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The rate of protein digestion affects protein gain differently during aging in humans

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In young men ingesting protein meals, slowly digested proteins (caseins: CAS) induce a higher protein gain than those that are rapidly digested (whey proteins: WP). Our aim was to assess whether or not this is true in elderly men receiving mixed meals. The effects of meals containing either CAS or two different amounts of WP (WP-iN: isonitrogenous with CAS, or WP-iL: providing the same amount of leucine as CAS) on protein metabolism (assessed by combining oral and intravenous leucine tracers) were compared in nine healthy, elderly (mean ± S.E.M. age 72 ± 1 years) and six young men (24 ± 1 years). In both age groups, WP-iL and WP-iN were digested faster than CAS ($P < 0.001$, ANOVA). Proteolysis was inhibited similarly whatever the meal and age groups (P = NS). Protein synthesis was higher with WP-iN than with CAS or WP-iL ($P < 0.01$), irrespective of age (P = NS). An age-related effect ($P < 0.05$) was found with postprandial leucine balance. Leucine balance was higher with CAS than with WP-iL ($P < 0.01$) in young men, but not in elderly subjects (P = NS). In isonitrogenous conditions, leucine balance was higher with WP-iN than with CAS ($P < 0.001$) in both age groups, but the magnitude of the differences was higher in the elderly men ($P = 0.05$). In conclusion, during aging, protein gain was greater with WP (rapidly digested protein), and lower with CAS (slowly digested protein). This suggests that a 'fast' protein might be more beneficial than a 'slow' one to limit protein losses during aging.

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The decline of lean body mass is a deleterious effect of aging. It has direct and indirect consequences (e.g. on the reduction of physical performance, loss of autonomy and increased susceptibility to illness; Roubenoff, 2000). An adequate dietary strategy could help to limit these losses.

In the postprandial period, the main regulators of protein metabolism are insulin and amino acid (AA) availability. Insulin acts through inhibition of proteolysis (Boirie *et al.* 2001) and stimulation of muscle protein synthesis (Biolo *et al.* 1999). The availability of AAs, which is reflected by plasma aminoacidaemia, is the major regulating factor of protein synthesis and oxidation (Gibson *et al.* 1996; Volpi *et al.* 1998). Since AA availability is affected by the protein digestion rate, this might explain the effects of nitrogen sources differing by their kinetics on postprandial protein gain (Boirie *et al.* 1997*a*; Dangin *et al.* 2001). Indeed, we have shown recently that in young men, proteins that are digested slowly, such as casein (CAS), induce a lower but more prolonged hyperaminoacidaemia and a higher postprandial leucine deposition than a rapidly digested protein fraction, such as whey protein (WP). This effect was independent of the AA composition of the dietary proteins (Dangin *et al.* 2001).

However, the relevance of these results for elderly nutrition is unclear. Indeed, in elderly subjects, indirect evidence suggests that the response of protein metabolism to AA availability is disturbed. First, the alteration of muscle protein synthesis in response to feeding, which has been detected both in old rats (Mosoni *et al.* 1995; Dardevet *et al.* 2002) and in elderly subjects (Welle *et al.* 1994), can be reversed by strong hyperaminoacidaemia (Volpi *et al.* 1998). Second, the specific anabolic response

This article is dedicated to Bernard Beaufrère, who died suddenly and unexpectedly on the 10th August 2002. As our mentor, we will miss his dynamism, his enthusiam and outstanding knowledge in the field of nutrition sciences, as well as his great humanity.

of muscle to leucine, observed in young rats, is blunted in older animals (Dardevet *et al.* 2000, 2002). Third, in elderly women, a 'pulse feeding' pattern (i.e. 80 % of the daily dietary protein consumed at noon) induces a higher nitrogen balance than a 'spread feeding' pattern (i.e. a daily protein intake evenly distributed over four meals; Arnal *et al.* 1999). In contrast, in young women, the spread diet tends to induce a better balance (Arnal *et al.* 2000). Since the pulse-feeding pattern very probably induces a higher hyperaminoacidaemia than the spread-feeding pattern (Wolever, 1994; Dangin *et al.* 2001), a different sensitivity of elderly and young subjects to AAs might partly explain these results. Taken together, these data suggest that in elderly subjects, protein synthesis could be resistant to AA availability and that high hyperaminoacidaemia (or leucinaemia) improves postprandial protein gain.

Thus, with respect to the kinetics of digestion of dietary proteins, a 'fast' protein might induce higher postprandial protein retention than a 'slow' one in elderly subjects by increasing AA availability. To verify this hypothesis, the effect of the protein digestion rate on whole-body postprandial protein metabolism was assessed in healthy elderly men and compared with the responses obtained in young adults. In both age groups, postprandial leucine kinetics were compared after ingestion of a mixed meal containing either CAS or an isonitrogenous amount of WP (WP-iN) or an amount of WP providing the same quantity of leucine (WP-iL) as CAS. This design was selected because both nitrogen and leucine have been shown to modulate protein retention (Pannemans *et al.* 1998; Dardevet *et al.* 2002).

METHODS

Materials

L-[1-^{13}C]Leucine (99 mol percent excess, MPE), L-[5,5,5-$^{2}H_3$] leucine (97 MPE) and sodium [^{13}C]bicarbonate (99 MPE) were obtained from Eurisotop (Gif-sur-Yvette, France). The isotopic and chemical purity of leucine was checked by gas chromatography–mass spectrometry (GCMS) and was tested for sterility and pyrogenicity before use.

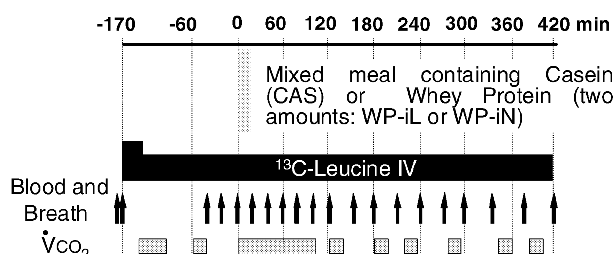

Figure 1. Experimental design

Young (n = 6) and elderly men (n = 9) ingested mixed meals containing different types of protein: casein (CAS: 0.48 g CAS (kg BM)$^{-1}$ and 296 μmol leucine (kg BM)$^{-1}$) or two amounts of whey protein (WP): one was isonitrogenous with CAS (WP-iN) and the other provided the same amount of leucine as CAS (WP-iL). CAS and WP were intrinsically labelled with [5,5,5,$^{2}H_3$]leucine. I.V., intravenous.

L-[5,5,5-$^{2}H_3$]Leucine was used to produce intrinsically labelled CAS and WP. Labelled proteins were obtained by infusing a cow with the deuterated tracer, collecting milk and purifying CAS and WP as described previously (Boirie *et al.* 1995). The leucine enrichments, checked by GCMS after hydrolysis, were 8.28 and 8.16 MPE for CAS and WP, respectively. Labelled protein fractions were mixed with their respective unlabelled fraction in order to obtain 10 μmol (kg body mass)$^{-1}$ of L-[5,5,5-$^{2}H_3$] leucine. The final enrichments were 3.37 MPE for CAS and WP-iL and 2.27 MPE for WP-iN. The proteins met chemical and bacteriological specifications for human consumption.

Subjects

Nine elderly (mean ± S.E.M. age, 72 ± 1 years; body mass (BM), 73.5 ± 3.1 kg; body mass index (BMI), 25.3 ± 1.0 kg m^{-2}; fat-free mass (FFM), 53.6 ± 1.5 kg; fat mass, 27.2 ± 1.2 % of BM) and six young healthy male volunteers (age, 24 ± 2 years; BM, 66.4 ± 1.6 kg; BMI, 21.1 ± 0.6 kg m^{-2}; FFM, 57.7 ± 1.9 kg; fat mass, 13.4 ± 1.3 % of BM) participated in the study. Subjects had a normal blood biochemical profile and physical condition and had no medical history of renal, cardiovascular, gastrointestinal or endocrine disease. The study was approved by our ethical committee (CCPPRB-Auvergne) and informed written consent was obtained from each participant after an explanation of the purpose, methodology and potential risks of the study. The study was in accordance with the Declaration of Helsinki.

Experimental design

Each subject was studied on three separate occasions differing only by the protein composition of the mixed meal. (1) Meal CAS contained 0.48 g CAS (kg BM)$^{-1}$ (~34 g), and provided 296 μmol leucine (kg BM)$^{-1}$. (2) Meal WP-iL contained 0.31 g WP (kg BM)$^{-1}$ (~22 g) and provided identical amounts of leucine as CAS (296 μmol leucine (kg BM)$^{-1}$). (3) Meal WP-iN was isonitrogenous with CAS (0.48 g WP (kg BM)$^{-1}$ (~34 g)) and provided 449 μmol leucine (kg BM)$^{-1}$. Thus, the amount of protein ingested was lower with WP-iL than with CAS and WP-iN. Conversely, the leucine intake was higher with WP-iN than with CAS and WP-iL. The CAS, WP-iL and WP-iN provided 0.20, 0.12 and 0.19 g (kg BM)$^{-1}$ of essential AAs, respectively. The volumes ingested (5.6 ml (kg BM)$^{-1}$) and the composition of the meals were otherwise identical: 0.75 g (kg BM)$^{-1}$ of carbohydrates (54 % sucrose, 46 % maltodextrins) and 0.13 g (kg BM)$^{-1}$ of fat (91 % sunflower oil, 9 % monoglycerides).

The meals were administered in a random order. Between two test meals, a wash-out period of at least 3 weeks was observed. During the 4 days preceding a test meal, the volunteers received a balanced diet (~30 kcal kg^{-1} day^{-1}; 16 % protein), and maintained their usual physical activity. The evening preceding the test meal, they consumed a standard meal providing 850 kcal and 16 % protein in the laboratory at 19.00 h. Thereafter, no food was allowed. The following day, at 07.30 h, a catheter was inserted retrogradely into a dorsal hand vein and used 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. After a priming dose of [^{13}C]bicarbonate (6 mg), a primed (4.2 μmol (kg BM)$^{-1}$) continuous intravenous (I.V.) infusion of L-[1-^{13}C]leucine (0.06 μmol (kg BM)$^{-1}$ min^{-1}) was started and was continued for 590 min (Fig. 1). After 170 min of infusion (t = 0 min), the test

meal was ingested within 5 min. Each meal provided 10 μmol (kg BM)$^{-1}$ of L-[5,5,5-$^2H_3$]leucine.

Blood and breath samples were collected (Fig. 1) before any infusion (−180, −170 min), before the meal when the I.V. tracer had reached an isotopic plateau (−40, −20 and 0 min), and after meal ingestion, at 20 min intervals until 120 min, then at 30 min intervals until 300 min and finally at 40 min intervals until 420 min. After centrifugation, the plasma samples were mixed with an internal standard (Norleucine), and analysed. Breath samples were collected in 10 ml Vacutainers (Becton Dickinson, Grenoble, France) for [^{13}C]CO_2 enrichment analysis. Total CO_2 production rates ($\dot{V}_{O_2}$) were measured by open-circuit indirect calorimetry (Deltatrac, Datex Ohmeda, Lyon, France) at regular intervals (Fig. 1).

Plasma insulin, glucagon and AA concentrations were measured before (−20 min) and after the meal (+20, +40, +60, +80, +120 and +300 min for hormones and +60, +120 and +240 min for AAs).

Analytical methods

Body composition was assessed by dual-energy X-ray absorptiometry (Hologic QDR-4500A, Waltham, MA, USA). Plasma L-[1-^{13}C]leucine, L-[5,5,5-$^2H_3$]leucine and ketoisocaproate (KIC) enrichments (MPE) were measured by GCMS (Hewlett-Packard 5971A) using tertiary-butyldimethylsilyl derivatives, as described previously (Boirie *et al.* 1996). Corrections for the ^{13}C and $^2H_3$ enrichments were applied according to Biolo *et al.* (1992). Leucine concentrations were measured by GCMS using norleucine as the internal standard. [^{13}C]CO_2 enrichments (Atom Percent Excess) were measured on a gas chromatography isotope ratio mass spectrometer (μGas system, Fisons Instruments, VG Isotech, Middlewich, UK). Plasma insulin and glucagon concentrations were measured by radioimmunoassay (CIS bio international, Gif-sur-Yvette, France). Total and essential plasma AA concentrations were measured by the ninhydrin method combined with ion-exchange chromatography (Biotech-Kontron, Saint-Quentin, France). Meals were analysed for leucine content and enrichment by GCMS, using norleucine as the internal standard and nitrogen content by Kjeldahl analysis.

Calculations

Protein metabolism parameters were estimated in non-steady-state conditions using oral and I.V. administration of leucine tracers. Leucine oxidation was calculated using KIC as the precursor pool because KIC is the immediate precursor of leucine decarboxylation (Matthews *et al.* 1981). For the other fluxes, calculations were performed using both plasma leucine MPE and KIC MPE as precursors. Although KIC MPE was probably more representative of the intracellular leucine enrichment, we present as the main results, calculations performed with leucine MPE because: (1) the conclusions were identical whatever the precursor pool used, (2) for some authors (Yu *et al.* 1990; Collin-Vidal *et al.* 1994), but not all (Matthews *et al.* 1993), dietary leucine is weakly transaminated on its first pass and thus it may be more appropriate to estimate dietary leucine rate of appearance, (3) it is the most classical approach used both in steady-state and non-steady-state conditions and (4) this allowed easier comparisons with our previous single-meals studies (Boirie *et al.* 1996, 1997*a*; Dangin *et al.* 2001) in which the same approach was selected. Results obtained with KIC are shown in the electronic archives.

The total leucine rate of appearance into the circulation (*Total Leu Ra*) is the sum of: (1) the rate of entry of exogenous (i.e. dietary) leucine (*Exo Leu Ra*), which is taken as an index of protein digestion rate, (2) the intravenously infused labelled leucine (*ir*) and (3) the rate of entry of endogenous leucine derived from protein breakdown (*Endo Leu Ra*). These parameters and splanchnic extraction of leucine (*Sp*; i.e. the fraction of dietary leucine taken up by the gut and the liver during its first pass) were calculated as follows:

$$Total\ Leu\ Ra = \frac{ir - pV\,C(t)\,\mathrm{d}E_{iv}/\mathrm{d}t}{E_{iv}\,t}, \quad (1)$$

$$Exo\ Leu\ Ra = \frac{Total\ Leu\ Ra\,E_{PO}(t) + pV\,\mathrm{d}E_{PO}/\mathrm{d}t}{E_{Prot}}, \quad (2)$$

$$Endo\ Leu\ Ra = Total\ Leu\ Ra - Exo\ Leu\ Ra - ir, \quad (3)$$

$$Sp = 100\left(\frac{Leu_{Prot} - AUC_{ExoLeuRa}}{Leu_{Prot}}\right), \quad (4)$$

where, pV (0.125) is the leucine pool size corrected for instant mixing. This constant is the same as used previously (Tessari *et al.* 1988; Boirie *et al.* 1996; Dangin *et al.* 2001). $C(t)$ represents the mean plasma leucine concentration between two sampling points. $\mathrm{d}E_{iv}/\mathrm{d}t$ corresponds to time-dependent variations of plasma leucine or KIC MPE of the intravenous tracer (^{13}C tracer), and $E_{iv}(t)$ is the mean plasma leucine or KIC MPE derived from the intravenous tracer between two consecutive time points. $E_{PO}(t)$ represents the mean plasma leucine or KIC MPE of the oral tracer between two time points, $\mathrm{d}E_{PO}/\mathrm{d}t$ is the time-dependent evolution of plasma leucine or KIC MPE of the oral tracer, and E_{Prot} corresponds to L-[5,5,5-$^2H_3$]leucine enrichment in dietary proteins. *Exo Leu Ra* is calculated according to Proietto's transposition of Steele's equations (Proietto *et al.* 1987). Leu_{Prot} is the amount of dietary leucine ingested. $AUC_{ExoLeuRa}$ represents the area under the curve (AUC) of *Exo Leu Ra* (calculated by the trapezoidal method). This corresponds to the amount of dietary leucine that appeared in the peripheral blood over 7 h after meal ingestion.

The total leucine rate of disappearance from the plasma (*Total Leu Rd*) corresponds to the sum of the fluxes of leucine oxidized (*Leu Ox*) and that utilized for protein synthesis (non-oxidative leucine disposal, *NOLD*). These parameters are calculated as follows:

$$Total\ Leu\ Rd = Total\ Leu\ Ra - pV\frac{\mathrm{d}C}{\mathrm{d}t}, \quad (5)$$

$$Leu\ Ox = \frac{\dot{V}_{CO_2}E_{CO_2}}{kE_{13C-KIC}}, \quad (6)$$

$$NOLD = Total\ Leu\ Rd - Leu\ Ox, \quad (7)$$

where, $\mathrm{d}C/\mathrm{d}t$ corresponds to the time-dependent variation of plasma leucine concentration. E_{CO_2} and $E_{13C\text{-}KIC}$ correspond to [^{13}C]CO_2 and L-[1-^{13}C]KIC MPE, respectively. k is a correcting factor for the incomplete recovery of CO_2 in the breath (0.8), as described previously (Boirie *et al.* 1997*a*; Raguso *et al.* 1999; Dangin *et al.* 2001).

Postprandial leucine balance was calculated over a 420 min period as follows:

$$Leucine\ balance = Leu\ In - AUC_{LeuOx}, \quad (8)$$

where *Leu In* and AUC_{LeuOx} correspond to the leucine intake (ingested + infused) and the amount of leucine oxidized over 7 h, respectively. The efficiency of postprandial protein utilization (PPUN) was estimated according to Millward *et al.* (2000, 2002).

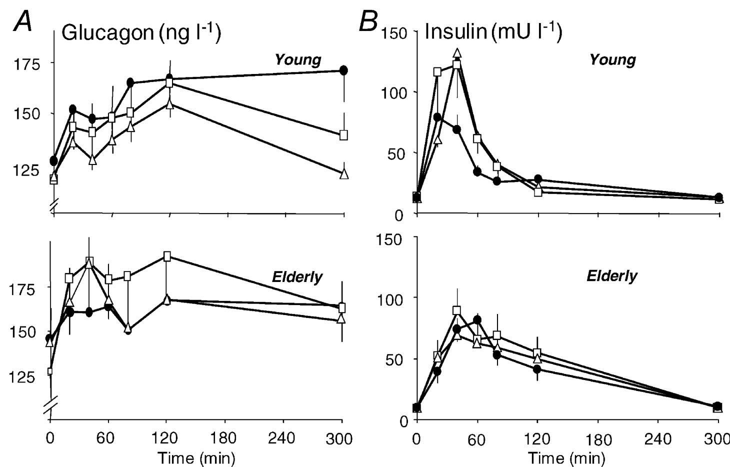

Figure 2. Effect of ingestion of CAS, WP-iL and WP-iN on plasma concentrations of glucagon and insulin

Plasma glucagon (*A*) and insulin concentrations (*B*) after ingestion of CAS (●), WP-iL (△) and WP-iN (□) by young ($n = 6$, upper graphs) or elderly men ($n = 9$, lower graphs). Values are means ± S.E.M.

PPUN was the ratio between a predicted nitrogen balance, based on the difference between postprandial and postabsorptive leucine balance, assuming a constant body protein leucine content of 625 mg (mg N)$^{-1}$ and nitrogen intake (mg N).

Each individual curve was characterized by its zenith or nadir (Y_{max}), by its AUC and by the time at which half the amount of *AUC* was obtained ($t_{½}$).

Statistical analysis

Results are expressed as means ± S.E.M. Statistical analyses (Statview, 5.0, Abacus Concepts, Berkeley, CA, USA) were performed in order to: (1) characterize the changes induced by meal ingestion (i.e. modifications from the postabsorptive state after every test meal); (2) assess the effect of the type of meal; and (3) determine the effect of aging.

Changes induced by meal ingestion. Individual curves were standardized by subtracting the average of the baseline values from each individual time point. A confidence interval ($\alpha = 0.05$) was calculated from the standard deviation of the *n* standardized baseline individual values within each experiment, and from the critical value of Student's distribution for ($n - 1$) degrees of freedom. The changes were declared significantly different from the baseline when all of the individual values fell outside this interval.

Effect of the type of meal within each age-group. Y_{max}, *AUC* and $t_{½}$ were compared by repeated-measures ANOVA. When an effect of the type of meal was detected ($P < 0.05$), the Least Significant Difference method was used to define which meal was different from the others.

Effect of age. The difference between CAS and WP-iL (CAS *vs.* WP-iL, identical amount of leucine) and between CAS and WP-iN (CAS *vs.* WP-IN, isonitrogenous meals) were first calculated. The effect of age on these differences was then assessed using ANOVA.

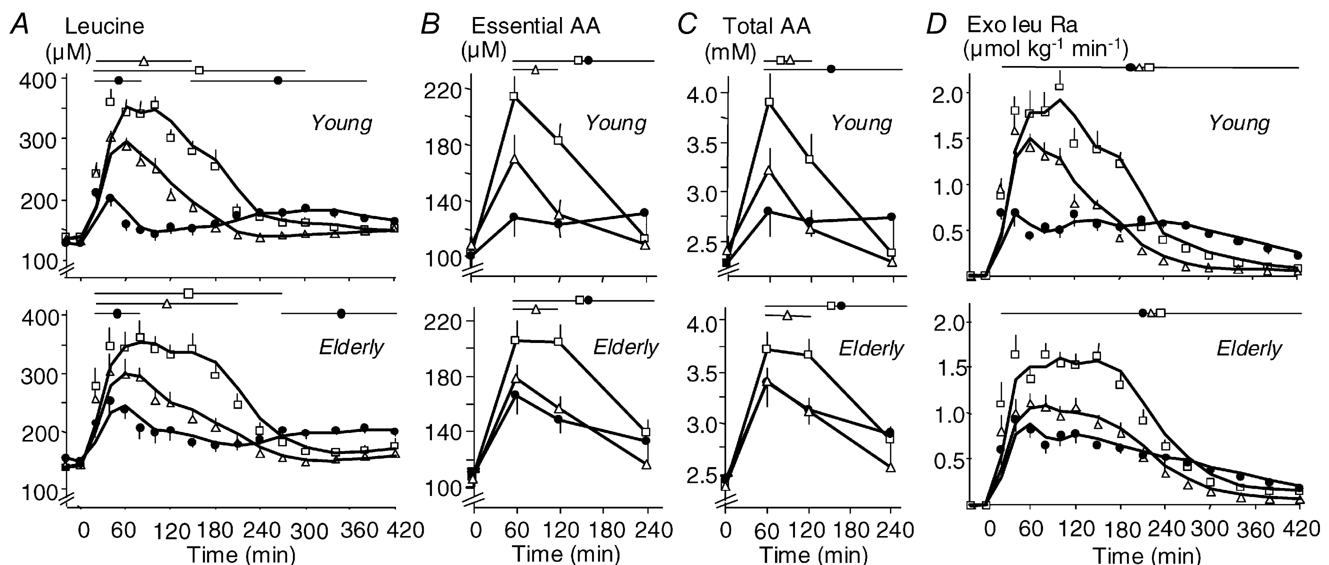

Figure 3. Effect of ingestion of CAS, WP-iL and WP-iN on plasma concentrations of leucine, amino acids (AAs) and the rate of appearance of exogenous leucine

Plasma concentrations of leucine (*A*), the sum of essential (*B*) and total AAs (*C*) and the rate of appearance of exogenous leucine (Exo Leu Ra, *D*) after ingestion of CAS (●), WP-iL (△) and WP-iN (□) by young ($n = 6$, upper graphs) or elderly men ($n = 9$, lower graphs). Values are means ± S.E.M. The lines at the top of the graphs indicate time-points where values are different ($P < 0.05$) from baseline within each study.

Table 1. Baseline and kinetics of leucine concentrations and fluxes after meal ingestion

		Young (*n*=6)			Elderly (*n*=9)			Age-effect
		WP-iN	CAS	WP-iL	WP-iN	CAS	WP-iL	Δ WP vs. CAS
[Leu]	Basal	140 ± 7	129 ± 4	140 ± 7	134 ± 8	146 ± 8	138 ± 8	
	Y_{max}	380 ± 14*	226 ± 8	314 ± 10*	401 ± 20*	270 ± 18	336 ± 24*	
	$t_{½}$	153 ± 3*	217 ± 3	161 ± 3*	160 ± 5*	207 ± 6	163 ± 5*	
Exo Leu Ra	Y_{max}	2.11 ± 0.19*	0.92 ± 0.07	1.78 ± 0.13*	2.01 ± 0.14*	1.15 ± 0.10	1.37 ± 0.07	†
	$t_{½}$	115 ± 5*	187 ± 7	92 ± 4*	131 ± 7*	159 ± 12	123 ± 10*	†
	AUC	336 ± 23*	206 ± 10	211 ± 4	355 ± 18*	218 ± 4	219 ± 6	
Endo Leu Ra	Basal	1.67 ± 0.08	1.72 ± 0.07	1.63 ± 0.06	1.65 ± 0.09	1.61 ± 0.09	1.62 ± 0.11	
	Y_{max}	1.11 ± 0.05	1.23 ± 0.07	1.15 ± 0.04	1.05 ± 0.07	1.05 ± 0.09	1.12 ± 0.09	
	$t_{½}$	221 ± 6	213 ± 3	216 ± 4	211 ± 6	219 ± 3	216 ± 2	
	AUC	599 ± 19	609 ± 33	600 ± 27	561 ± 36	557 ± 28	568 ± 36	
NOLD	Basal	1.33 ± 0.06	1.37 ± 0.05	1.27 ± 0.02	1.35 ± 0.09	1.27 ± 0.08	1.22 ± 0.08	
	Y_{max}	2.46 ± 0.27*	1.86 ± 0.16	2.01 ± 0.06	2.69 ± 0.39*	1.76 ± 0.11	1.87 ± 0.10	
	$t_{½}$	179 ± 7*	210 ± 2	184 ± 4*	168 ± 4*	199 ± 5	182 ± 6*	†
	AUC	616 ± 51	579 ± 35	535 ± 20	621 ± 56*	521 ± 24	522 ± 22	
Leu Ox	Basal	0.39 ± 0.02	0.39 ± 0.03	0.41 ± 0.03	0.35 ± 0.02	0.40 ± 0.02	0.37 ± 0.03	
	Y_{max}	1.88 ± 0.10*	0.89 ± 0.05	1.50 ± 0.11*	1.73 ± 0.11*	1.18 ± 0.12	1.50 ± 0.19*	†
	$t_{½}$	160 ± 4*	195 ± 7	147 ± 2*	172 ± 7*	188 ± 7	168 ± 9*	†
	AUC	440 ± 19*	289 ± 15	340 ± 12*	424 ± 18*	347 ± 15	361 ± 17	†

Meals ingested contained casein (CAS) or two different amounts of whey proteins (WP): WP isonitrogenous with CAS (WP-iN) or WP that provided the same amount of leucine as CAS (WP-iL). Results are presented as means ± S.E.M. Basal, baseline values; Y_{max}, zenith or nadir value; AUC: postprandial area under the curve; $t_{1/2}$, time to reach half AUC (in minutes); [Leu], plasma leucine concentration (μM); Exo Leu Ra, exogenous (dietary) leucine rate of appearance; Endo Leu Ra, endogenous leucine rate of appearance (i.e. proteolysis); NOLD, non-oxidative leucine disposal (i.e. protein synthesis); Leu Ox, total leucine oxidized. Flux values are in μmol (kg fat-free mass)$^{-1}$ min^{-1}, except Exo Leu Ra (μmol kg^{-1} min^{-1}). Statistical analyses were performed by ANOVA to assess the differences related to the type of meals; within age groups (*$P < 0.05$) and to age (†$P < 0.05$).

RESULTS

Tracer enrichments

Figures and details concerning leucine, KIC and CO_2 enrichments are provided as Supplementary material with the online version of this paper, and can be found at:

http://www.jphysiol.org/cgi/content/full/549/2/635.

Plasma concentrations of hormones and amino acids

Glucagon and insulin increased after meals ingestion (Fig. 2*A* and *B*). For glucagon, the increase and the shape of the curves were similar among meals and age groups (Fig. 2*A*). In contrast, for insulin (Fig. 2*B*), age affected the differences between WP meals and CAS. In young subjects, insulin concentrations were higher ($P < 0.01$) 20 and 40 min after WP-iL and WP-iN than after CAS. This was not the case in elderly men, for whom the shape of the curve was similar among meals.

Before meal ingestion, leucine, essential and total AA concentrations were similar between groups (Table 1, Fig. 3*A*, *B* and *C*). In the postprandial period, these parameters increased differently with the different meals ($P < 0.05$). WP-iL induced a higher leucine Y_{max} ($P < 0.001$), a shorter $t_{½}$ ($P < 0.001$) and a faster return to baseline of leucine concentration than CAS (Fig. 3*A*, Table 1) despite the identical leucine content of these meals. Also, and as expected, leucine concentrations were higher after WP-iN than after CAS, since WP contained more leucine than CAS (Fig. 3*A*, Table 1).

Essential and total AA concentrations were higher ($P < 0.01$) 60 min after WP-iN than after CAS, despite similar AA intakes (Fig. 3*B* and *C*). Then, from 120 to 240 min after the meal, AA concentrations decreased more rapidly after WP-iN than after CAS (Fig. 3*B* and *C*). In young men, essential AA concentrations were higher 60 min after WP-iL than after CAS, despite a lower AA intake (Fig. 3*B*). No significant effect of age was detected, but the shapes of the curves were slightly different (Fig. 3*A*, *B* and *C*). The slope of the decrease seemed to be more rapid with CAS and slower with WP-iL and WP-iN in elderly men than in young men.

Protein digestion rate

Leucine derived from the meals appeared rapidly in the plasma, with differences related to the type of meal ingested (Fig. 3*D*, Table 1). With identical leucine intakes, WP-iL had faster absorption than CAS in both age groups ($t_{½}$: $P < 0.01$) and a higher peak leucine appearance ($P < 0.001$ in the young; P = NS in the elderly). With

WP-iN (higher leucine intake), the Y_{max} ($P < 0.001$) and $t_{½}$ ($P < 0.05$) of the exogenous leucine rate of appearance were again higher and shorter, respectively, than with CAS (Table 1). There was an effect of age, since the differences between the WP meals and CAS (WP-iL – CAS and WP-iN – CAS) were smaller in the old group (Y_{max}: $P < 0.05$; $t_{½}$: $P < 0.01$) than in the young one. That effect of age was apparently related to both a slower rate of digestion of WP and a faster digestion rate of CAS in the elderly population. As expected, the total dietary leucine appearing in the peripheral circulation over 7 h (AUC, Table 1) was higher with WP-iN than with the two other meals. When normalized for leucine intake, there was no effect of age or the meal. Indeed, no difference was found on the percentage of ingested leucine taken up by the splanchnic area during its first pass (Young: WP-iN, 31 ± 4 %; WP-iL, 34 ± 1 %; CAS, 36 ± 3 %; Elderly: WP-iN, 25 ± 4 %; WP-iL, 31 ± 2 %; CAS, 32 ± 1 %). The conclusions drawn were similar when the rate of appearance of exogenous leucine was calculated using KIC MPE (Supplementary material, Fig. 8*A* and Table 2).

Whole-body protein metabolism

In both the postabsorptive and postprandial states, Endo Leu Ra, an index of whole-body proteolysis, was similar whatever the meal and age group (Table 1, Fig. 4*A*, P = NS). All the meals induced a persistent inhibition of Endo Leu Ra, with a maximal decrease at 30–40 % below baseline. Again, similar results were observed when calculations were performed with KIC MPE (Supplementary material, Fig. 8*B* and Table 2).

In the postabsorptive period, NOLD, an index of whole-body protein synthesis, was similar in all groups (Table 1). However, in the postprandial period, the type of meal affected NOLD (Y_{max} and $t_{½}$, $P < 0.01$): WP-iN induced a higher Y_{max} and a shorter $t_{½}$ than CAS ($P < 0.01$). Although WP-iL induced shorter $t_{½}$ than CAS ($P < 0.01$), Y_{max} values were not different (P = NS, Fig. 4*B* and Table 1). There was no effect of age on Y_{max} and *AUC* (P = NS), but $t_{½}$ values were shorter in elderly men than in young men ($P < 0.01$). Similar effects were detected when NOLD was calculated using KIC MPE (Supplementary material, Fig. 8*C* and Table 2).

Total leucine oxidation responded differently to meals ($P < 0.01$, Table 1, Fig. 4*C*). WP-iL and WP-iN induced a higher Y_{max} ($P < 0.05$), a shorter $t_{½}$ ($P < 0.01$) and a greater AUC than CAS ($P < 0.05$, except WP-iL *vs.* CAS in the older group, P = NS). Age affected the differences between WP meals and CAS on Y_{max}, $t_{½}$ and AUC (Table 1). The differences of AUC between WP-iN and CAS (Elderly: 77 ± 22 μmol (kg FFM)$^{-1}$; Young: 151 ± 26 μmol (kg FFM)$^{-1}$) and between WP-iL and CAS (Elderly: 14 ± 17 μmol (kg FFM)$^{-1}$; Young: 50 ± 12 μmol (kg FFM)$^{-1}$) were smaller in elderly subjects than in young men ($P < 0.05$).

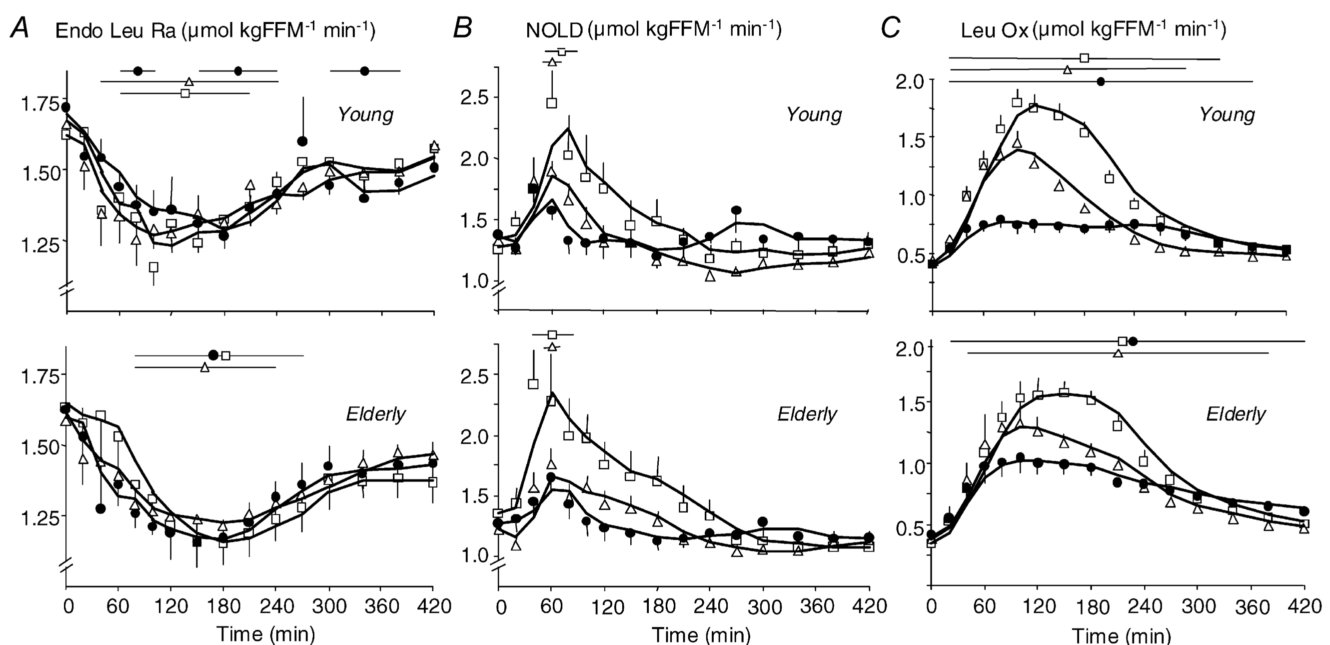

Figure 4. Effect of ingestion of CAS, WP-iL and WP-iN on the rate of appearance of endogenous leucine, non-oxidative leucine disposal (NOLD) and leucine oxidation

A, rate of appearance of endogenous leucine (Endo Leu Ra; i.e. proteolysis). *B*, NOLD (i.e. protein synthesis) and *C*, leucine oxidation (Leu Ox) after ingestion of CAS (●), WP-iL (△) and WP-iN (□) by young (n = 6, upper graphs) or elderly men (n = 9, lower graphs). Results are means ± S.E.M. FFM: fat free mass (kg). The lines at the top of the graphs indicate time-points where values are different from baseline within each study ($P < 0.05$).

Journal of Physiology

Postprandial leucine balance was also modulated by the type of meal ($P < 0.001$). In addition, age ($P < 0.05$) affected the differences between WP meals and CAS (Fig. 5). Indeed, when considering the meals that provided identical amounts of leucine (WP-iL *vs.* CAS, Fig. 5*A*), CAS (88 ± 15 μmol (kg FFM)$^{-1}$) induced a higher leucine balance than WP-iL (39 ± 9 μmol (kg FFM)$^{-1}$, $P < 0.01$) in young adults ($n = 6$). In contrast in the older group ($n = 9$), CAS (70 ± 17 μmol (kg FFM)$^{-1}$) was not more efficient than WP-iL (53 ± 18 μmol (kg FFM)$^{-1}$, P = NS).

When considering the isonitrogenous condition (WP-iN *vs.* CAS, Fig. 5*B*), postprandial leucine balance was higher ($P < 0.001$) with WP-iN (Young: 133 ± 11 μmol (kg FFM)$^{-1}$, $n = 6$; Elderly: 174 ± 20 μmol (kg FFM)$^{-1}$, $n = 9$) than with CAS (see values above), in both populations. However, the magnitude of the difference between WP and CAS was smaller for the young (+44 ± 9 μmol (kg FFM)$^{-1}$) than for the elderly subjects (+104 ± 19 μmol (kg FFM)$^{-1}$, $P = 0.05$).

Finally, when the results are expressed as PPUN, no significant difference was detected between meals in the younger group ($n = 6$, P = NS), while in the older group WP meals were more efficient than CAS ($n = 9$, $P = 0.01$). Age did not affect PPUN of the WP meals (no age effect), while in elderly men, CAS was less efficient than in the young (CAS: Young *vs.* Elderly, $P < 0.05$).

DISCUSSION

The present study was designed to assess the impact of dietary protein digestion rate on protein metabolism in elderly subjects. For this purpose, we compared the effects induced by a mixed liquid meal containing either CAS or WP, taken as protocols for 'slow' and 'fast' digested proteins, respectively (Mahé *et al.* 1991; Boirie *et al.* 1997*a*; Dangin *et al.* 2001). We show that the effects of protein digestion rate on postprandial protein gain change with age. Indeed, in elderly subjects, in contrast to young adults, a fast protein induced a higher postprandial leucine balance than a slow protein.

The methodological approach was similar to that reported previously (Boirie *et al.* 1997*a*), but with adaptations. In our former studies, each volunteer had to be studied on two separate occasions to test the effect of a given meal. On one occasion, L-[1-^{13}C]leucine-labelled protein was given orally, while on another occasion, that tracer was infused intravenously to assess whole-body leucine oxidation. In the present study, intrinsically L-[5,5,5-^{2}H$_3$]leucine-bound proteins were produced and administered with the meal, and L-[1-^{13}C]leucine was infused. Using this approach it is possible to assess whole-body protein metabolism parameters and the metabolic fate of dietary proteins in a single experiment, thereby reducing the number of studies carried out on an individual volunteer and producing more homogeneous results.

A major change from our previous studies was the addition of carbohydrate and fat to the meal (Boirie *et al.* 1997*a*; Dangin *et al.* 2001). Non-protein energy sources (fat or carbohydrates) are known to decrease the rate of gastric emptying (Jian *et al.* 1986; Calbet & MacLean, 1997) and might have suppressed the differences in digestion rate between CAS and WP. This was not the case: WP was still more rapidly absorbed and induced higher

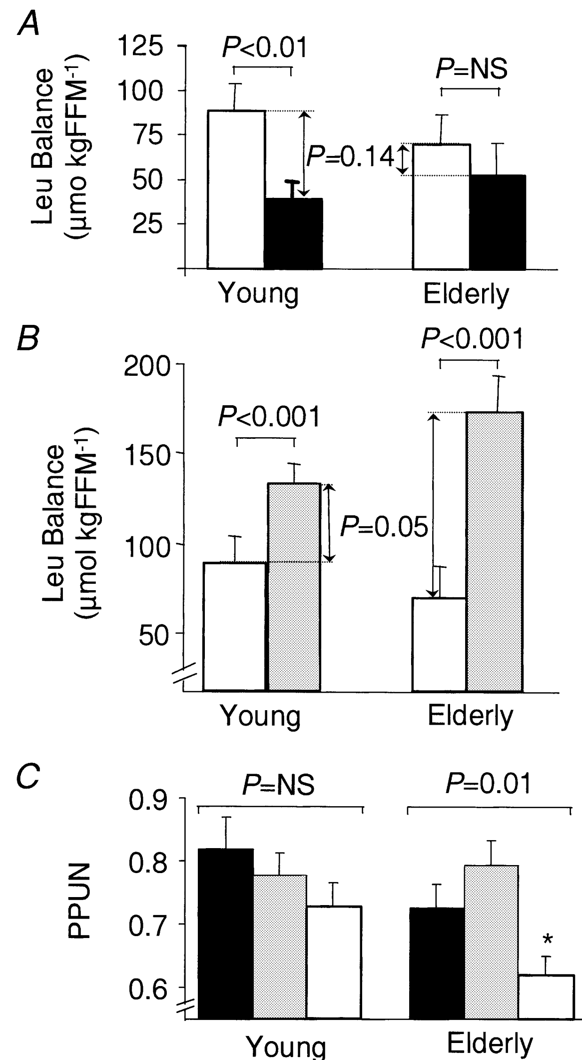

Figure 5. Effect of ingestion of CAS and WP on leucine balance in elderly and younger men

Leucine (Leu) balance over 420 min after ingestion of CAS (white bars) and of WP providing either the same amount of leucine as CAS (black bars: WP-iL; *A*) or the same quantity of protein as CAS (grey bars: WP-iN; *B*), and efficiency of posptrandial protein utilization (PPUN; *C*). Meals were ingested by young ($n = 6$; left) and elderly men ($n = 9$; right). FFM, fat-free mass (kg). The results are presented as means ± S.E.M. The lines at the top of the graphs indicate differences between CAS and WP within the age group. *Significant differences between age groups within each meal ($P < 0.05$).

Journal of Physiology

and more transient hyperaminoacidaemia than CAS. However, in young subjects the differences between these two proteins were less marked than when the protein was given alone (Boirie *et al.* 1997*a*; Dangin *et al.* 2001). This was mostly due to a slower WP digestion rate in the presence of carbohydrate or fat (Jian *et al.* 1986; Calbet & MacLean, 1997). The differences between WP and CAS were attenuated by age: the rate of digestion of WP tended to be slower and that of CAS faster in elderly than in young subjects. The slowing down of the WP digestion rate may be explained by an age-related decrease of the gastric emptying rate (Clarkston *et al.* 1997; Cook *et al.* 1997). The acceleration of the CAS digestion rate may be due to an age-related reduction in gastric acid secretions (Korkushko *et al.* 1992; Morihara *et al.* 2001); this decrease might alter the clotting of CAS, maintaining it in a liquid form, which is emptied more rapidly than in the solid form (Achour *et al.* 2001).

In elderly subjects, leucine splanchnic extraction was not higher than in the young subjects, and was much lower than reported previously in steady-state conditions in a similar population (Boirie *et al.* 1997*b*). It is unlikely that this discrepancy is due to potential errors in the calculations and/or to the use of a non-steady-state approach. The calculations depend on the variations in size of the truly active metabolic pool of leucine (i.e. pV), which may be not accurate and may fluctuate over the time course of the study. However, as discussed by Boirie *et al.* (1996), variations of pV over a wide range do not affect the amount of dietary leucine appearing in the peripheral blood. In addition, splanchnic extractions of our previous studies with slowly digested meals (Boirie *et al.* 1997*a*; Dangin *et al.* 2001) as well as the present results obtained in young men are consistent with those observed in a similar population in a steady-state condition (Matthews *et al.* 1993; Boirie *et al.* 1997*b*). This discrepancy is more likely to be due to differences in the BMI of elderly subjects between the two studies. Indeed, because it has been reported that high splanchnic extractions are found only in elderly subjects with a large BMI (Boirie *et al.* 1997*b*), it may be possible that the lower BMI of our older group would result in lower leucine splanchnic extractions.

We confirm a nitrogen-sparing effect of energy (Pellet & Young, 1992), since postprandial leucine balance was higher with the mixed meals (this study) than with proteins alone (Boirie *et al.* 1997*a*; Dangin *et al.* 2001). However, the apparent AA-sparing effect of energy was more pronounced with WP (results of WP alone were derived from Dangin *et al.* (2001) and are expressed in μmol kg^{-1}: 6 ± 19; WP + energy (this study): 115 ± 10) than with CAS (CAS alone: 38 ± 19; CAS + energy: 76 ± 13). The more beneficial effect of CAS was thus reversed with energy. This effect was mediated by a better inhibition of proteolysis (probably due to carbohydrate-induced hyperinsulinaemia) rather than by a greater stimulation of protein synthesis (Boirie *et al.* 2001). Indeed, energy added to WP did not affect the protein synthesis, but induced a strong and persistent inhibition of proteolysis that was not present with WP alone (Boirie *et al.* 1997*a*). By contrast, energy added to CAS had lower impact on proteolysis because CAS alone had already decreased that parameter (Boirie et al. 1997*a*) and, as previously, did not modify protein synthesis.

The most important result was that postprandial protein gain induced by CAS and WP, differed with age. In young men, and in agreement with our previous results (Boirie *et al.* 1997*a*), CAS induced a higher postprandial leucine balance than WP, with meals providing identical amount of leucine (CAS *vs.* WP-iL, Fig. 4*A*). This was not the case in the elderly subjects since CAS and WP-iL resulted in similar balances. When considering isonitrogenous meals (Fig. 4*B*), leucine balance was higher with WP-iN than with CAS in both age groups. This was foreseeable because WP-iN provided more leucine than CAS, a factor known to improve leucine balance (Dardevet *et al.* 2002). The key point is that an effect of age was again detected; the magnitude of the difference between WP-iN and CAS was 58 % larger in elderly than in young subjects. This age-related effect was also confirmed when expressing the data as PPUN, as proposed by Millward *et al.* (2002). This mode of expression compensates for the difference in AA composition between proteins. In young men, PPUN was similar with every meal, while in elderly subjects, PPUN was higher with WP-iL and WP-iN than with CAS. In fact, this difference was due to a decreased efficiency of CAS with age, rather than to an increased efficiency of WP. Therefore, whatever the pair of studies examined and the way of expression, there was an age-related effect of the rate of digestion on postprandial protein gain. WP, a fast protein, seemed to be more efficient than CAS, a slow one, in elderly subjects, at least in the short term.

The modifications responsible for a lower efficiency of CAS and/or a greater utilization of WP during aging are unclear. In the absence of clear-cut differences in the rates of whole-body synthesis or breakdown, it is difficult to ascribe the final differences of leucine balance to an alteration of one of these two parameters. It is tempting to incriminate protein synthesis in particular at the muscle level (the main tissue affected by aging). Indeed, it has been shown that impairment of protein synthesis of old muscle (Welle *et al.* 1994; Mosoni *et al.* 1995) after meal ingestion could be normalized by high levels of AA (Mosoni *et al.* 1993; Volpi *et al.* 1998). This suggests that AA availability becomes critical in elderly subjects. If so, it is possible that the slow rate of delivery of AA from CAS may be rate limiting for muscle protein synthesis with aging. This hypothesis was also proposed to explain the specific age-related effect of the pulse diet pattern (Arnal *et al.* 1999).

Another possibility is a resistance of old muscle to leucine-induced stimulation of mRNA translation initiation (Kimball & Jefferson, 2002). Indeed, stimulation of protein synthesis by a meal was blunted in muscle from old rats, but a normal response could be obtained with leucine supplementation that provoked a higher increment in leucine availability (Dardevet *et al.* 2002). In contrast, in young rats, stimulation of muscle protein synthesis was submaximal after meal ingestion, and leucine supplementation did not have additive effects. Therefore, in elderly subjects, high leucine concentrations, such as those induced by WP meals, might possibly be required to overcome a 'leucine resistance' that could exist with CAS. However, we failed to observe an age-specific impairment of protein synthesis in response to CAS (i.e. to low variations in AA and/or leucine concentrations). This is not surprising since protein synthesis was measured at the whole-body level and since muscle proteins only represent approximately 27 % of whole-body protein synthesis (Nair *et al.* 1988).

In conclusion, we have demonstrated that whey protein and casein affect protein gain differently during aging. In elderly men, when consuming mixed meals, there was a greater utilization of WP, a rapidly digested protein, than CAS, a slowly digested protein, compared with the younger men. Variation of AA and/or leucine availability is probably responsible for this difference. Therefore, a fast protein might be more beneficial than a slow protein to improve postprandial protein gain and consequently to limit the body protein losses of elderly subjects. Studies examining the long-term effects of protein digestion rate on nitrogen balance are needed.

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Supplementary material

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and contains material entitled:

Postprandial protein metabolism during aging: raw data and calculations using KIC as a precursor