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Am J Physiol Endocrinol Metab 280:340-348, 2001.

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The digestion rate of protein is an independent regulating factor of postprandial protein retention

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Received 23 June 2000; accepted in final form 16 October 2000

Dangin, Martial, Yves Boirie, Clara Garcia-Rodenas, Pierre Gachon, Jacques Fauquant, Philippe Callier, Olivier Ballèvre, and Bernard Beaufrère. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* 280: E340–E348, 2001.—To evaluate the importance of protein digestion rate on protein deposition, we characterized leucine kinetics after ingestion of “protein” meals of identical amino acid composition and nitrogen contents but of different digestion rates. Four groups of five or six young men received an L-[1-¹³C]leucine infusion and one of the following 30-g protein meals: a single meal of slowly digested casein (CAS), a single meal of free amino acid mimicking casein composition (AA), a single meal of rapidly digested whey proteins (WP), or repeated meals of whey proteins (RPT-WP) mimicking slow digestion rate. Comparisons were made between “fast” (AA, WP) and “slow” (CAS, RPT-WP) meals of identical amino acid composition (AA vs. CAS, and WP vs. RPT-WP). The fast meals induced a strong, rapid, and transient increase of aminoacidemia, leucine flux, and oxidation. After slow meals, these parameters increased moderately but durably. Postprandial leucine balance over 7 h was higher after the slow than after the fast meals (CAS: 38 ± 13 vs. AA: -12 ± 11 , $P < 0.01$; RPT-WP: 87 ± 25 vs. WP: 6 ± 19 $\mu\text{mol/kg}$, $P < 0.05$). Protein digestion rate is an independent factor modulating postprandial protein deposition.

amino acid turnover; postprandial protein anabolism; milk protein; stable isotopes

SHIFTING FROM THE POSTABSORPTIVE (overnight fasting) to the fed state is characterized by protein deposition, as a result of coordinated changes in protein synthesis, breakdown, and amino acid oxidation (4, 17, 19, 28, 31, 33, 40). The main regulators of these metabolic pathways are hormones, primarily insulin (10, 15), and substrates, primarily amino acids (18, 38, 40). Amino acid availability depends directly on both the quantity (17, 31) and the quality (30, 33) of the dietary source of nitrogen. In addition, we have recently suggested the

existence of a relationship between the regulation of postprandial protein kinetics and the rate of digestion and absorption of dietary proteins (5). Using milk protein fractions intrinsically labeled with L-[1-¹³C] leucine, we demonstrated that whey proteins are more rapidly digested than native micellar casein. Most importantly, postprandial leucine oxidation was significantly higher after ingestion of whey proteins than after ingestion of casein despite identical leucine intakes. Therefore, the postprandial leucine balance, an index of protein deposition, was better with casein than with whey (5). Taken together, our results suggested that, in young adults, “slow” proteins (e.g., casein) fare better than “fast” proteins (e.g., whey) with respect to postprandial protein gain. This new concept might represent an original and adjunctive dietary strategy to prevent protein losses in various pathological circumstances (16).

However, the relative importance of protein digestion rate on protein metabolism requires further investigation. The amino acid composition of a dietary protein is a major factor affecting its biological value. The amount of dietary nitrogen also affects postprandial protein anabolism. In our previous studies, we closely controlled the leucine contents of the different meals (5). However, both the total nitrogen content and the amino acid composition of the whey proteins and of the casein were different. This might have influenced the previous results and warranted further examination.

Therefore, the main objective of the present set of studies was to compare postprandial whole body protein kinetics, and mainly protein deposition, after ingestion of various protein meals having identical amino acid composition but differing only by their rate of digestion. Our primary end point was postprandial leucine balance. For that purpose, casein (a slow protein) was compared with a mixture of free amino acids that mimicked the amino acid composition of casein

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Table 1. Amino acid composition of the meals

	AA	CAS	Whey Proteins
Aspartic acid	3.3	3.3	6.0*
Asparagine	2.1	3.2	
Threonine	4.1	4.0	3.3
Serine	3.8	5.0	3.7
Glutamine	12.7	11.6	21.9†
Glutamic acid	9.2	9.2	
Proline	10.6	10.1	10.2
Glycine	1.5	1.7	1.5
Alanine	2.7	2.7	2.5
Valine	6.3	6.0	5.3
Cysteine	0.2	0.3	ND
Methionine	2.9	2.7	2.4
Isoleucine	4.9	4.6	3.9
Leucine	9.2	8.9	11.1
Tyrosine	5.0	5.2	4.6
Phenylalanine	4.8	4.8	4.4
Lysine	7.6	7.3	6.0
Histidine	2.6	2.7	2.3
Arginine	3.3	3.3	2.6
Tryptophan	1.5	1.5	ND

Values are in g/100 g powder. AA, single meal made of free amino acids mimicking casein composition; CAS, single meal made of casein; whey proteins, single meal made of whey proteins (WP) and 13 small meals ingested every 20 min totaling 30 g of whey proteins (RPT-WP); ND, not determined. *Sum of aspartic acid and asparagine; †sum of glutamic acid and glutamine.

but acted as a fast-digested meal. The second pair of studies compared the effects of a single whey protein meal (a fast protein) with repeated small feeding of whey (same amount of protein but mimicking a slow protein). Thus, in each pair of studies, the slow and fast meals being compared had both identical nitrogen content and amino acid composition.

MATERIALS AND METHODS

Materials

L-[1-¹³C]leucine [99 mole percent excess (MPE)], L-[5,5,5-²H₃] leucine (97 MPE), and sodium [¹³C]bicarbonate (99 MPE) were obtained from MassTrace (Woburn, MA). Isotopic and chemical purity of leucine were checked by gas chromatography-mass spectrometry (GC-MS). Solutions of tracers were tested for sterility and pyrogenicity before use and were prepared in sterile pyrogen-free water. During each experiment, the tracers were membrane filtered through 0.22-μm filters.

Two bovine milk protein fractions (casein and whey proteins) were separated by microfiltration and ultrafiltration techniques as already described (6). Their amino acid composition was determined by liquid chromatography (amino acid analyzer Beckman 6300) after acid hydrolysis (or alkaline hydrolysis for tryptophan content). A mixture of free amino acids (Ajinomoto, Hamburg, Germany) was made very close to that of casein amino acid composition (Table 1). The mixture of amino acids, casein, and whey protein fractions were tested for microbiological quality.

Subjects

Twenty-two healthy young male volunteers were divided into four groups. They were 25 ± 1 yr old and had normal body weight (mass 66.8 ± 1.2 kg; body mass index 21.5 ± 0.3 kg/m²; means ± SE). Each subject had a normal physical

examination without any medical history of renal, cardiovascular, or endocrine disease. During the 4 days before the study, the subjects maintained their usual physical activity and protein-energy intake of ≈159 kJ·kg⁻¹·day⁻¹ with protein comprising 16% of total energy intake. The purpose and the potential risks of the study were fully explained, and written informed consent was obtained from each participant. The experimental study was approved by the local ethics committee.

Experimental Protocol

On the day of the experiment, a catheter was retrogradely inserted into a dorsal vein of the hand and used for arterial-ized blood sampling after introduction of the hand into a ventilated box heated to 60°C. A second catheter was inserted into a vein of the contralateral arm for tracer infusion. After a priming dose of sodium [¹³C]bicarbonate (6 mg), a primed (3.9 μmol/kg) continuous (0.06 μmol·kg⁻¹·min⁻¹) infusion of L-[1-¹³C]leucine commenced and was continued for 580 min (Fig. 1). After 160 min of infusion, one of the following liquid meals was administered: a single meal composed of 30 g of free amino acids mimicking casein composition (AA, n = 6); a single meal composed of 30 g of casein (CAS, n = 6); a single meal composed of 30 g of whey proteins (WP, n = 5); a sequence of 13 small whey protein meals given every 20 min during 240 min and totaling 30 g of protein (RPT-WP, n = 5).

All of the meals were prepared on the day of the protocol by adding 285 ml of water to the protein or amino acid powders. The single meals (CAS, AA, and WP studies) were ingested in <5 min. To evaluate the digestion rate, L-[5,5,5-²H₃]leucine (12.9 μmol/kg body wt) was included in the AA and RPT-WP meals (in the latter case, the tracer was divided among the 13 meals). The goal of the experimental design was to compare the protocols in pairs: AA vs. CAS and WP vs. RPT-WP; in each of the study pairs, one meal (AA or WP) was intended to

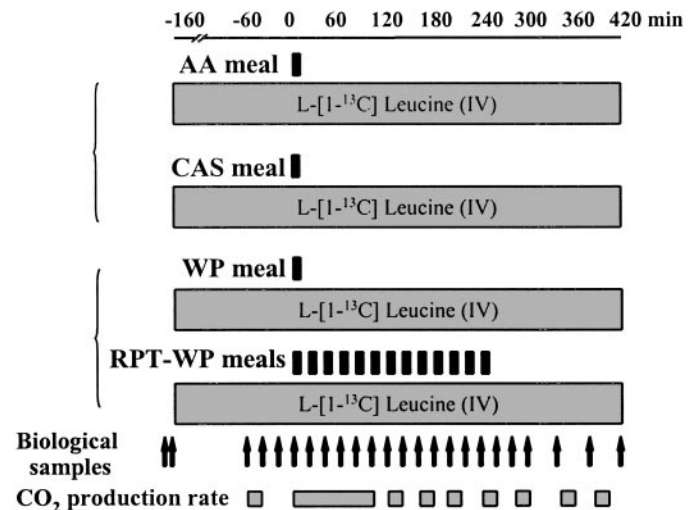


Fig. 1. Experimental protocol composed of four different studies performed in two pairs of experiments (in brackets). In the first pair of studies, volunteers ingested either a single meal composed of 30 g of free amino acids mimicking casein composition (AA) or 30 g of casein (CAS). In the second pair, the subjects received either a single meal composed of 30 g of whey proteins (WP) or 13 small meals ingested every 20 min (during 240 min) composed of whey proteins and totaling 30 g (RPT-WP). Within each set of studies, amino acid composition and nitrogen content of the meals were the same.

be fast, whereas the other one (CAS or RPT-WP) was intended to be slow. The leucine contents of the CAS and AA meals were identical [289 ± 8 and $289 \pm 15 \mu\text{mol/kg}$, respectively, not significant (NS)] but lower ($P < 0.01$) than the leucine contents of the WP and RPT-WP meals (359 ± 18 and $393 \pm 15 \mu\text{mol/kg}$, respectively, NS). The leucine content was slightly higher in the RPT-WP than in the WP meals, because two consecutive batches of whey fractions were used.

Blood and breath samples were taken before any infusion (-170 and -160 min, respectively), before the meal when L-[^{13}C]leucine had reached a plateau (-60 , -40 , -20 , and 0 min), and after meal ingestion at 20-min intervals for the first 300 min and at 40-min intervals for the last 120 min (Fig. 1). The plasma supernatant was separated, an internal standard was added (norleucine), and the sample was kept at -20°C until further analysis. Breath samples were collected and stored in 10-ml Vacutainers (Beckton-Dickinson, Grenoble, France) until [^{13}C]CO₂ enrichment analysis. Total CO₂ production rates were measured in the postabsorptive state and after meal ingestion for 20 min by open-circuit indirect calorimetry (Deltatrac, Datex, Geneva, Switzerland). Plasma insulin and amino acid concentrations were also measured before the meal (-20 min) and after the meal ($+60$ min for insulin, $+120$ and $+300$ min for insulin and amino acid, respectively).

Analytical Methods

Plasma [^{13}C]leucine and ketoisocaproate (KIC) and [$^2\text{H}_3$]leucine enrichments were measured by selected ion monitoring electron impact GC-MS (Hewlett-Packard 5971A, Palo Alto, CA) using *tert*-butyldimethylsilyl derivatives as previously described (8). The L-[^{13}C]leucine enrichments in the RPT-WP and AA meals were also determined. Plasma leucine concentrations were measured on the same runs with an internal standard. Corrections for the ^{13}C and $^2\text{H}_3$ enrichments were applied according to Biolo et al. (4). [^{13}C]CO₂ enrichments were measured on a gas isotope ratio-mass spectrometer (μGas system, Fisons Instruments, VG Isotech, Middlewich, England). In preliminary studies, it was confirmed that diets did not induce any modification of the natural ^{13}C abundance in CO₂ (data not shown).

Plasma insulin concentrations were measured by radioimmunoassay (Schering, CIS Bio International, Gif-sur-Yvette, France) and plasma amino acid concentrations by liquid chromatography (Biotech-Kontron; St-Quentin, France).

Calculations

Leucine fluxes were calculated from the time-dependent evolution of plasma leucine and KIC enrichments and concentrations in the nonsteady state by use of a precursor pool model as previously described (8).

The rate of total leucine appearance in the peripheral circulation (Tot Leu R_a) is the sum of the entry rate of endogenous leucine derived from protein breakdown (Endo Leu R_a) plus the intravenously infused labeled leucine plus the entry rate of exogenous (i.e., dietary) leucine (Exo Leu R_a). The latter is equal to zero before the meal, and then Tot Leu R_a equals Endo Leu R_a. Similarly, total leucine rate of disappearance from plasma (Tot Leu R_d) is the sum of the fluxes of leucine, either oxidized (Tot Leu Ox) or utilized for protein synthesis (total nonoxidative leucine disposal or total NOLD).

The equations used were previously reported in detail (8). Briefly, Tot Leu R_a was calculated from the intravenous tracer (^{13}C]leucine) infusion rate, corrected for its time-dependent appearance into the plasma, and from the plasma

leucine enrichment of the intravenous tracer. In the AA and RPT-WP studies, Exo Leu R_a (plasma entry rate of dietary leucine) was calculated from the simultaneous enrichments of the infused (^{13}C]leucine) and of the ingested ($[5,5,5\text{-}^2\text{H}_3\text{]}leucine$) tracers, according to the transposition of Steele's equations by Proietto et al. (35). The fraction of dietary leucine taken up by the gut and the liver during its first pass (splanchnic extraction of leucine) was also estimated as $\{[\text{leucine ingested}] - \text{area under the curve (AUC) of Exo Leu R}_a\} / \text{leucine ingested} \times 100$. Endo Leu R_a, an index of whole body protein breakdown, was obtained by subtracting Exo Leu R_a and the tracer infusion rate from Total Leu R_a. In the CAS and WP single-meal studies, no oral tracer was given (see DISCUSSION); therefore, Exo Leu R_a, splanchnic extraction, and Endo Leu R_a were not calculated for these two meals.

Postabsorptive and postprandial Tot Leu Ox and NOLD were measured in the four studies. Tot Leu Ox was obtained by dividing the [^{13}C]CO₂ production rate by the plasma [^{13}C]KIC enrichment. Tot Leu R_d was calculated as Tot Leu R_a minus the time-dependent changes in leucine pool size. Total NOLD was therefore Tot Leu R_d minus Tot Leu Ox. Postprandial leucine balance was finally calculated over a 420-min period by subtracting the integrated AUC of Tot Leu Ox from the leucine intake (leucine ingested + leucine infused). AUC was calculated by the trapezoidal method.

In all the calculations, the constants for leucine distribution volume (0.5 l/kg), correction factor of the pool size for instant mixing (0.25), and CO₂ recovery factor (0.8) were the same as those previously utilized (5, 36, 39).

As described above, leucine oxidation was calculated using KIC as the precursor pool, because KIC is the immediate precursor of leucine decarboxylation (26). The choice of leucine or KIC as precursor for the other calculations is more controversial, because KIC could be more representative of the intracellular leucine enrichment. On the other hand, it is more logical to use the oral tracer enrichment to calculate Exo Leu R_a, because the dietary leucine is little transaminated on its first splanchnic pass (11, 42). Then, leucine enrichment was used all over the calculation of the fluxes (except oxidation) for the sake of homogeneity. In addition, this allowed easier comparisons with our previous single-meal studies (5, 8) in which the same approach was selected.

Statistical Analyses

Statistical analyses were performed (Statview, 4.02, Abacus Concept, Berkeley, CA) to 1) characterize leucine kinetics modification after a test meal (i.e., changes from the post absorptive state) and 2) compare the protocols two-by-two (i.e., AA vs. CAS and WP vs. RPT-WP).

Changes induced by meal ingestion. Individual curves were standardized by subtracting the mean of the baseline values (-40 to 0 min) from each individual time point. To assess modifications in leucine kinetics induced by meal ingestion, a confidence interval was calculated for each test meal by use of the baseline values as follows: mean $\pm T\alpha \times \text{SD}$, where SD is the standard deviation of the n baseline individual values, and $T\alpha$ is the critical value of the Student's distribution for $(n - 1)$ degrees of freedom. The changes were declared significantly different ($\alpha = 0.001$) from the baseline when all the individual values fell outside of this interval.

Comparison between the slow and fast meals. Each individual curve was also characterized by its zenith or nadir (Y_{max}) by the time at which Y_{max} was observed (T_{max}) and by the AUC when appropriate. Because Y_{max} occurred at variable times after the meal ingestion, the mean Y_{max} values

Table 2. Maximum value and time of occurrence of postprandial variation for plasma leucine concentrations and fluxes

	AA			CAS			WP			RPT-WP		
	Basal	Y_{max}	T_{max}	Basal	Y_{max}	T_{max}	Basal	Y_{max}	T_{max}	Basal	Y_{max}	T_{max}
[Leu]	137 ± 6	446 ± 25‡	40 ± 6†	121 ± 6	230 ± 8	253 ± 52	115 ± 6	449 ± 25†	68 ± 10‡	118 ± 9	321 ± 16	224 ± 28
Tot Leu R_a	1.6 ± 0.1	3.7 ± 0.1‡	37 ± 7*	1.6 ± 0.1	2.2 ± 0.1	147 ± 41	1.6 ± 0.1	3.9 ± 0.2‡	80 ± 11‡	1.6 ± 0.1	2.8 ± 0.1	204 ± 31
Exo Leu R_a		2.2 ± 0.2	43 ± 13								1.8 ± 0.2	136 ± 36
Endo Leu R_a	1.5 ± 0.1	1.2 ± 0.1	120 ± 32	1.5 ± 0.1			1.6 ± 0.1			1.6 ± 0.1	0.9 ± 0.1	160 ± 37
Tot Leu Ox	0.4 ± 0.1	1.5 ± 0.1‡	97 ± 10†	0.4 ± 0.1	0.8 ± 0.1	197 ± 31	0.4 ± 0.1	1.7 ± 0.1†	112 ± 15†	0.4 ± 0.1	1.1 ± 0.1	220 ± 33
NOLD	1.2 ± 0.1	2.4 ± 0.1‡	60 ± 6*	1.2 ± 0.1	1.5 ± 0.1	123 ± 51	1.2 ± 0.2	2.6 ± 0.1‡	112 ± 15	1.3 ± 0.1	1.9 ± 0.1	164 ± 48

Results are means ± SE. Y_{max} , mean zenith or nadir value for each subject; T_{max} , mean of time to Y_{max} for each subject (in min); [Leu], plasma leucine concentrations in $\mu\text{mol/l}$; Tot Leu R_a , total leucine rate of appearance; Exo Leu R_a , dietary (exogenous) leucine rate of appearance; Endo Leu R_a , endogenous leucine rate of appearance (reflection of proteolysis); Tot Leu Ox, total leucine oxidation. NOLD, nonoxidative leucine disposal (reflection of protein synthesis). Flux values are in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. AA significantly different from CAS; WP significantly different from RPT-WP (* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$; t -test).

(Table 2) may be different from the mean values at a given time point, as shown on Figs. 3–5. To compare Y_{max} , T_{max} , and AUC between CAS and AA, and between WP and RPT-WP, we used (unpaired) one-tailed Student's t -tests, because we expected modifications in the same directions as the one previously reported with the same model (5). A Wilcoxon rank sum test was also performed on the same parameters. Because the P values were always close to those calculated with the Student's test, only the latter are provided. The results are expressed as means ± SE.

RESULTS

Plasma Insulin and Amino Acid Concentrations

Plasma insulin concentration increased moderately 60 min after the AA meal ($+6.9 \pm 2.6 \mu\text{U/ml}$ above baseline, $P < 0.05$) but not after the CAS meal (AA vs. CAS, $P < 0.05$). Similarly, it increased after the WP

meal ($+14.9 \pm 3.2 \mu\text{U/ml}$, $P < 0.01$) but not during the RPT-WP meal (WP vs. RPT-WP, $P < 0.01$). The increase was slightly more pronounced after WP than after AA ($P < 0.05$).

Leucine concentrations increased after each meal. The AA and WP (fast) meals induced a higher and faster increase than their respective CAS and RPT-WP counterparts (see Y_{max} and T_{max} in Table 2, and curves on Fig. 2A). In addition, this elevation was more transient for the fast meals than for the slow meals: it started at 20 min and lasted until 180–200 min after the AA and WP meals, whereas for the CAS and RPT-WP meals the elevation started at 20–40 min and continued until 420 min (time points significantly different from baseline values, Fig. 2A). The variations above baseline of the sum of total and essential plasma

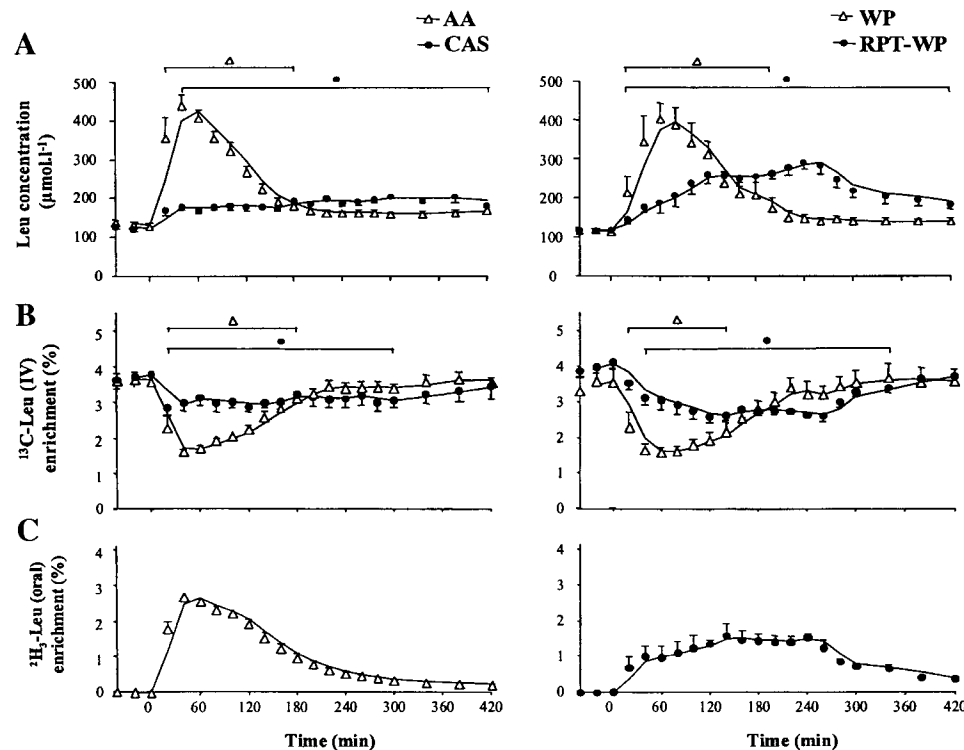


Fig. 2. Evolution of plasma leucine (Leu) concentration (A) and plasma leucine enrichments (B and C) after meal ingestion. Meals ingested by the volunteers were AA, CAS, WP, or RPT-WP. Between AA and CAS meals (left) and between WP and RPT-WP meals (right), protein digestion rates were different ("fast" or "slow"), but amino acid profiles were identical. Δ , Fast meals (AA or WP); \bullet , slow meals (CAS or RPT-WP). B: time-dependent evolution of plasma [^{13}C]leucine enrichments (iv tracer) for the fast and slow meals, respectively. C: evolution of plasma [^3H]leucine enrichments (oral tracer added for AA and RPT-WP meals, respectively). Results are expressed as means ± SE. Lines at the top of each graph indicate a significant difference ($P < 0.001$) from baseline within each study. Moving averages were calculated on 2 consecutive time points.

Table 3. Baseline plasma amino acid concentration and %variations above baseline 120 or 240 min after meal ingestion

	Baseline, $\mu\text{mol/l}$	%Variation Above Baseline							
		AA		CAS		WP		RPT-WP	
		120	240	120	240	120	240	120	240
SEA	860 ± 19	$77 \pm 11^{*\dagger}$	$16 \pm 5^{*\dagger}$	$32 \pm 6^\dagger$	$35 \pm 6^\dagger$	$92 \pm 6^\dagger$	$9 \pm 6^*$	$75 \pm 15^\dagger$	$66 \pm 12^\dagger$
STA	$2,436 \pm 51$	$43 \pm 15^\dagger$	$5 \pm 5^*$	22 ± 10	$23 \pm 6^\dagger$	$48 \pm 11^\dagger$	-3 ± 3	$36 \pm 13^\dagger$	$28 \pm 6^\dagger$

Results are means \pm SE. Baseline values are calculated from the basal values from all 22 subjects. %Variations above basal are calculated using the corresponding baseline value of each subject. SEA, sum of essential amino acids (minus tryptophan); STA, sum of total amino acids analyzed. *Significant difference ($P < 0.05$, t -test) between AA and CAS, and between WP and RPT-WP, at 120 or 240 min; \dagger significant increase ($P < 0.05$, t -test) above baseline within each study.

amino acids are listed in Table 3. They confirmed a marked but transient increase of essential and nonessential amino acids after the fast meals. By contrast, the increases were persistent at 240 min after the slow meals.

Leucine Enrichments

In all studies (Fig. 2B), the intravenous tracer enrichments (^{13}C leucine) reached a plateau before meal ingestion. Thereafter, plasma ^{13}C leucine enrichment was reduced for 140–180 min after the fast meal ingestion. This decrease was less pronounced but lasted at least for 340 min after the slow meal consumption. Plasma ^{13}C KIC enrichments (data not shown) followed the same patterns as the plasma ^{13}C leucine enrichments. In the two studies that included $[5,5,5\text{-}^2\text{H}_3]$ leucine as an oral tracer (AA and RPT-WP), the increase in plasma enrichment was high and transient

after the AA meal but was persistent and low after the RPT-WP meal (Fig. 2C).

Leucine R_a

The patterns of Tot Leu R_a paralleled those of leucine concentrations: Tot Leu R_a increased rapidly but transiently after the AA and WP meals, whereas a plateau was achieved after the CAS or RPT-WP meals (Fig. 3B). The Y_{max} values were significantly higher with the AA and WP than with the CAS or RPT-WP meals, whereas the corresponding T_{max} values were shorter (Table 2).

As stated in *Calculations*, Exo Leu R_a and Endo Leu R_a were measured only during the AA and RPT-WP studies. The dietary leucine (Exo Leu R_a) appeared in the plasma immediately (20 min) after ingestion of both meals (Fig. 3A). However, after AA ingestion, the peak of absorption was achieved rapidly (Table 2) and

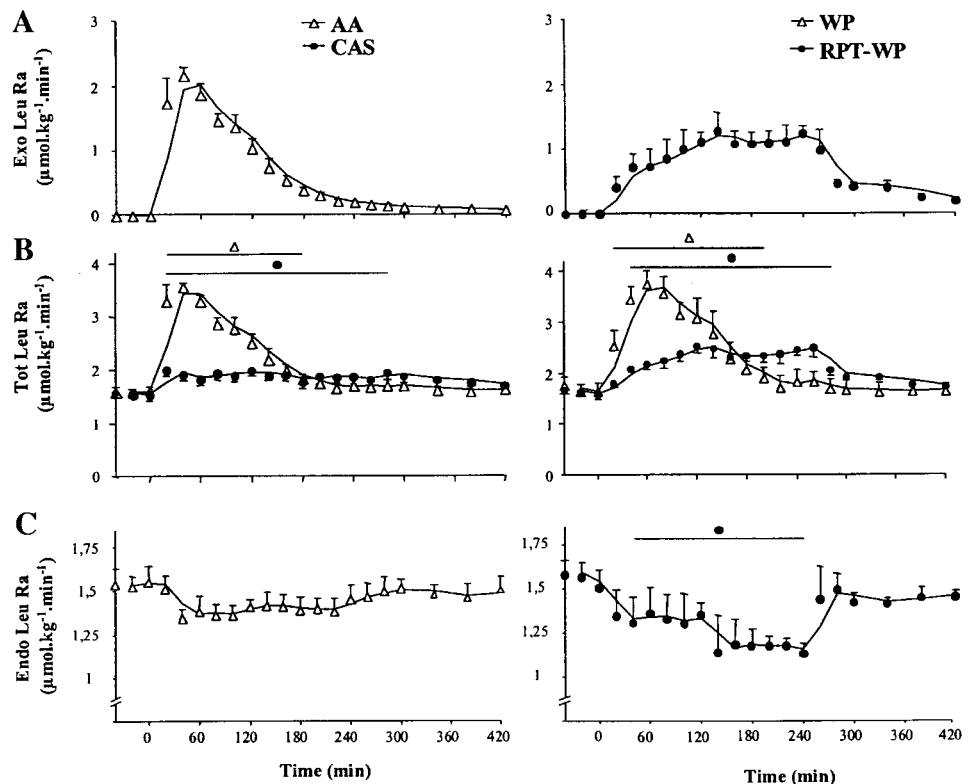


Fig. 3. Time-dependent evolution of exogenous (Exo Leu R_a ; A), total (Tot Leu R_a ; B), and endogenous leucine rates of appearance (Endo Leu R_a ; C) after meal ingestion. Meals ingested by the volunteers were AA, CAS, WP, or RPT-WP. Between AA and CAS meals (left) and between WP and RPT-WP meals (right), protein digestion rates were different (fast or slow), but amino acid profiles were identical. Δ , Fast meals (AA or WP); \bullet , slow meals (CAS or RPT-WP). Results are expressed as means \pm SE. Lines at the top of each graph indicate a significant difference ($P < 0.001$) from baseline within each study. Moving averages were calculated on 2 consecutive time points.

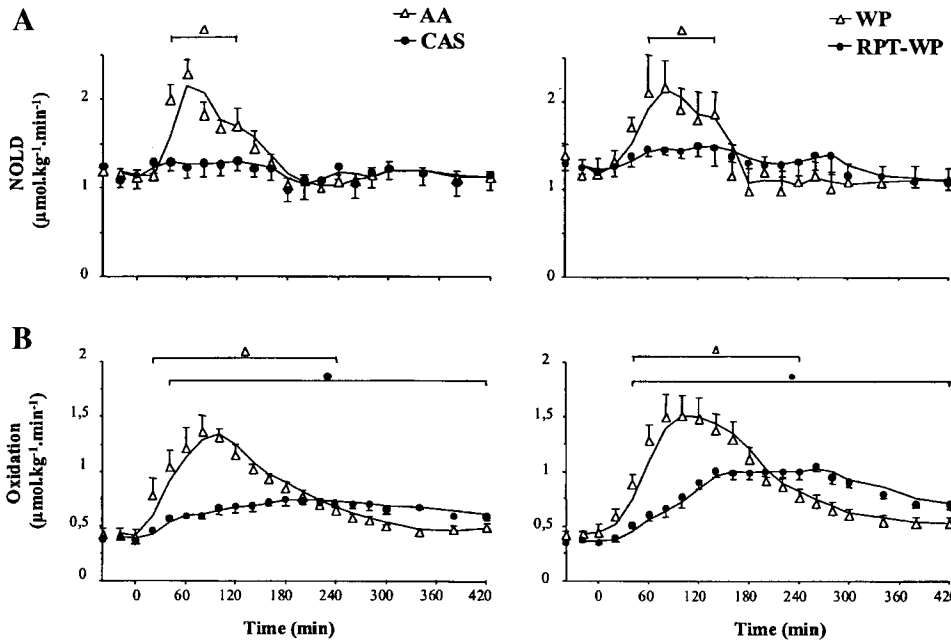


Fig. 4. Time-dependent evolution of total nonoxidative leucine disposal (NOLD), i.e., protein synthesis (A) and total leucine oxidation (B) after meal ingestion. Between AA and CAS meals (left) and between WP and RPT-WP meals (right), protein digestion rates were different (fast or slow), but amino acid profiles were identical. Δ , Fast meals (AA or WP); \bullet , slow meals (CAS or RPT-WP). Results are expressed as means \pm SE. Lines at the top of each graph indicate a significant difference ($P < 0.001$) from baseline within each study. Moving averages were calculated on 2 consecutive time points.

Exo Leu R_a was close to the basal value after 260 min (260 min: $0.16 \pm 0.02 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Conversely, after RPT-WP, Exo Leu R_a peaked at 136 min (Table 2 and Fig. 3A) and remained high for 340 min (340 min: $0.43 \pm 0.07 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The integrated values (AUC) of Exo leu R_a were 257.9 ± 13.5 and $312.0 \pm 27.7 \mu\text{mol}/\text{kg}$ for AA and RPT-WP, respectively. Splanchnic extraction of the dietary leucine was 10.9 ± 1.5 and $20.7 \pm 3.6\%$ for AA and RPT-WP, respectively.

Endo Leu R_a , an index of whole body protein breakdown, decreased after the AA and RPT-WP meals (Fig. 3C and Table 2). However, after AA ingestion, the postprandial inhibition was moderate ($-0.34 \pm 0.07 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ below baseline). After the RPT-WP meal, this inhibition was more pronounced ($-0.69 \pm 0.13 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P < 0.05$ vs. AA) and more prolonged (for 40–240 min).

Leucine Rates of Utilization

NOLD, an index of whole body protein synthesis, dramatically rose from 40 to 120 min after the AA meal and from 60 to 140 min after the WP meal (Fig. 4A and Table 2). By contrast, this increase was transient and moderate after either the CAS or RPT-WP meals and occurred at variable times after the meals. Therefore, the stimulation of NOLD, as assessed by the Y_{max} values, was more marked ($P < 0.001$, Table 2) after the fast meals than after the slow meals.

After protein ingestion, Tot Leu Ox increased in all studies (Fig. 4B). Again, the shapes of the curves were clearly different depending on the type of meal. A peak was clearly identified after the fast meals: Tot Leu Ox were significantly different from baseline from 20 (AA meal) or 40 min (WP meal) until only 240 min. After the CAS and RPT-WP meals, Tot Leu Ox was stimulated to a lesser extent but for a longer time (significant until 420 min). The peak values (Y_{max} , Table 2) were

higher after the AA and WP meals than after their respective CAS and RPT-WP counterparts. The AUCs, which represent the total postprandial leucine losses over 7 h, were different between AA ($325 \pm 17 \mu\text{mol}/\text{kg}$) and CAS ($275 \pm 9 \mu\text{mol}/\text{kg}$, $P < 0.05$ vs. AA) but only tended to be higher with WP ($377 \pm 28 \mu\text{mol}/\text{kg}$) than with RPT-WP ($344 \pm 12 \mu\text{mol}/\text{kg}$, $P = 0.15$).

Finally, and as shown in Fig. 5, postprandial leucine balance was higher after the CAS ($38 \pm 13 \mu\text{mol}/\text{kg}$) than after the AA meal ($-12 \pm 11 \mu\text{mol}/\text{kg}$, $P < 0.01$). It was also better after the RPT-WP ($87 \pm 25 \mu\text{mol}/\text{kg}$) than after the WP meal ($6 \pm 19 \mu\text{mol}/\text{kg}$, $P < 0.05$).

DISCUSSION

Our data demonstrate that the rate of absorption of a high biological value dietary nitrogen source affects postprandial leucine deposition independently of amino acid composition. In the first pair of studies, free amino acids and native casein were taken as paradigms for the fast and slow proteins, respectively. The

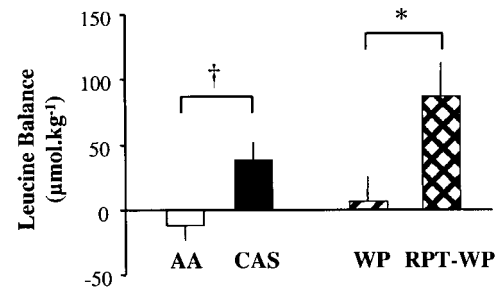


Fig. 5. Postprandial leucine balance over 420 min after meal ingestion. Between AA and CAS meals (left) and between WP and RPT-WP meals (right), protein digestion rates were different (fast or slow), but amino acid profiles were identical. Statistical difference between AA and CAS and between WP and RPT-WP studies are indicated (* $P < 0.05$, † $P < 0.01$; *t*-test).

rate of absorption of the AA meal was assessed by adding free [$^2\text{H}_3$]leucine into the meal and measuring its appearance into the plasma. We confirmed that a free amino acid mixture is rapidly absorbed by the gut, as previously shown (37). Assessment of the digestion rate of casein raised a more difficult problem. Adding an oral free tracer to the CAS meal would have been useless, because we had previously demonstrated that only an intrinsically labeled dietary protein is appropriate for such a purpose (8). Unfortunately, [$^2\text{H}_3$]leucine-labeled casein was not available. Using [^{13}C]leucine-labeled casein was not adequate either. Because a reliable measurement of leucine oxidation was needed to calculate leucine balance, the [^{13}C]leucine label had to be given intravenously in a continuous manner. A combination of oral [^{13}C]leucine (as labeled casein) and intravenous [^{14}C]leucine would have been ideal, but radioactive tracers are not allowed for such studies in France. However, despite the absence of direct measurement of the casein digestion rate, we have good evidence that casein did behave as a slow protein. First, we (5) and others (24, 25) had previously demonstrated this fact by means of various approaches. Second, in our previous studies (5), we had always observed a close relationship between the measured rate of digestion of a protein and the pattern of either plasma leucine concentration, Tot Leu R_a , or [^{13}C]CO $_2$ R_a . In the present CAS experiment, the postprandial evolution of these three parameters was quite consistent with a slow protein pattern, since they exhibited a pseudo-plateau. It is likely that the rate-limiting step of such a slow absorption is due to coagulation of casein in the stomach (24, 25). There is also a possibility that active opioid regulatory peptides, contained in the sequence of some of the casein fractions, could play a role by modulating gastrointestinal motility (12).

In our second pair of experiments, we actually compared two modes of administration of the same milk protein fraction, i.e., native whey. Again, leucine oxidation and balance were the most important variables; therefore, no tracer was included within the single WP meal for the same aforementioned reasons. However, the patterns of postprandial changes of leucine concentration, Tot Leu R_a , and oxidation again demonstrate that a single WP meal is indeed a fast meal, in keeping with previous data from our group (5, 8) and from others (25). To mimic a slow meal, small boluses were given every 20 min. Such a mode of feeding (4, 17, 28, 29, 31) was shown previously to result in a plateau equivalent to what is obtained with a continuous nasogastric feeding (11, 19, 27, 41). Steady state was indeed achieved after 1.5–2 h of feeding for leucine concentrations and for the oral and intravenous tracer enrichments. In steady-state conditions, free [^{13}C]leucine added to protein induced identical enrichments and leucine fluxes to those observed with an intrinsically [^{13}C]leucine-labeled protein (29). Therefore, it is likely that the free tracer added to the RPT-WP meals does represent the digestion rate of the meal, at least during the steady-state period. The pur-

pose of the interruption of feeding at 240 min was to mimic the progressive return to baseline observed with casein. This was generally achieved in particular for the [^{13}C]leucine and KIC enrichments and for leucine oxidation. Thus we believe that our study design allowed adequate comparison of fast and slow meals of identical amino acid composition.

The postprandial period involves modifications of protein breakdown and synthesis and of amino acid oxidation. Protein breakdown, as assessed by Endo Leu R_a , is a component of Tot Leu R_a together with the dietary leucine flux (Exo Leu R_a). Because the latter was measured only in the AA and RPT-WP experiments (see above), we had access to protein breakdown only in these two situations. After a fast meal made of free amino acids, protein breakdown was transiently and moderately inhibited. By contrast, in a previous study, a casein meal inhibited protein breakdown by 30% and for 7 h (5). In the second set of studies, the inhibition of protein breakdown induced by the slow meal (RPT-WP) was consistent with similar decreases reported in numerous continuous-feeding studies performed with (7) or without an oral tracer (4, 17, 32, 40). Again, this contrasts with the absence of inhibition observed in our previous studies of a single WP meal (5). The inhibition of protein breakdown after the RPT-WP meal is unlikely to be due to insulin, because no insulin increase was detectable (although the peak of secretion could have been missed). In this respect, the addition of carbohydrates to a protein meal would certainly enhance the inhibition of protein breakdown. Amino acids are also known to inhibit protein breakdown (10, 18, 40), even in the presence of basal insulin levels (10). Although the hyperaminoacidemia after RPT-WP was not very high (and in any case lower than after a fast meal), it was prolonged over 4–5 h. This would suggest that a significant duration of hyperaminoacidemia is necessary to inhibit protein breakdown.

The most striking differences between the fast and slow meals had to do with protein synthesis, as assessed by NOLD, and with amino acid oxidation. Stimulation of synthesis was dramatic and immediate after the fast meals, whereas it was absent after the slow meals. Such unique responses to the fast and slow meals are most likely due to different levels of amino acid availability. Indeed “fast proteins,” which strongly increased amino acid availability (as reflected by plasma aminoacidemia), probably induced a stimulation of protein synthesis. By contrast, “slow proteins,” which modified plasma amino acid concentration to a much slower extent, were not associated with a stimulation of protein synthesis. In accordance with this theory, it has been demonstrated that significant stimulation of protein synthesis requires at least a twofold increase of plasma aminoacidemia above basal level (10, 18, 38). Results obtained with continuous feeding are also consistent with this interpretation. Indeed, protein intakes $>1.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, which lead to hyperaminoacidemia (11, 19), generally induce a stimulation of protein synthesis (19, 31, 32), whereas it is seldom seen for low protein intakes (17, 28, 31, 32).

However, in the present study, stimulation of protein synthesis did not result in a better leucine balance. Indeed, leucine oxidation was also stimulated more by the fast than by the slow meals, which was likely due to direct stimulation of the rate-limiting enzymes of amino acid oxidation by the amino acids themselves. The absence of a relationship between a better balance and a higher synthesis also emphasizes the importance of the inhibition of protein breakdown in the postprandial utilization of proteins (14).

Our results suggest that a slowly digested protein induces a better postprandial utilization than a fast one. The efficiency of postprandial protein utilization can be calculated on the basis of leucine balance, as proposed by Millward et al. (30). In brief, Δ leucine balance (postprandial balance – postabsorptive balance) is used to predict a Δ protein nitrogen balance assuming a tissue protein leucine content of 3.93 mmol leucine/g N. This predicted nitrogen balance is then divided by nitrogen intake. With these calculations, we confirm a better ($P < 0.05$) protein utilization over 7 h after the slow meals (CAS, 0.78 ± 0.04 ; RPT-WP, 0.80 ± 0.07) than after the fast meals (AA, 0.62 ± 0.06 ; WP, 0.66 ± 0.03). Obviously, studies in other populations and dealing with more complex meals and examining longer-term changes are needed. In this respect, it will be particularly important to assess the effects of nonprotein energy sources, because they could affect both digestion rate (9, 20, 23) and protein metabolism (3, 21, 34). It was important, however, to reinforce the initial data (5) obtained with two proteins strongly differing not only by their rate of digestion but also by their amino acid composition.

More generally, our results support the idea that the chronology of amino acid availability is an important and independent factor modulating nitrogen retention. Substrate availability can vary with time by feeding slow and fast meals, as in the present study, but it can also be modified by changing the repartition of dietary proteins (or protein/energy) during the day. In young women, nitrogen balance was higher when the daily protein intake was spread fairly evenly over three meals than when spread over two meals, with one meal containing no protein (22). In young men, whole body leucine balance was higher with a diet given over 24 h than with three discrete meals (13). More recently, Arnal et al. (2) showed that, in elderly women, protein retention was better with a protein pulse-feeding pattern than with a spread-feeding pattern. It is tempting to equate pulse feeding with a fast protein condition and spread feeding with a slow one. Thus, extrapolating from Arnal's study, the fast feeding would give a better balance than a slow one in older populations. However, the same authors further demonstrated that, in young people, the opposite effect was observed, i.e., a trend for a better nitrogen balance with a spread feeding than with a pulse feeding (1). The latter result is more in accord with the present study. It also emphasizes the fact that age may be an important determinant of the mechanisms of nitrogen retention. There are numerous differences among all these studies, such

as sex and age, the nature and amount of the protein and nonprotein energy intake, or the methods used. Collectively, however, these results support the idea that postprandial nitrogen retention is dependent on the time and level (the two being related) of amino acid availability.

In conclusion, we have demonstrated that, in addition to protein quantity and amino acid composition, the protein digestion rate is an independent factor modulating postprandial protein deposition. The concept of slow and fast proteins offers a number of applications in elderly people or in patients with wasting disorders (e.g., protein-energy malnutrition, critically ill patients). It could also be applied to circumstances where excessive amino acid concentrations have to be avoided while preserving the anabolic action of dietary proteins (e.g., renal diseases, hepatic encephalopathy).

We wish to thank P. Rousset, L. Morin, J. Vuichoud, and M. Genest for their technical and nursing assistance, S. Corny for tracer preparations, M.-O. Gailing of Nestlé (France), for help, and K. Short for stylistic and grammatical corrections.

This study was supported by Nestlé and by Grant 96G0174 from the Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche and Grant 443/95 from the Association Nationale de la Recherche Technique.

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