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Original article

"Fast proteins" with a unique essential amino acid content as an optimal nutrition in the elderly: Growing evidence

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SUMMARY

Background & aims: Adequate protein intake is crucial to maintain body protein content in elderly subjects, but quality of dietary proteins should be also considered since amino acid composition and rate of protein digestion modulate amino acid availability. This study investigates whether the efficacy of optimal protein intake levels for protein retention in the elderly is influenced by protein quality.
Methods: We investigated the effect of a 10-day adequate-protein (AP) or high-protein (HP) diet together with the protein source as caseins (CAS) or soluble milk proteins (PRO) on whole-body (WB) protein synthesis (PS) and protein breakdown (PB) in 4 groups of healthy elderly men (mean ± SEM: 71.8 ± 24.4 yr). The study consisted of two periods of 4 h each: a post-absorptive period and a post-prandial period. The fed state was defined by consumption every 20 min and for 4 h, of either 15 g or 30 g of PRO or CAS. Steady-state WB and splanchnic leucine kinetics were measured using a continuous infusion of L-[1-13C]leucine in the postabsorptive state and L-[1-13C]leucine infusion plus oral L-[5,5,5-2H3]leucine in the postprandial state.
Results: WB PS was stimulated by feeding only with HP diets, whereas WB PB corrected for splanchnic extraction showed a similar pattern of post-feeding decrease in all groups. Consequently, net leucine balance was greater in the postprandial state after HP meals than after AP meals, with PRO meals leading to a better postprandial leucine balance (3.63 ± 0.16 μmol kg FFM^{-1} min^{-1} *vs.* 2.77 ± 0.21 μmol kg FFM^{-1} min^{-1} for PRO HP and CAS HP, respectively; $P = 0.005$).
Conclusion: Postprandial protein retention was better improved in elderly men by an increase in protein intake when the protein supplementation was provided as fast-digesting proteins that induce high leucine availability.

1. Introduction

The elderly population is rapidly expanding worldwide, making innovative strategies to decrease the prevalence of age-related disorders and maintain elderly quality-of-life a major socio-economic and public health challenge. Optimal nutritional intake strategies have been shown to improve health outcomes, but the crucial factor in aging subjects is to get the right dietary strategy to preserve protein homeostasis.[1]

Elderly people tend to consume less than the recommended dietary allowance of protein (RDA = 0.8–1.0 g kg body wt^{-1} d^{-1} depending of the country considered), likely resulting in an accelerated body protein loss[2] and impaired physiological functions. The RDA may be slightly higher for elderly people than for young people, but the impact of different levels of protein intake and intrinsic protein quality in elderly subjects has attracted little attention from the research community.[3]

Dietary proteins need to be considered not only quantitatively but also qualitatively, as their properties could provide a basis for

Non-standard abbreviations: FSR, Fractional Synthesis Rate.

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Table 1
Physical characteristics of the subjects.

	All subjects ($n = 31$)	PRO HP ($n = 8$)	PRO AP ($n = 8$)	CAS HP ($n = 8$)	CAS AP ($n = 7$)
Age (yr)	71.8 ± 2.4	72.8 ± 1.1	71.1 ± 0.8	71.5 ± 0.5	72.0 ± 1.1
Body weight (kg)	73.6 ± 1.2	74.9 ± 2.5	70.4 ± 2.4	73.5 ± 2.3	75.9 ± 2.8
BMI (kg/m^2)	24.9 ± 0.3	24.9 ± 0.9	24.3 ± 0.5	25.1 ± 0.7	25.4 ± 0.6
FFM (kg)	52.0 ± 0.9	51.5 ± 1.2	51.3 ± 1.7	53.9 ± 1.3	51.2 ± 3.4
Fat mass (%)	28.8 ± 1.4	30.9 ± 2.6	26.3 ± 2.1	26.0 ± 3.2	33.0 ± 3.6
Fasting insulin (μUI/ml)	14.4 ± 1.5	11.0 ± 1.8	15.1 ± 2.7	14.9 ± 1.3	16.7 ± 4.9

PRO HP: high protein meal with a soluble milk protein source; PRO AP: adequate protein meal with a soluble milk protein source; CAS HP: high protein meal with a casein source; CAS AP: adequate protein meal with a casein source; FFM: fat-free mass. Values are means ± SEMs. Fat mass and FFM were evaluated from specific equations based on resistance value obtained on a monofrequency (50 kHz) bioelectrical impedance analyzer (16).

innovative strategies to limit body protein loss during aging. The anabolic action of dietary proteins depends on their composition in terms of essential amino acids,[4] notably leucine which is the most potent branched-chained amino acid (BCAA) acting not only as a substrate for protein synthesis but also as a signal to activate translation.[5] The time-course of daily protein administration (spread vs. pulse protein pattern)[6] and digestion rate (slow vs. fast proteins concept)[7] have recently been defined as major regulatory factors of postprandial protein metabolism. Modulating protein feeding pattern may be a more attractive option than simply increasing protein intake when the goal is to improve protein turnover and retention in the elderly. Likewise, the rate of dietary amino acid delivery within the body is an important and independent factor modulating protein retention.[7,8] By feeding "slow" or "fast" protein meals, a previous study demonstrated that protein digestion rate differentially affects anabolic response in young and older adults,[9] as only faster-digesting proteins promote anabolism in elderly subjects.[9] Thus, in addition to quantity and amino acid profile, protein digestion rate should now be considered an independent factor modulating postprandial protein deposition.

However, it is not known whether the effect of protein digestion rate on WB protein metabolism would be different according to adequate or highdietary protein intake in elderly subjects. We thus investigated the effect of a 10 day-adequate-protein (1.0 g kg body wt^{-1} d^{-1}) or high-protein (1.2 g kg body wt^{-1} d^{-1}) diet on WB protein synthesis and breakdown both in the postabsorptive and postprandial states in elderly men. We hypothesized that the efficacy of protein intake on WB protein retention would be influenced by protein quality, with "fast" proteins inducing high leucine availability.

2. Materials and methods

2.1. Subjects

Thirty-one healthy men aged 71.8 ± 2.4 yrs participated in the study. The physical characteristics of the subjects are listed in Table 1. Each subject had a normal blood biochemical profile and physical condition without any medical history of renal, cardiovascular, endocrine, digestive, hepatic, inflammatory or currently-evolving disease. Subjects were excluded from the study if they had undergone any surgery in the 3 months before the study. None of the subjects were under medication liable to affect the parameters under study (i.e. corticosteroids, β-adrenergic blockers and anticoagulants) or modify intestinal protein absorption. Subjects did not consume vegetarian diets and did not take any nutritional supplements (vitamins, minerals, polyols, fibre) the three months before and during the study. All subjects were sedentary (no participation in any regular exercise program) and were asked not to change their level of physical exercise before and during the study.

The study protocol was approved by the Ethical Review Board of the Human Nutrition Research Center at Clermont-Ferrand, France, in accordance with the Declaration of Helsinki. Each volunteer gave his written informed consent after being explained the purposes, methodology and potential risks of the study.

2.2. Experimental protocol

The study was designed as a double-blind, randomized trial in order to compare two different types and amounts of milk proteins (See Experimental design on Fig. 1). The isotopic study was

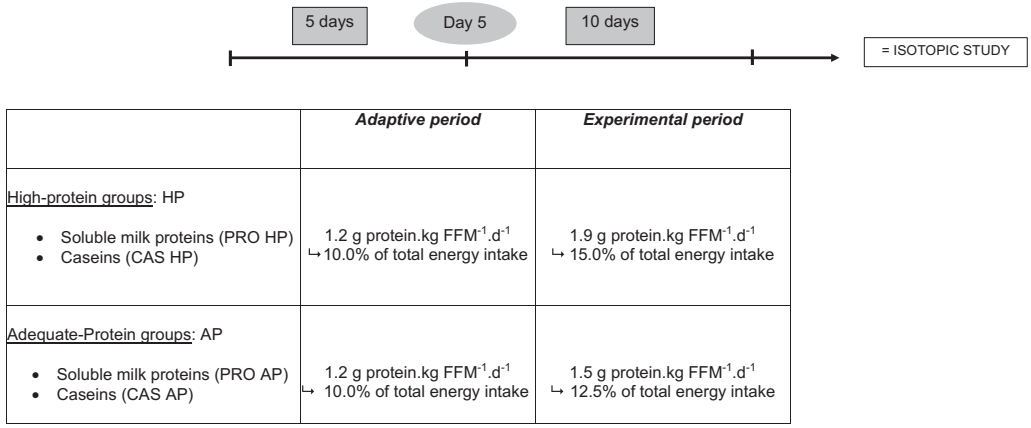

Fig. 1. Experimental design. The isotopic study was preceded by two consecutive periods with food intake control: −5-d adaptive period where subjects received a controlled diet providing 1.2 g protein kg FFM^{-1} d^{-1} representing 10.0% of total energy intake. −10-d experimental period where subjects consumed daily a drinkable dairy product containing either soluble milk proteins (PRO) or caseins (CAS). Daily protein intake represented 15.0% of total energy intake (1.9 g protein kg FFM^{-1} d^{-1}) for the HP groups (CAS HP and PRO HP) or 12.5% of total energy intake (1.5 g protein kg FFM^{-1} d^{-1}) for the AP groups (CAS AP and PRO AP). Daily protein intake was expressed with regard to weight of fat-free mass (FFM), evaluated from specific equations based on the resistance value obtained on a monofrequency (50 kHz) bioelectrical impedance analyzer (16).

preceded by two consecutive periods of food intake control: a 5-d adaptive period and a 10-d experimental period. During the adaptive period, subjects received a controlled diet providing 1.2 g protein kg FFM^{-1} d^{-1}. (i.e. 0.8 g kg body wt^{-1} d^{-1}) representing 10.0% of total energy intake. This 5-d adaptive period allows to control the diet and to have thereafter uniform metabolic responses between subjects. As one of the objectives of this study was to evaluate the effect of a 10-days diet supplemented with two types and two quantities of proteins on protein metabolism in healthy elderly, during the following 10-d experimental period, protein intake was made greater than during the adaptive period by consuming a drinkable dairy product containing either soluble milk proteins (Prolacta®) or caseins. Prolacta® is as a concentrate of native whey proteins extracted directly from skimmed cow milk, and not from whey, with an amino acid composition superior to conventional whey proteins. Half the volunteers consumed caseins ('CAS' groups) and the other half consumed soluble milk proteins ('PRO' groups). Furthermore, two different amounts of proteins were compared for each protein tested, i.e. adequate ('AP' groups) or high ('HP' groups) protein doses. Thus, volunteers were randomly distributed into 4 groups as follows: (1) PRO AP ($n = 8$); (2) PRO HP ($n = 8$); (3) CAS AP ($n = 7$); (4) CAS HP ($n = 8$). Protein intake increased to 1.9 g protein kg FFM^{-1} d^{-1} (i.e. 1.2 g kg body wt^{-1} d^{-1}) in HP groups representing 15.0% of total energy intake, and to 1.5 g protein kg FFM^{-1} d^{-1} (i.e. 1.0 g kg body wt^{-1} d^{-1}) in AP groups representing 12.5% of total energy intake, respectively. Therefore, the total quantity of proteins (including CAS and PRO) consumed per day during the experimental phase was: 97.85 g d^{-1} for PRO HP, 102.40 g d^{-1} for CAS HP, 76.95 g d^{-1} for PRO AP and 76.80 g d^{-1} for CAS AP.

The increase in protein intake during the 10-d experimental period was compensated in order to make the adaptive and experimental periods isoenergetic.

2.3. Food consumption

Before the start of the study, food consumption was assessed by interview with a dietitian to determine dietary patterns and estimate energy and protein intakes. Food intake was customized for each volunteer (3 or 4 meals daily composed of usual food products) according to personal energy intake based on estimated resting energy expenditure (REE) multiplied by an activity factor of 1.6. REE was determined using Black's equation.[10] Estimated energy intake was close to usual intake as assessed by the previous dietary inquiry.

Furthermore, subjects were asked to complete a food-intake questionnaire during the 15-day period of controlled diet where they reported the type and amount of each food consumed. The data were analyzed by the dietitian using French computerized nutrient databases in order to control dietary compliance by the volunteers.

3. Materials

3.1. Dairy products

The milk proteins were incorporated into a drinkable dairy product marketed by Lactalis (Laval, France) that contained water, cream milk, milk proteins as either Prolacta® (soluble milk proteins rich in leucine at up to 12% of total amino acid content) or caseins (leucine up to 9% of total amino acid content), sucrose, glucose syrup, pectin, guar and aromas. Prolacta® is a specific ingredient produced using a two-step cold membrane process directly from pasteurized milk (Lactalis Industry, Bourgbarre, France). The soluble milk proteins extracted by this specific and innovative process are consequently native and named virgin whey proteins.

The product was stored in bottles sealed and coded by subject number according to a randomization list. The drinkable dairy product met all bacteriological specifications for human consumption. The bottles were delivered to the investigators in boxes indicating shelf-life date. Each box contained a 10-day supply for each subject.

3.2. Tracers

L-[1-^{13}C]leucine (99 mol percent excess, [MPE]), L-[5,5,5-$^{2}H_3$] leucine (98 MPE) and sodium [^{13}C]bicarbonate (99 MPE) were obtained from Eurisotop (Gif-sur-Yvette, France). Tracer preparation was performed as previously described.[11]

Isotopic determination of whole body protein turnover.

After the period of adaptation and supplementation, subjects went to the GCRC (General Clinical Research Center) for testing after an overnight fast of at least 12 h. The study took place over one day (8 h) and consisted of two periods of 4 h each: a basal, i.e. post-absorptive, period and a postprandial period. The post-absorptive phase, has allowed studying the baseline effect of the two protein strategies on protein metabolism. The fed state was defined by consumption every 20 min and for 4 h, of either soluble milk proteins (PRO - soluble milk proteins rich in leucine at 12% of total amino acid content) or caseins (CAS - leucine at 9% of total amino acid content). Although this situation did not reflect the circadian rhythm of food intake, the use of a repeated consumption of protein beverages was necessary to reach a steady state and to calculate kinetics of protein metabolism especially in the splanchnic area.

The quantity of proteins and leucine ingested by each group during the infusion study is as follows:

- AP: 15 g of proteins corresponding to 1.38 g of leucine for CAS and 1.80 g of leucine for PRO
- HP: 30 g of proteins corresponding to 2.76 g of leucine for CAS and 3.60 g of leucine for PRO

The beverages were isonitrogenous and isocaloric.

After a priming dose of [^{13}C]bicarbonate (6 mg/5 mL within 1 min), a primed (8.4 μmol kg FFM^{-1} min^{-1}) intravenous (i.v.) infusion of L-[1-^{13}C]leucine was followed by continuous infusion maintained for the next 480 min (0.14 μmol kg FFM^{-1} min^{-1}), as previously described.[12] After 240 min, a semi-liquid diet was administered in small aliquots given every 20 min for the four remaining hours (from 240 to 480 min). L-[5,5,5-$^{2}H_3$]leucine was added to the meal in order to obtain an oral administration rate of 0.10 μmol kg FFM^{-1} min^{-1}[12]. We adapted the tracer perfusion rate to be able to reach the steady-state regardless of the protein in the beverage.

Blood and breath samples were collected for the determination of plasma isotopic enrichment and AA and insulin concentrations as previously described.[12]

3.3. Analytical methods

To correct protein metabolism data for fat-free mass (FFM), body composition was estimated by a monofrequency (50 kHz) bioelectrical impedance analyser (BIA-101A; RJL Systems, Inc, Detroit). The specific equation from Deurenberg et al.[13] was applied for fat and FFM estimations. Leucine kinetics were normalized for fat free mass to consider between-group differences in body composition.

Plasma L-[1-^{13}C]leucine, L-[5,5,5-$^{2}H_3$]leucine and ^{13}C-ketoisocaproate (KIC) enrichments (MPE) were measured by selected ion monitoring-electron impact GC–MS (Hewlett–Packard 5971A, Hewlett–Packard, Palo Alto, CA) using tertiary-butyldimethylsilyl derivatives, as described previously.[14] Corrections for the ^{13}C and

$^{2}H_3$ enrichments were applied according to Biolo et al.[15] $^{13}CO_2$ enrichments (Atom Percent Excess [APE]) were measured on a gas chromatography–combustion–isotope ratio mass spectrometer (μGas system, Fisons Instruments, VG Isotech, Middlewich, UK). Plasma insulin concentrations were measured by ELISA. Total and essential plasma AA concentrations were measured using ion-exchange chromatography with ninhydrin detection (Biotech-Kontron, Saint-Quentin, France). Meals were analyzed for leucine content and enrichment by GCMS using norleucine as internal standard. Nitrogen content was analyzed by Kjeldahl analysis.

3.4. Calculations

Protein metabolism parameters were estimated in steady-state conditions using oral and i.v. administration of leucine tracers as previously described.[12]

Dietary leucine is the quantity of leucine provided from the meal proteins including the oral tracer (L-[5,5,5-$^{2}H_3$]leucine).

Leucine intake is the sum of the dietary leucine and the i.v-infused labelled leucine (L-[1-^{13}C]leucine).

The total rate of leucine appearing in the systemic circulation (Total Leu Ra), also named leucine flux, is the sum of the rate of entry of exogenous leucine (Leucine intake) and the rate of entry of endogenous leucine derived from protein breakdown (Endo Leu Ra), and was calculated from plasma isotope dilution of [^{13}C]leucine at steady state as follows:

$$\text{Total Leu Ra}\left(\mu\text{mol kg FFM}^{-1}\ \text{min}^{-1}\right) = \text{F}[^{13}\text{C}]\text{leu}/(\text{leu}[^{13}\text{C}]\text{MPE} \times 0.01) \quad (1)$$

where [^{13}C]leu MPE is plasma [^{13}C]leucine enrichment (% MPE) and F[^{13}C]leu is [^{13}C]leucine infusion rate (μmol kg FFM^{-1} min^{-1}) corrected for isotopic purity.

Splanchnic extraction of leucine (Sp) represents the fraction of dietary leucine taken up by the gut and the liver during its first pass[16] and was calculated as follows:

$$\text{Sp}(\%) = [1 - (\text{Leu Ra}[^{13}\text{C}]/\text{Leu Ra}[^{2}\text{H}_3])] \times 100 \quad (2)$$

where Leu Ra [^{13}C] and [$^{2}H_3$] are leucine fluxes calculated according to equation [1].

Leucine oxidation (Leu Ox) was calculated as follows:

$$\text{Leu Ox} = {}^{13}\text{CO}_2\ \text{excretion}/([^{13}\text{C}]\text{KIC MPE} \times 0.01) \quad (3)$$

where $^{13}CO_2$ excretion (μmol kg FFM^{-1} min^{-1}) is the product of CO_2 production and $^{13}CO_2$ APE, corrected for incomplete recovery by a factor of 0.70 in the postabsorptive state and 0.82 in the fed state.[12]

Nonoxidative leucine disposal (NOLD) is an index of WB protein synthesis calculated as follows:

$$\text{NOLD}\left(\mu\text{mol kg FFM}^{-1}\ \text{min}^{-1}\right) = \text{Total Leu Ra} - \text{Leu Ox} \quad (4)$$

Endogenous leucine production (Endo Leu Ra) is an index of WB protein breakdown that needs to consider the amount of exogenous leucine reaching the body's free plasma leucine pool. It is thus calculated as follows:

$$\text{Endo Leu Ra}\left(\mu\text{mol.kg FFM}^{-1}\ \text{min}^{-1}\right) = \text{Total Leu Ra} - \text{Post} - \text{splanchnic leucine delivery} \quad (5)$$

where Post-splanchnic leucine delivery = Corrected dietary leucine + F[C]leu with Corrected dietary leucine = Dietary leucine X [1-(Sp X 0.01).

Finally, net leucine balance was calculated over a 480 min period as follows:

$$\text{Leucine balance} = \text{Leucine intake} - \text{Leu Ox} \quad (6)$$

3.5. Statistical analysis

Results are expressed as means ± SEMs. For plasma amino acid concentrations, each individual curve was characterized by its zenith (Y_{max}), by the time lag to Y_{max} (T_{max}), and by area under the curve (AUC) at the postprandial state (between timepoint 240 and timepoint 480 min). Because Y_{max} occurred at variable times after meal ingestion for each individual, the mean Y_{max} values may differ from the mean values at a given timepoint.

Leucine kinetics between postabsorptive and postprandial state were compared using a two-way ANOVA with group (PRO or CAS, AP or HP) and time as factors in order to discriminate between effect of diets, effect of time, and their interaction. A $P < 0.05$ was considered as significant. Both the effect of protein source and the effect of protein amount on Y_{max}, T_{max}, AUC and leucine kinetics were assessed using a two-way ANOVA with protein source (PRO or CAS) and protein amount (AP or HP) as factors in order to discriminate between effect of protein source, effect of protein amount, and their interaction. When the two-way ANOVA gave statistical differences, a post hoc Fisher's test was applied in order to define which meal was different from the others.

4. Results

4.1. Subject characteristics

The characteristics of the subjects are given in Table 1. Subjects from each group presented similar anthropometrical characteristics. Age, body weight, body mass index (BMI), fat free mass (FFM) and fat mass did not differ among groups. Fasting plasma insulin concentrations for each individual on the experimental day were not different between groups.

According to the food-intake questionnaire completed by each subject during the 15-day controlled diet period, protein intake was significantly greater during the experimental period than in the adaptive period, increasing from 11.0% to 12.5% in the AP groups and from 11.0% to 15.5% of total energy intake in the HP groups. As expected, protein intakes increased to a larger extent in the experimental period for the HP groups but were similar within each experiment (AP or HP) irrespective of the protein source provided.

4.2. Plasma insulin and amino acid concentrations

In the postabsorptive state, there were no significant diet effects on plasma insulin concentration, as shown in Table 1. Plasma insulin concentration increased moderately for all groups after the beginning of meal ingestion ($P < 0.01$) but this change was more pronounced after PRO HP meals than after PRO AP and CAS AP meals ($P < 0.05$, data not shown). In the postprandial state, there were no significant effects of protein source within each protein quantity group.

In the postabsorptive state, EAAs (Essential Amino Acids), BCAAs (Branched Chain Amino Acids, e.g. Ile, Leu, Val) and leucine concentrations were similar between groups whatever the amount of proteins and protein source (Fig. 2). In the postprandial state, HP meals induced higher Y_{max} than AP meals for EAAs ($2.1.10^3 \pm 79$ μM *vs.* $1.8.10^3 \pm 91$ μM for PRO HP and PRO AP, respectively, $P < 0.02$, and $1.8.10^3 \pm 144$ μM *vs.* $1.4.10^3 \pm 65$ μM for CAS HP and CAS AP, respectively, $P < 0.01$) and BCAAs ($1.3.10^3 \pm 49$ μM *vs.* $1.0.10^3 \pm 60$ μM for PRO HP and PRO AP, respectively, $P < 0.03$, and

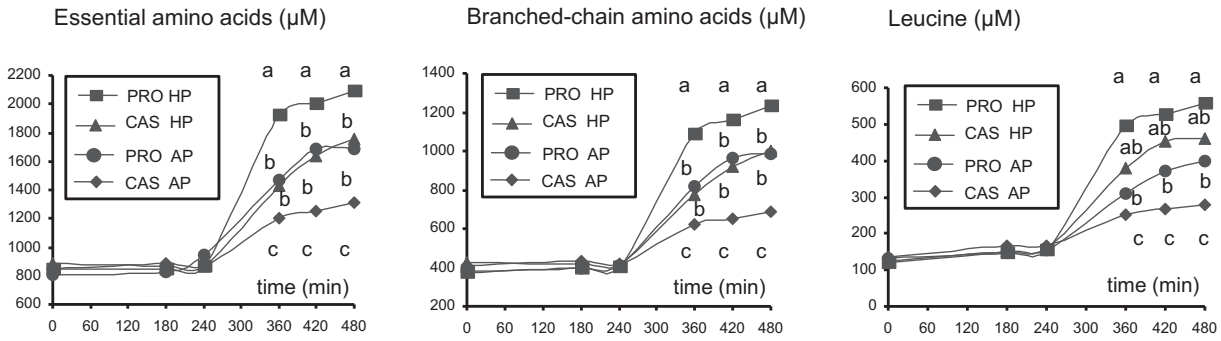

Fig. 2. Plasma amino acid concentrations in the postabsorptive and the postprandial state. Data are means for the plasma essential amino acid (EAA), branched-chain amino acid (BCAA) and leucine concentrations at the postabsorptive and postprandial state. PRO HP: high protein meal with a soluble protein source; PRO AP: adequate protein meal with a soluble protein source; CAS HP: high protein meal with a casein source; CAS AP: adequate protein meal with a casein source. $P < 0.05$.

$1.0.10^3 \pm 101$ μM *vs*. $0.7.10^3 \pm 40$ μM for CAS HP and CAS AP, respectively, $P < 0.01$). However, AUC values for both EAAs and BCAAs were greater with HP meals than AP meals only when PRO was the protein source (PRO HP *vs*. PRO AP, $P < 0.01$ and CAS HP vs. CAS AP, P=NS, data not shown). Moreover, PRO meals always induced greater Y_{max} and AUC values than both CAS meals (PRO HP *vs*. CAS HP, $P < 0.02$, and PRO AP *vs*. CAS AP, $P < 0.01$, Fig. 2). Concerning postprandial leucine concentrations, PRO meals also induced greater Y_{max} and AUC values than CAS meals (PRO HP *vs*. CAS HP, $P < 0.0005$, and PRO AP *vs*. CAS AP, $P < 0.002$, Fig. 2). Furthermore, a higher casein intake (CAS HP meal) only increased the Y_{max} for EAAs, BCAAs and leucine to the same extent as with PRO AP meals.

4.3. Leucine enrichments

The intravenous tracer enrichments ([^{13}C]leucine) reached a plateau in the last hour of the postabsorptive state, just before meal ingestion (data not shown). Thereafter, plasma [^{13}C]leucine enrichment fell after the beginning of meal ingestion due to dilution of the isotopic pool from the leucine contained in the meal (data not shown). We adapted the tracer perfusion rate to be able to reach the steady-state regardless of the protein in the beverage. [^{13}C]leucine enrichment also reached a plateau in the last hour of the fed state. This, the decrease in the fed state was less pronounced after CAS meals than after PRO meals (data not shown). This decrease was also less pronounced after AP meals than after HP meals (data not shown).

4.4. Rates of leucine appearance

In both the postabsorptive and fed states, the Endo Leu Ra index of WB proteolysis was similar whatever the diet provided (Table 2). The meals induced a pronounced inhibition of Endo Leu Ra, which decreased by 73% and 81% for HP diets (PA *vs*. PP, $P = 0.01$ and $P = 0.002$ for PRO HP and CAS HP, respectively) and by 18% and 46% for PRO AP and CAS AP, respectively (PA *vs*. PP, P=NS and $P = 0.01$ for PRO AP and CAS AP, respectively). Nevertheless, there were no differences in the amplitude of diet-induced Endo Leu Ra inhibition between the different diet groups.

4.5. Rates of leucine utilization

In both the postabsorptive and postprandial states, the NOLD index of WB protein synthesis was similar whatever the diet (Table 2). However, NOLD was significantly stimulated by feeding only in HP diet treatments (PA *vs*. PP, $P = 0.01$ and $P = 0.001$ for PRO HP and CAS HP, respectively). In contrast, this increase was moderate and not significant after either PRO AP or CAS AP meals.

In the postabsorptive period, Tot Leu Ox was similar in all groups (Table 2). After protein ingestion, Tot Leu Ox increased in all cases but with different response patterns according to meal. Tot Leu Ox

Table 2
Baseline and kinetics of leucine concentrations and fluxes after meal ingestion.

		PRO		CAS		ANOVA
		AP	HP	AP	HP	$p < 0.0001$
[Leu] (μM)	Basal	122 ± 9	116 ± 10	134 ± 5	132 ± 11	NS
	PP	431 ± 28^{a}	527 ± 30^{ab}	265 ± 12^{b}	360 ± 37^{c}	Q/S
	AUC (10^3)	84.7 ± 5.5^{a}	102.3 ± 5.7^{b}	56.9 ± 2.1^{c}	67.2 ± 5.6^{c}	Q/S
Tracer infusion rate	(μmol kg FFM^{-1} min^{-1})	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.30	NS
Leucine intake	(μmol kg FFM^{-1} min^{-1})	2.67 ± 0.06^{a}	5.37 ± 0.16^{b}	2.27 ± 0.17^{c}	3.96 ± 0.14^{d}	Q/S
Endo Leu Ra (μmol kg FFM^{-1} min^{-1})	PA	1.59 ± 0.06	1.73 ± 0.19	1.60 ± 0.06	1.55 ± 0.43	NS
	PP	1.38 ± 0.36	0.59 ± 0.43*	0.85 ± 0.20*	0.29 ± 0.26*	NS
NOLD (μmol kg FFM^{-1} min^{-1})	PA	1.48 ± 0.05	1.60 ± 0.18	1.49 ± 0.07	1.43 ± 0.03	NS
	PP	1.66 ± 0.24	1.76 ± 0.20*	1.47 ± 0.10	1.67 ± 0.06*	NS
Leu Ox (μmol kg FFM^{-1} min^{-1})	PA	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.01	0.25 ± 0.01	NS
	PP*	1.16 ± 0.08^{a}	1.76 ± 0.11^{b}	0.80 ± 0.08^{c}	1.21 ± 0.17^{ad}	Q/S
Splanchnic extraction	%	51 ± 0.50	52 ± 0.50	60 ± 0.50	63 ± 0.40	NS ($p = 0.06$)

PRO: soluble milk protein source; CAS: casein source; AP: adequate protein meal; HP: high protein meal; [Leu]: Leucine plasma concentration; Y_{max}: Maximal plasma concentration; AUC: Area under the curve; PA: Postabsorptive state; PP: Postprandial state; FFM: Fat-free mass. Values are means ± SEMs; $n = 8$ for PRO AP, PRO HP, CAS HP groups and $n = 7$ for CAS AP groups. Two-way repeated-measures ANOVA was performed to discriminate among the effects of quantity (Q) and source (S) of protein. Individual means were compared using a post hoc Fisher's test. Postabsorptive and postprandial state were compared using unpaired *t*-tests a two-way ANOVA with group (PRO or CAS, HP or AP) and time as factors in order to discriminate between effect of diets, effect of time, and their interaction. *$P < 0.05$ time effect.
Means sharing the same superscript letter are not significantly different from each other (a, b, c, d different for $p < 0.05$).

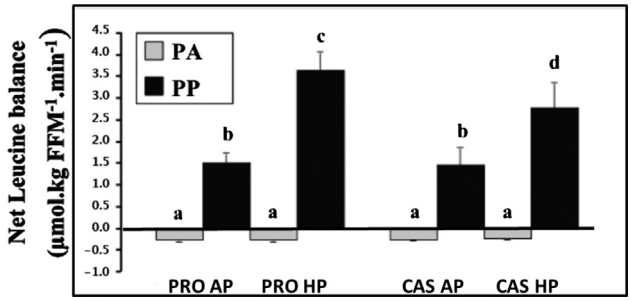

Fig. 3. Net leucine balance in the postabsorptive and the postprandial state. Statistical analysis was performed on change in endpoint values between postabsorptive (PA) and postprandial (PP) state. Data are means ± SEM; $n = 8$ for PRO AP, PRO HP, CAS HP groups and $n = 7$ for CAS AP groups. PRO AP: adequate protein meal with a soluble protein source; PRO HP: optimal protein meal with a soluble protein source; CAS AP: adequate protein meal with a casein source; CAS HP: optimal protein meal with a casein source. Values with different superscript letters are significantly different: $a < b < d < c$, $P < 0.05$ (two-way repeated-measures ANOVA followed by a Fisher's test).

values were higher after PRO meals than CAS meals whatever the amount of proteins considered. Concerning protein source, PRO HP meals significantly increased Tot Leu Ox in the fed state to a larger extent than PRO AP ($P < 0.001$), whereas CAS HP failed to stimulate Tot Leu Ox more than CAS AP ($P = 0.06$).

Finally, net leucine balance in the postprandial state was higher after HP meals than after AP meals (Fig. 3). Cross-comparing the protein sources revealed that PRO meals induced a better postprandial leucine balance only when a large amount of proteins was provided (3.63 ± 0.16 µmol.kg FFM^{-1} min^{-1} *vs.* 2.77 ± 0.21 µmol kg FFM^{-1} min^{-1} for PRO HP and CAS HP, respectively, $P = 0.005$).

For HP meals, splanchnic extraction of dietary leucine was 52.4 ± 4.8% (PRO HP) and 62.8 ± 3.9% (CAS HP). For AP meals, splanchnic extraction of the dietary leucine was 51.4 ± 4.9% (PRO AP) and 59.5 ± 5.0% (CAS AP). According to these values, there were no significant effects of either protein source or protein amount. Nevertheless, when expressed in absolute terms taking dietary leucine into account, splanchnic extraction was higher for HP meals than AP meals ($P < 0.05$).

5. Discussion

This study demonstrates that postprandial protein retention due to an increase in protein intake (from 1.2 to 1.9 g protein kg FFM^{-1} d^{-1}) is greater in elderly men when a fraction of the dietary proteins is provided by soluble milk proteins rather than caseins. However, the higher amount of casein produced a greater postprandial protein retention compared to both AP groups. In addition, because we used the same proteins during the study day and the 10 day-intervention period, we cannot exclude an effect of the 10 day-diet on the postprandial protein retention, although postpabsorptive net protein balance was not different among the groups.

Stimulated protein synthesis is rarely observed in most studies during the fed state and in steady-state conditions.[15] The fact that this was also the case here in the AP groups raises the issue of isotope methodology. Previous studies have reported that feeding failed to stimulate protein synthesis when dietary proteins were provided in moderate quantity.[17,18] Our experimental conditions were not favourable to reproducing a fast protein pattern. Nevertheless, we still observed the positive effect of soluble milk proteins on postprandial protein anabolism in elderly subjects, probably due to either leucine content or the remaining effect of fast proteins, which still persisted after the end of the diet. Despite steady-state conditions, we succeeded in inducing a sufficient increase of postprandial aminoacidemia with HP meals. Focussing on plasma EAAs and BCAAs, HP meals induced greater Y_{max} than AP meals whatever the protein source considered, due to the greater amount of protein provided. Accordingly, in our study, HP meals induced an increase in the responsiveness of protein synthesis to the fed state, in agreement with several previous studies observing unchanged whole-body protein synthesis in response to feeding except when protein intake was particularly high.[17,19]

Not only the quantity but also the quality of protein intake is an important factor to be considered. Here, the two different protein meals (PRO and CAS) were isonitrogenous but were not matched for AA composition. PRO meals induced greater postprandial leucine Y_{max} and AUC values than CAS meals. The provision of a larger amount of leucine within the diet could stimulate protein anabolism in the postprandial period in the elderly. Likewise, Koopman et al.[20] observed a stimulation of whole-body protein synthesis with the co-ingestion of proteins and a large quantity of leucine together with carbohydrate, whereas previous studies failed to increase whole-body protein synthesis and net leucine balance in the postprandial state after moderate quantities of dietary leucine.[21] Thus the leucine signal appears to be particularly important, but the threshold needs to be determined.

Furthermore, with respect to their digestion behaviours, the two major bovine milk protein fractions, i.e. soluble milk proteins and caseins, have quite different digestion patterns. Soluble milk proteins remain in a liquid form in the stomach and thus strongly increase amino acid availability as they are rapidly absorbed through the gut. In contrast, for slow proteins such as caseins that clot at low pH in the stomach, the increase in amino acid availability is lower.[7] The magnitude and duration of changes in AA availability determine the anabolic effects of the protein. Moreover, the effects of delivery profile of dietary AA, i.e. whether using "slow" or "fast" proteins, are age-dependent. Only faster-digesting proteins (and not the slow proteins) were able to promote whole-body anabolism in the elderly subjects.[9] It was hypothesized that fast proteins could be able to overflow the splanchnic tissue extraction whereas slow proteins are unable to escape it.

In our dose–response study, increasing protein intake using a fast protein-based dietary product (PRO HP group) tended to decrease splanchnic extraction. As there was no difference between AP and HP groups whatever the protein source considered, it was possible to pool AP and HP results together in order to compare splanchnic extraction following either PRO or CAS consumption. Taken as a whole, splanchnic extraction was significantly greater in CAS groups than PRO groups (52 ± 3% *vs.* 61 ± 3%, respectively; $P < 0.05$). There was also an increase in plasma AA concentrations, especially leucine, in the PRO groups. These results suggest a better availability of leucine for peripheral tissues when the proteins are soluble milk proteins, and PRO HP meals led to a greater net leucine balance in postprandial state than CAS HP meals.

Since it has been proven that excess leucine is able to overcome the age-related resistance to muscle protein synthesis,[22] nutritional manipulation to increase leucine availability could be beneficial in maintaining the postprandial stimulation of protein synthesis during aging. Long-term but sequential EAA supplementation with excess leucine may be a useful tool for the prevention and treatment of sarcopenia.[23] In particular, as previously shown,[23] our data indicate that a higher proportion of leucine within an isonitrogenous protein source stimulated protein synthesis to a greater extent in older adults. Likewise, recent human trials concluded that leucine supplementation during feeding improves muscle protein synthesis in the elderly,[24] independently of an overall increase in other AA[22]. Whether this anabolic effect is maintained through leucine-rich diet supplementation is still open to debate.

To conclude, daily administration of a drinkable dairy product containing large amount of proteins is able to promote an increase

in protein intake and postprandial protein anabolism in elderly men. Protein retention was better achieved when soluble milk proteins were provided rather than caseins. Consequently, improving the quality of protein by combining digestion rate and leucine content may be a more attractive option than simply increasing the quantity of protein intake (whatever their biological value) to improve protein retention in elderly subjects. The anabolic effect achieved using a drinkable dairy product may be a new way for fast recovery in frail hospitalized patients suffering from protein-energy malnutrition.

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Author contributions

Céline Gryson contributed to the study concept and design, acquisition of subjects and/or data, analysis and interpretation of data, and preparation of the manuscript. Stéphane Walrand and Yves Boirie contributed to the study concept and design, analysis and interpretation of data, and preparation of the manuscript. Christophe Giraudet, Paulette Rousset and Carole Migné contributed to data acquisition, analysis and interpretation. Cécile Bonhomme and Pascale le Ruyet contributed to the study concept and preparation of the manuscript.

Conflict of interest

Céline Gryson, Stéphane Walrand and Yves Boirie declared they have received support including grants, contracts or subcontracts, and gifted experimental diets by Lactalis. Cécile Bonhomme and Pascale Le Ruyet are employed at Lactalis Group.

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