher in the pulse than in the spread groups (−0.24 ± 0.02 and −0.33 ± 0.03 μmol·kg $FFM^{-1}\cdot min^{-1}$, respectively). In the fed state, Leu R_a tended to be more stimulated ($P = 0.06$) when women were previously fed the pulse diet.

The stimulation of Leu Ox in the fed state was not different in the two groups. NOLD was increased in the fed state when women had been fed the pulse diet but not the spread diet. No significant effect of the spread or

Table 3. *Plasma free amino acids and urea concentrations during [^{13}C]leucine infusion*

	Young		Elderly				Variance Analysis Significant Effects
	PA	Fed	PA	Fed	RSD_1	RSD_2	
Urea							
Spread	22.9	23.0	28.4	31.9	7.7	3.2	A, N, A×N
Pulse	22.4	21.9	30.3	33.5			
His							
Spread	65.6	86.6	69.9	83.3	10.1	8.0	A, P, A×P, N
Pulse	65.4	77.5	55.8	61.8			
Ile							
Spread	42.9	78.5	44.4	97.6	15.4	13.1	N, A×N
Pulse	41.3	76.7	35.4	91.6			
Leu							
Spread	124.9	175.9	134.7	208.3	28.7	23.5	A, N, A×N ($P = 0.06$)
Pulse	119.4	171.6	117.8	197.3			
Lys							
Spread	150.5	256.7	169.9	268.8	46.6	30.7	P, N
Pulse	153.9	241.8	138.7	208.3			
Phe							
Spread	45.3	70.0	42.2	77.8	9.6	6.1	P, N
Pulse	42.2	62.8	47.2	65.5			
Thr							
Spread	112.8	148.2	99.9	149.4	34.3	16.7	N
Pulse	123.8	157.8	98.4	146.2			
Tyr							
Spread	38.9	71.7	54.5	98.5	20.5	14.8	A, N
Pulse	45.4	77.2	41.7	90.8			
Val							
Spread	179.8	250.7	190.9	288.8	33.9	25.7	A, N, A×N
Pulse	175.5	242.5	175.3	280.5			

Values are means and RSD. Urea concentrations are expressed in cg/l and essential amino acid concentrations in μmol/l. Data were analyzed as described in METHODS and in Table 2. Effects reported are significant ($P < 0.05$).

Table 4. *Leucine kinetics*

	Young				Elderly						
	Spread		Pulse		Spread		Pulse				Variance Analysis Significant Effects
n	PA 8	Fed 8	PA 8	Fed 8	PA 7	Fed 6	PA 7	Fed 6	RSD_1	RSD_2	
Leu R_a	2.00	2.76	1.86	2.77	2.00	3.02	1.79	2.83	0.39	0.15	N, A × N
Leu Ox	0.54	1.36	0.41	1.03	0.43	1.30	0.37	1.12	0.22	0.16	P, N
NOLD	1.45	1.41	1.45	1.74	1.54	1.69	1.43	1.71	0.36	0.14	N, N × P
Endogenous Leu R_a	1.84	0.39	1.71	0.52	1.81	0.73	1.63	0.50	0.37	0.19	N, A × N
Splanchnic extraction		33.7		28.6		33.8		31.1	13.0		
Corrected Endo Leu R_a	1.84	1.14	1.71	1.11	1.81	1.44	1.63	1.18	0.20	0.15	P, N, A × N
Leu balance	−0.39	1.02	−0.27	1.22	−0.26	0.96	−0.20	1.21	0.23	0.16	P, N

Values are means and RSD, expressed in μmol leucine·kg FFM^{-1}·min^{-1}, except for splanchnic extraction expressed in %; *n*, no./group. R_a, appearance rate or flux; Ox, oxidation; NOLD, nonoxidative leucine disposal; corrected Endo Leu R_a, endogenous leucine R_a corrected for splanchnic extraction. Effects reported are significant ($P < 0.05$).

the pulse diets was detected on corrected Endo Leu R_a inhibition (Fig. 3). Finally, fed-state Leu balance was higher in the pulse group than in the spread group (+1.22 ± 0.04 and +0.99 ± 0.08 μmol·kg FFM^{-1}·min^{-1}, respectively).

DISCUSSION

The present study was undertaken to determine whether the mechanisms involved in the improvement of protein anabolism observed in elderly women fed a

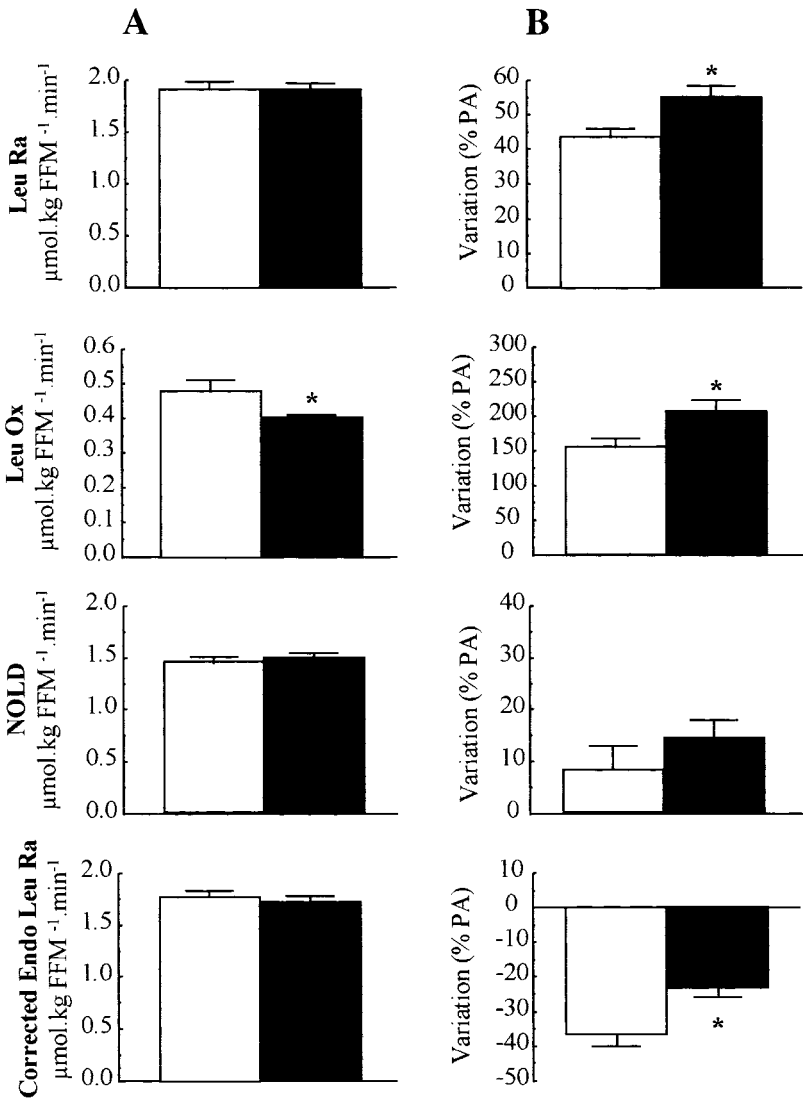

Fig. 2. Effect of age on leucine rate of appearance (R_a), leucine oxidation (Leu Ox), nonoxidative leucine disposal (NOLD), and endogenous leucine R_a corrected for splanchnic extraction (corrected Endo Leu R_a). Values are means ± SE. Data are analyzed using a 2-way ANOVA (age and protein feeding pattern) in the postabsorptive (PA) state (*A*) and in responses to feeding in steady-state conditions (*B*). Only comparisons between elderly and young women are reported: $n = 16$ and $n = 13$, respectively, in young and elderly groups. *Significantly different from young group, $P < 0.05$.

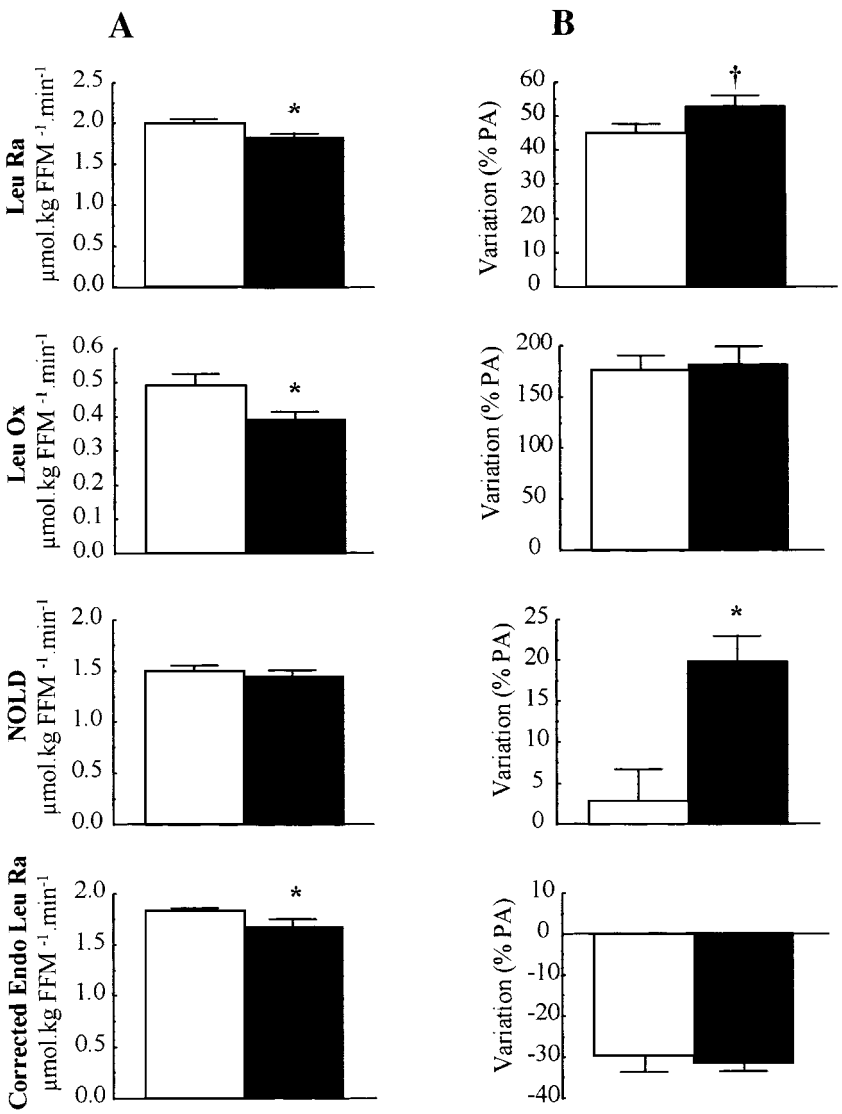

Fig. 3. Effect of diet on leucine R_a, Leu Ox, NOLD, and corrected Endo Leu R_a. Values are means ± SE. Data are analyzed using a 2-way ANOVA (age and protein feeding pattern) in PA state (*A*) and in responses to feeding in steady-state conditions (*B*). Only comparisons between pulse and spread groups are reported; $n = 15$ and $n = 14$, respectively, in spread and pulse groups. Significantly different from spread group: $*P < 0.05$; $\dagger P = 0.06$.

protein pulse pattern (1) are durable after the dietary treatment (i.e., the pulse or the spread patterns) has ended. Thus we determined protein synthesis and breakdown on the day after the end of dietary treatments both in a basal (postabsorptive) state and in response to the same moderate stimulus (fed state). Our results demonstrated that the metabolic adaptation induced by the pulse pattern continues and is detectable on postabsorptive protein losses and on fed state protein gains.

Indeed, this specific pulse pattern was able to induce after 14 days, in young as in elderly women, a higher postabsorptive leucine balance than the spread pattern because of a lower protein breakdown. This may be the consequence of the evening meal, which was a low-protein meal in the pulse pattern (providing 14% of total daily intake vs. 28% for the spread pattern), because low-protein diets are known to induce a reduction of postabsorptive protein losses by decreasing protein breakdown (20). The effect of this low-protein evening meal may also be involved in the low postabsorptive leucine oxidation with the pulse pattern. It could be caused by a decrease in the active form of the liver branched-chain α-keto acid dehydrogenase, as described in meal-fed compared with ad libitum-fed rats (5). Moreover, the protein feeding pattern had no significant effect on NOLD during the postabsorptive phase.

In contrast, a significant increase in whole body protein synthesis during the fed state was observed only in women previously fed the pulse feeding pattern. The induction of the fed state was characterized by a moderate increase of the aminoacidemia (i.e., 53% for the sum of the essential amino acids), which was not different between the pulse and the spread groups. However, a higher increase (>50%) of plasma free amino acid levels was shown to be necessary to induce a postprandial increase in the whole body protein synthesis in young adults (15). Such a stimulation is scarcely observed in most studies of the fed state in steady-state conditions (3, 4, 18, 19), and it was also the case in the spread groups. Only the pulse pattern induced an increase in the responsiveness of NOLD to the fed state in both young and elderly women. This suggests that,

one day after the end of the pulse diet, a moderate increase in plasma amino acid concentration remained effective in activating protein synthesis. Furthermore, leucine oxidation was lower during the fed state in the pulse than in the spread groups. This result is in agreement with the sparing effect of a 3-discrete-meal pattern vs. a 10-small-meal pattern (11). Thus fed-state leucine balances were higher in the pulse groups in both young and elderly women. This is in keeping with the improvement of nitrogen balance described in elderly women during the dietary period (1). However, this was not in agreement with nitrogen balance data obtained in young women, because no significant effect of the protein feeding pattern was observed on protein retention (2). In addition, during dietary treatments, protein turnover was higher with the pulse pattern, whereas the previous protein feeding patterns had no effect on Leu R_a in the present study. These discrepancies could be due to the fact that the effects observed during dietary treatments resulted from the interaction between the chronic modifications of protein metabolism and the nutrient flux, which is specific to each diet. In contrast, the results reported in the present paper focus only on chronic metabolic modifications that can be detected in a steady state. Thus the pulse pattern induced similar chronic modifications of protein turnover in both young and elderly women. It could be speculated that, in young women, an efficient response of protein metabolism to the nutrient flux of the pulse pattern could rapidly occur during the dietary treatments, leading to the lack of effect observed with that pattern (2). It will now be of great interest to verify this hypothesis by performing the same measurements for each age group during dietary treatments in non-steady-state conditions.

The present study also reveals some other modifications of the metabolic responses to feeding during aging. The higher increase of glycemia and insulinemia observed in the same fed state in the elderly than in young women is in keeping with the insulin resistance of glucose metabolism that occurs during aging (12). It is associated with a lower inhibition of corrected Endo Leu R_a. Methodological issues must be raised concerning the accurate measurement of Endo Leu R_a in the fed state. Calculation of splanchnic extraction allows a more accurate estimation of protein breakdown, because dietary leucine sequestered by splanchnic tissues during the first pass cannot reach the metabolic pool where [^{13}C]leucine is infused. Corrected Endo Leu R_a takes into account this splanchnic uptake of dietary leucine (23). Leucine splanchnic extraction was measured using free [$^2H_3$]leucine given orally with the liquid meal. This assumed that leucine in the whey protein was as rapidly absorbed as free [$^2H_3$]leucine. Although this was probably not the case, the resulting error in the leucine flux determination is probably minor, because the leucine of whey proteins is readily available (6), and the 4 h of constant feeding allowed a continuous and steady amount of leucine to enter into the body pool. This correction for splanchnic retention provides the means to detect a significant effect of age on proteolysis during feeding, which was less inhibited in elderly women; this was also observed in elderly men (7). In young adults, it was shown in leg muscles that insulin acts predominantly on protein breakdown, resulting in decreasing amino acid release (17). In the present study, the reduction of proteolysis inhibition during the fed state, leading to a higher increase of plasma free amino acids, was observed in elderly women despite the higher increase of insulinemia. Thus, as it has already been suggested in other experiments in humans (8, 14) and in rats, at muscle level (10, 13), an insulin resistance of protein metabolism could develop during aging.

In conclusion, our data demonstrated some age-related dysregulation of protein breakdown during the fed state. Moreover, this experiment clearly shows that protein turnover modifications induced in elderly women fed the protein pulse pattern for 14 days persist 1 day after subjects have stopped this diet. These modifications provide an explanation for the mechanisms implied in the positive effect of the pulse pattern observed previously in elderly women (1). It improves the responsiveness of protein synthesis to the fed state and shows its better protein-sparing effect in the postabsorptive period.

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Address for reprint requests: P. Patureau Mirand, UEMA, INRA Clermont-Ferrand-Theix, 63122 St Genès Champanelle, France.

Address for correspondence: M. A. Arnal, UEMA, INRA, 63122 St Genès Champanelle, France (E-mail: arnalma@clermont.inra.fr).

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