

# Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement<sup>1-4</sup>

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## ABSTRACT

**Background:** The in vivo quality of milk protein fractions has seldom been studied in humans.

**Objective:** Our objective was to compare the postprandial utilization of dietary nitrogen from 3 [<sup>15</sup>N]-labeled milk products: micellar caseins (MC), milk soluble protein isolate (MSPI), and total milk protein (TMP).

**Design:** The macronutrient intakes of 23 healthy volunteers were standardized for 1 wk, after which time the subjects ingested a meal containing MC (*n* = 8), MSPI (*n* = 7), or TMP (*n* = 8). [<sup>15</sup>N] was measured for an 8-h period in plasma amino acids, proteins, and urea and in urinary urea.

**Results:** The transfer of dietary nitrogen to urea occurred earlier after MSPI ingestion than after MC and TMP ingestion, and concentrations remained high for 8 h, concomitantly with higher but transient hyperaminoacidemia and a higher incorporation of dietary nitrogen into plasma amino acids. In contrast, deamination, postprandial hyperaminoacidemia, and the incorporation of dietary nitrogen into plasma amino acids were lower in the MC and TMP groups. Finally, total postprandial deamination values were 18.5 ± 2.9%, 21.1 ± 2.8%, and 28.2 ± 2.9% of ingested nitrogen in the TMP, MC, and MSPI groups, respectively.

**Conclusions:** Our results confirm the major role of kinetics in dietary nitrogen postprandial utilization and highlight the paradox of MSPI, which, despite its high Protein Digestibility Corrected Amino Acid Score, ensures a rate of amino acid delivery that is too rapid to sustain the anabolic requirement during the postprandial period. Milk proteins had the best nutritional quality, which suggested a synergistic effect between soluble proteins and caseins. *Am J Clin Nutr* 2006;84:1070-9.

**KEY WORDS** Milk proteins, humans, amino acid kinetics, protein metabolism, deamination

## INTRODUCTION

The nutritional utilization of total milk protein has been studied in both animals (1-3) and humans (4, 5). Specific isotope methods previously developed to follow dietary nitrogen in the different experimentally accessible body compartments in humans (6-8) have shown that total milk protein (TMP) is of particularly excellent nutritional value in humans with a true digestibility and a net postprandial protein utilization of 95-96%

and 74%, respectively (6, 9). Often considered as reference proteins, dairy proteins cover a wide variety of compounds, with respect to both their composition and abundance in milk.

Cow milk contains 2 major protein fractions: casein and whey protein (10). Caseins are phosphate-containing proteins that occur as micelles in the native form, precipitate at pH 4.6, and are represented by 4 major classes:  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -, and  $\kappa$ -caseins. Milk soluble proteins remain in solution at pH 4.6 and constitute a heterogeneous group of proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, serum albumin, immunoglobulins, lactoferrin, and other minor fractions). As well as their physicochemical differences, caseins and milk soluble proteins greatly differ regarding their amino acid (AA) composition, the latter of which contains higher concentrations of total sulfur AAs (methionine and cysteine), lysine, threonine, and tryptophan.

Moreover, during digestion in the intestine, the behavior of caseins and milk soluble proteins differs markedly. Milk soluble proteins are rapidly evacuated from the stomach, whereas caseins clot or precipitate under the acidic gastric pH, which delays AA delivery to the gut (11). These differences in AA delivery kinetics are responsible for varying effects on whole-body protein metabolism in humans ingesting either casein or whey protein (12, 13), as has also been shown when the metabolism of milk and soy protein metabolism is compared (7). However, the precise postprandial metabolic fate of dietary AAs and nitrogen after the ingestion of either casein or milk soluble protein remains unclear.

Thus, the objective of the present study was to precisely characterize the kinetics of dietary nitrogen transfer into blood nitrogen compounds (serum AAs, proteins, and urea) and urinary urea

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**TABLE 1**  
Characteristics of the subjects<sup>1</sup>

	Group		
	TMP (n = 3M, 5F)	MC (n = 3M, 5F)	MSPI (n = 2M, 5F)
Age (y)	27.1 ± 7.8	31.4 ± 5.9	24.3 ± 6.6 <sup>2</sup>
BMI (kg/m <sup>2</sup> )	22.0 ± 2.0	22.1 ± 2.2	21.5 ± 3.1
Body weight (kg)	63.6 ± 11.3	64.0 ± 13.0	61.9 ± 9.3
TBW (L)	37.5 ± 9.4	35.5 ± 7.4	36.4 ± 8.6
Fat mass (kg)	20.8 ± 8.0	24.5 ± 6.1	20.9 ± 8.7

<sup>1</sup> All values are  $\bar{x} \pm SD$ . TBW, total body water; TMP, total milk protein; MC, micellar casein; MSPI, milk soluble protein isolate.

<sup>2</sup> Significantly different from the MC group,  $P < 0.05$  (one-way ANOVA, Bonferroni post hoc test).

in humans fed a meal containing either [<sup>15</sup>N]-labeled casein in a micellar form (MC), [<sup>15</sup>N]-labeled milk soluble protein isolate (MSPI), or [<sup>15</sup>N]-labeled microfiltered milk, ie, total milk protein (TMP). Before the metabolic exploration, the diet of the subjects had been strictly standardized for 1 wk, because the habitual protein intake is known to influence the metabolic fate of protein (9).

## SUBJECTS AND METHODS

### Subjects

Twenty-three men ( $n = 8$ ) and women ( $n = 15$ ) were included in this study. All participants were certified as being in good health after undergoing routine biochemical tests and a thorough examination performed by the medical staff of the CRNH. The purpose and the potential risks of the study, as well as the constraints due to the obligatory dietary standardization period, were fully explained to the subjects. Written informed consent was obtained from all participants, and the protocol was approved by the Institutional Review Board of Saint-Germain-en-Laye Hospital. We distributed the volunteers into 3 groups so that body composition did not differ significantly from one group to another.

### Main characteristics of the subjects

Body composition did not significantly differ between the groups (Table 1). Age was the sole characteristic to show a statistical difference, the MC group being older than the MSPI group.

### Adaptation period

During the week preceding the day of the experiment, the subjects had to follow an adaptation diet at home, adjusted to their body weight as verified during the prestudy medical examination. Dietary notebooks containing daily menus and the specific quantities of food to be consumed at each meal were delivered to all subjects, as were kitchen scales (accurate to the nearest 2 g) and daily record sheets. The subjects were asked to ensure strict compliance with their respective diets but were allowed some equivalent food exchanges. They also had to adhere to 3 main meals per day and were allowed one snack per day. The daily menus were designed to provide 138 kJ · kg<sup>-1</sup> · d<sup>-1</sup>, comprising 1.3 g protein · kg<sup>-1</sup> · d<sup>-1</sup>, 4.2 g carbohydrate · kg<sup>-1</sup> · d<sup>-1</sup>, and 1.2 g fat · kg<sup>-1</sup> · d<sup>-1</sup>. The subjects exhibited globally good

compliance with the standardization diet (data not shown) on the basis of their protein ingestion, which ranged from 1.24 g · kg<sup>-1</sup> · d<sup>-1</sup> in the MC group to 1.27 g · kg<sup>-1</sup> · d<sup>-1</sup> in the TMP group, as computed from the dietary records.

### [<sup>15</sup>N]-labeled milk products

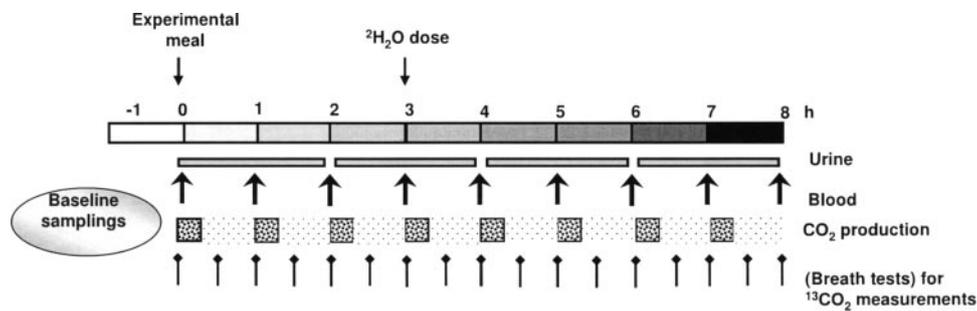
Milk was [<sup>15</sup>N]-labeled in the Milk Production Unit (UPL, Unité de Production du Lait, INRA, Saint-Gilles, France) by giving 100 g/d of [<sup>15</sup>N]H<sub>4</sub>SO<sub>4</sub> [12 atom% (AP)] isotope enrichment (Euriso-top, Saint-Aubin, France) for 7 d to 3 lactating cows via a rumen fistula. When the isotopic enrichment plateau was reached, milk from 3 consecutive milkings was pooled, defatted, and then microfiltered on a 1.4- $\mu$ m membrane (GP7 Sterilox equipment; Paul Exekia, Bazet, France) to produce microfiltered milk (TMP). About 5% of the initial milk volume was retained in the bacterial retentate. This retentate, which had the same protein composition as the permeate, was used to concentrate and purify proteins as follow. The proteins were diafiltered with osmosed water, and the permeate was concentrated and microfiltered on a 0.1- $\mu$ m membrane (S7 pilot), which allowed for the separation of micellar caseins (in the retentate) from milk soluble proteins (in the permeate), the latter being further concentrated by ultrafiltration on a 5-kDa membrane (TIA, Bollene, France). The final isotopic enrichment of milk collected ranged from 0.6365 to 0.6682 AP. The isotopic enrichment of the test products was 0.6336 ± 0.0002, 0.6452 ± 0.0014, and 0.6485 ± 0.0009 AP in the MSPI, MC, and TMP groups respectively.

### Test meals

Test meals contained TMP from microfiltered milk ( $n = 8$ ), native caseins in a micellar form (MC;  $n = 8$ ), or MSPI ( $n = 7$ ). The MC and MSPI meals were supplemented with lactose to reach the same level as that in the TMP meal (skim microfiltered milk). Meals were isoenergetic and isonitrogenous: 996 kJ and 23.3 g protein for the TMP meal, 993 kJ and 23.2 g protein for the MC meal, and 983 kJ and 22.6 g protein for the MSPI meal. These semisynthetic meals were dissolved in water to a final volume of 500 mL and with 83 mg [<sup>13</sup>C]phenylalanine was added as an extrinsic marker of AA deamination.

### Experimental design

The experimental design is illustrated in Figure 1. After fasting overnight, the subjects were admitted to the hospital on the morning of the experiment, and a catheter was placed in a superficial forearm vein for blood sampling. Baseline samples were then collected: blood, urine, and breath test. Total carbon dioxide production was measured by open-circuit indirect calorimetry. A plastic ventilated canopy connected to a pump, a flow meter, and a nondispersive infrared carbon dioxide analyzer (URAS 4G; Hartman & Brown, Rueil-Malmaison, France) was placed on the head and neck of the subjects while they were in a reclining position. Data points were collected every second for 20-min periods. Basal glycemia was measured immediately with a portable refractometer (Encore glucometer; Bayer Diagnostics, Puteaux, France). The subjects were required to consume their liquid meal within 15 min. They were then monitored for 8 h. Blood samples were withdrawn every 30 min for the first 3 h and then hourly for the remaining 5 h. The subjects were asked to rest quietly in their beds throughout the duration of the experiment to measure carbon dioxide production during discontinued periods



**FIGURE 1.** Experimental design illustrating the times that blood and urine samples were collected and carbon dioxide production and breath  $^{13}\text{CO}_2$  were measured in fasting subjects. After the subjects ingested the experimental meals, blood was collected every 0.5 h over 3 h and then every 1 h over 5 h. Urine was collected every 2 h. Carbon dioxide production was monitored 20 min/h, and the breath tests were performed every 30 min.

of 20 min every hour. Expired air was sampled every 30 min for 8 h after meal ingestion. The subjects were asked to void every 2 h, and the volume of total urine was measured. Three hours after the experimental meal, each subject was also given an accurately weighed dose ( $80 \pm 6$  mg/kg body wt) of deuterated water ( $^2\text{H}_2\text{O}$ , 99.94 AP; Euriso-Top, Gif-sur-Yvette, France) to determine total body water (TBW). For each blood sample, glycemia was determined immediately, and plasma or serum was obtained by centrifugation ( $3000 \times g$ , 15 min,  $4^\circ\text{C}$ ), portioned, and frozen at  $-20^\circ\text{C}$  until analyzed.  $\gamma$ -Aminobutyric and  $\alpha$ -amino adipic acids were added to one aliquot to act as an internal standard for the subsequent measurement of serum AA concentrations. Urine samples were stored at  $-4^\circ\text{C}$  with thymol crystals and paraffin as preservatives to be processed within the next 48 h or frozen immediately at  $-20^\circ\text{C}$  depending on the analysis.

## Analytic procedures

### Plasma and urinary urea measurements

Serum urea, urinary creatinine, and urinary urea were measured by using an enzymatic method (Dimension Automate; Dupont de Nemours, Les Ulis, France).

### Urea and amino acid extraction

Serum proteins were precipitated by using sulfosalicylic acid (Sigma Aldrich, St-Quentin Fallavier, France). The pellets were frozen at  $-20^\circ\text{C}$  and then freeze-dried until subsequent measurement of the nitrogen content and  $^{15}\text{N}$  enrichment. Serum urea was isolated by using a  $\text{Na}^+$  form of the cation exchange resin (Biorad Dowex, mesh 100–200; Interchim, Montluçon, France), as described by Preston and McMillan (14) in the presence of urease (EC 3.5.1.5) for 2 h at  $30^\circ\text{C}$ .

Ammonia was extracted from the urine on a cation exchange resin (Biorad Dowex, mesh 100–200; Interchim, Montluçon, France). Urea was further extracted after hydrolysis with urease by using the same protocol as used for serum urea. Resins were washed 5 times with distilled water and stored at  $4^\circ\text{C}$  before being eluted with a 2.5 mol/L  $\text{KHSO}_4$  solution for isotopic determination.

### Individual serum amino acid measurements

Serum AA concentrations were determined by using an HPLC system (Bio-Tek Instruments, St Quentin en Yvelines, France) combined with postcolumn ninhydrin derivatization and monitored by using Kromasystem 2000 software (Biotek, St-Quentin en Yvelines, France). Separation was performed

on a cation exchange resin (MCIGEL CK10F; Interchim, Montluçon, France). All AAs were detected at 540 nm, except for proline (440 nm).

### Isotopic analyses

**$^{15}\text{N}$  enrichment of AAs, protein, and urea.** Percentages of nitrogen and  $^{15}\text{N}$  enrichment were measured by using isotope ratio mass spectrometry (IRMS) (Optima, Fisons Instruments, Manchester, United Kingdom) coupled with an elemental analyzer (NA 1500 series 2, Fisons Instruments) with atropina (Carlo Erba Instruments, Fisons, Arcueil, France) as the standard, as previously described (8). Briefly, aliquots (serum protein, urea, and AA and urinary urea and ammonia) were burned in the elemental analyzer. Carbon dioxide and water were trapped, and the isotope ratio in nitrogen was measured by using calibrated nitrogen gas as the  $^{15}\text{N}/^{14}\text{N}$  reference. The AP and the AP excess (APE) of each sample were then calculated by subtracting the baseline AP measured in each subject.

**$^{13}\text{C}$  Enrichment in breath tests.** Measurement of  $^{13}\text{CO}_2$  in exhaled air was carried out by using standard mass spectrometric methods with an IRMS (Multiflow-IRMS Isoprime; Micromass, Manchester, United Kingdom).  $^{13}\text{C}$  Abundance was measured against a reference standard (PDB). The percentage recovery of  $^{13}\text{CO}_2$  per time interval was defined as the ratio of expired  $^{13}\text{C}$  to the  $^{13}\text{C}$  content of the meal. Their sum over all time intervals gave the cumulative percentage of  $^{13}\text{CO}_2$  recovery.

**$^2\text{H}$  Isotopic enrichment in urine.** Deuterium enrichments were measured in a baseline urine sample and in urine collected between 1 and 3 h after ingestion of the  $^2\text{H}_2\text{O}$  dose. At that time, isotopic equilibrium had been reached, as checked by deuterium determinations in both urine and plasma for 5 h after dose ingestion in 5 subjects (data not shown). Isotopic measurements were analyzed by using an isotope ratio mass spectrometer coupled with a gas-chromatography device (Multiflow-IRMS Isoprime) in 200- $\mu\text{L}$  urine samples after a 120-min equilibration between the water hydrogen of the samples and the gas hydrogen of a helium + hydrogen mixture in the presence of platinum as a catalyst.  $^2\text{H}_2$  Enrichment was then analyzed by using hydrogen gas as the reference.

### Calculations

TBW was calculated as follows:

$$\text{TBW (kg)} = \frac{^2\text{H}_2\text{O dose (g)}}{1000} \times \frac{(E_{\text{dose } ^2\text{H}_2\text{O}} - E_{\text{basal urine}})/(E_{1-3\text{h urine}} - E_{\text{basal urine}})}{1.04} \quad (1)$$

where  $E_{\text{dose}}^{2\text{H}_2\text{O}[r]}$  is the isotopic enrichment of the oral dose of  $^2\text{H}_2\text{O}$ ,  $E_{\text{basal urine}}$  is the isotopic enrichment of the basal urine sample, and  $E_{1-3\text{h urine}}$  is the isotopic enrichment of a urine sample collected between 1 and 3 h after ingestion of the oral dose of  $^2\text{H}_2\text{O}$ .

Calculated values for TBW were corrected for deuterium exchanges with nonaqueous hydrogen in the body (1.04 factor) (15). Fat-free mass was calculated by dividing TBW by an average hydration factor (0.736). Body fat was calculated as the difference between body weight and fat-free mass.

The rate of phenylalanine oxidation was calculated according to the following equation:

$$\begin{aligned} \% \text{ of oxidized Phe} &= \text{amount of expired CO}_2 \\ &\times (E_{\text{sample } t} - E_{\text{basal}}) / \text{amount of ingested } [^{13}\text{C}_2] \times 100 \end{aligned} \quad (2)$$

where  $E_{\text{sample } t}$  is the  $[^{13}\text{C}]$  enrichment of the sample at time point  $t$ , and  $E_{\text{basal}}$  is the  $[^{13}\text{C}]$  enrichment of the basal sample ( $t_0$ ).

### Incorporation of exogenous nitrogen into nitrogen body pools

The time course of dietary nitrogen incorporation into the different pools monitored (serum free AA pool and proteins, body urea, urinary urea) was evaluated by using the following general equation:

$$N_{\text{exo pool } t} = N_{\text{tot pool } t} \times (E_t - E_0) / (E_{\text{meal}} - E_0) / N_{\text{ingested}} \times 100 \quad (3)$$

where  $N_{\text{tot pool } t}$  is the nitrogen content of the pool (mmol nitrogen) at each time point  $t$ ,  $E_t$  is the  $[^{15}\text{N}]$  enrichment (expressed as AP) in the pool sampled at time  $t$ ,  $E_0$  is the baseline  $[^{15}\text{N}]$  enrichment,  $E_{\text{meal}}$  is the  $[^{15}\text{N}]$  enrichment of the meal, and  $N_{\text{ingested}}$  is the nitrogen content (mmol) of the meal.

For urinary urea,  $N_{\text{tot}}$  was calculated as the product of the urinary urea nitrogen concentration and the volume of urine excreted.  $N_{\text{tot}}$  in the serum free AA or protein pool was determined as the serum concentration of free  $\alpha$ -amino nitrogen or protein nitrogen multiplied by the serum volume, estimated to be 5% of body weight (16).

The exogenous nitrogen recovered in the urea body pool was calculated assuming that urea was uniformly distributed throughout the TBW and according to the following equation:

$$\begin{aligned} N_{\text{exo body urea}} &= C_{\text{urea}} \times \text{TBW} \times 0.92 \\ &\times (E_t - E_0) / (E_{\text{meal}} - E_0) / N_{\text{ingested}} \times 100 \end{aligned} \quad (4)$$

where 0.92 is the correction factor for the water content of plasma.

Nitrogen recovered in the different pools was always expressed as the percentage of ingested nitrogen during our study, except for AA, for which it was expressed as % of ingested nitrogen.

### Statistics

The results are expressed as means  $\pm$  SDs. Differences in body composition and compliance with the standardization diet between subjects were tested by one-way analysis of variance using the general linear model procedure under SAS (version 6.11;

SAS Institute Inc, Cary, NC). Differences between groups during the 8 h after meal ingestion were tested by repeated-measures analysis of variance using the MIXED procedure under SAS. For each parameter, 5 different covariance structures for random statements [Compound Symmetry (CS), Unstructured, Auto Regressive 1, Auto Regressive Moving Average 1 [ARMA (1,1)] and Toeplitz] were tested, and the most appropriate matrix was then selected. Post hoc tests were performed by using the Bonferroni test.  $P$  values  $<0.05$  were considered to be statistically significant. For glycemia and aminoacidemia, statistical analyzes were done by using the crude values, but the results were represented as variations from baseline data. In each group, differences from baseline values were tested with a  $t$  test. The differences between AA peak values among groups were tested with a one-way ANOVA using the general linear model procedure and a Bonferroni post hoc test under SAS.

## RESULTS

### Blood glucose and serum urea and amino acid concentrations

The postmeal responses of glycemia and urea were not influenced by the dietary protein source as shown in **Figure 2**. When compared with the baseline values, ingestion of the meal was followed by a marked increase in total AAs (**Figure 3**). In the MSPI group, this immediate postmeal hyperaminoacidemia was followed by marked hypoaminoacidemia from the sixth hour after the meal, whereas aminoacidemia slowly decreased to reach the baseline value in the MC group. In the TMP group, aminoacidemia remained above baseline at 8 h. The striking hyperaminoacidemia observed in the MSPI group 1 h after the meal was mainly due to an increase in indispensable AAs (IAAs) (**Figure 3**) and especially branched-chain AAs (BCAAs) (**Figure 3**). IAAs and BCAAs in the MSPI group increased significantly more than those measured in the TMP and MC groups. Peak values for BCAA were  $553 \pm 66 \mu\text{mol/L}$  in the MC group,  $577 \pm 77 \mu\text{mol/L}$  in the TMP group, and  $780 \pm 121 \mu\text{mol/L}$  in the MSPI group ( $P = 0.0002$ ). In the MSPI group, BCAA returned to baseline value within 4 h of ingestion, whereas they remained at  $\approx 150 \mu\text{mol/L}$  above baseline in both the TMP and MC groups. Dispensable AAs (DAAs) showed a moderate increase in the postmeal period, with the peak value occurring 1 h after ingestion of the meal in all groups. In the MSPI group, DAAs significantly decreased below the baseline value from the sixth hour after the meal. This decrease in DAAs was the main determinant of hypoaminoacidemia (**Figure 3**).

### Exogenous nitrogen transfer to serum AAs, protein, and urea

The time courses of  $[^{15}\text{N}]$  enrichment in serum AAs, protein, and urea nitrogen pools are shown in **Figure 4**. A significant meal  $\times$  time interaction was observed whatever the nitrogen pool ( $P < 0.05$ ). The transfer of  $[^{15}\text{N}]$  into the AA nitrogen pool peaked 2 h after ingestion of the meal in the MSPI group, whereas it remained very stable between 2 and 6 h in the MC and TMP groups (**Figure 4**). The highest peak observed in the MSPI group was followed by a marked decline, whereas the ratio of dietary nitrogen to total nitrogen remained stable until 8 h in the other 2 groups. Time courses of  $[^{15}\text{N}]$  enrichment in serum proteins are shown in **Figure 4**. The APE ratio was highest in the MSPI group



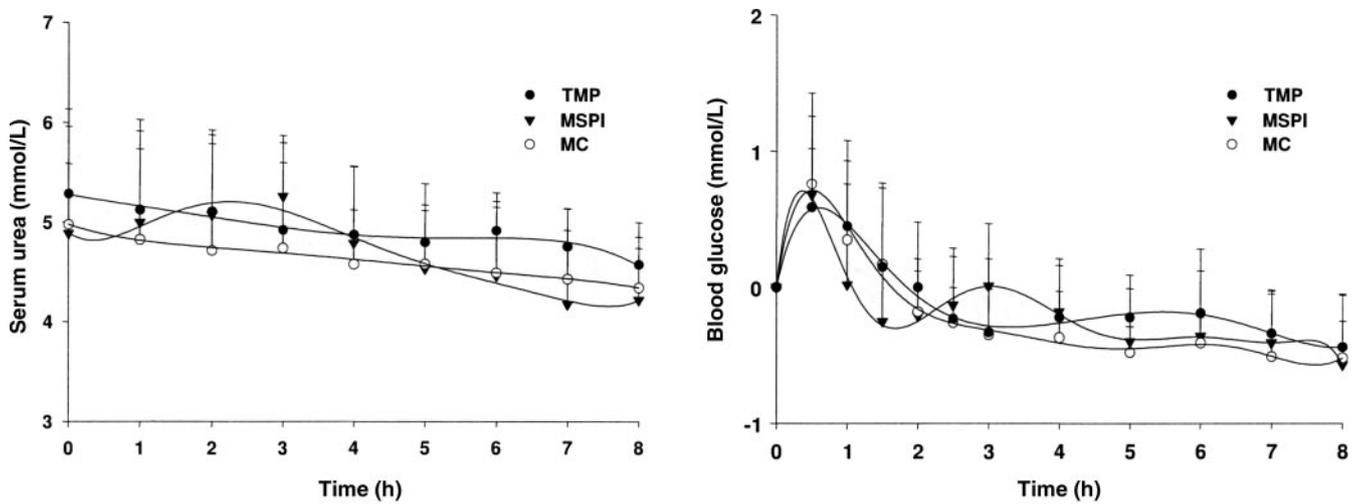


FIGURE 2. Mean ( $\pm$ SD) serum urea and blood glucose concentrations above baseline in the subjects after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). No significant effect of time, meal, or their interaction was observed as tested by a mixed-model analysis [ARMA (1,1) matrix] with time as a repeated measure.

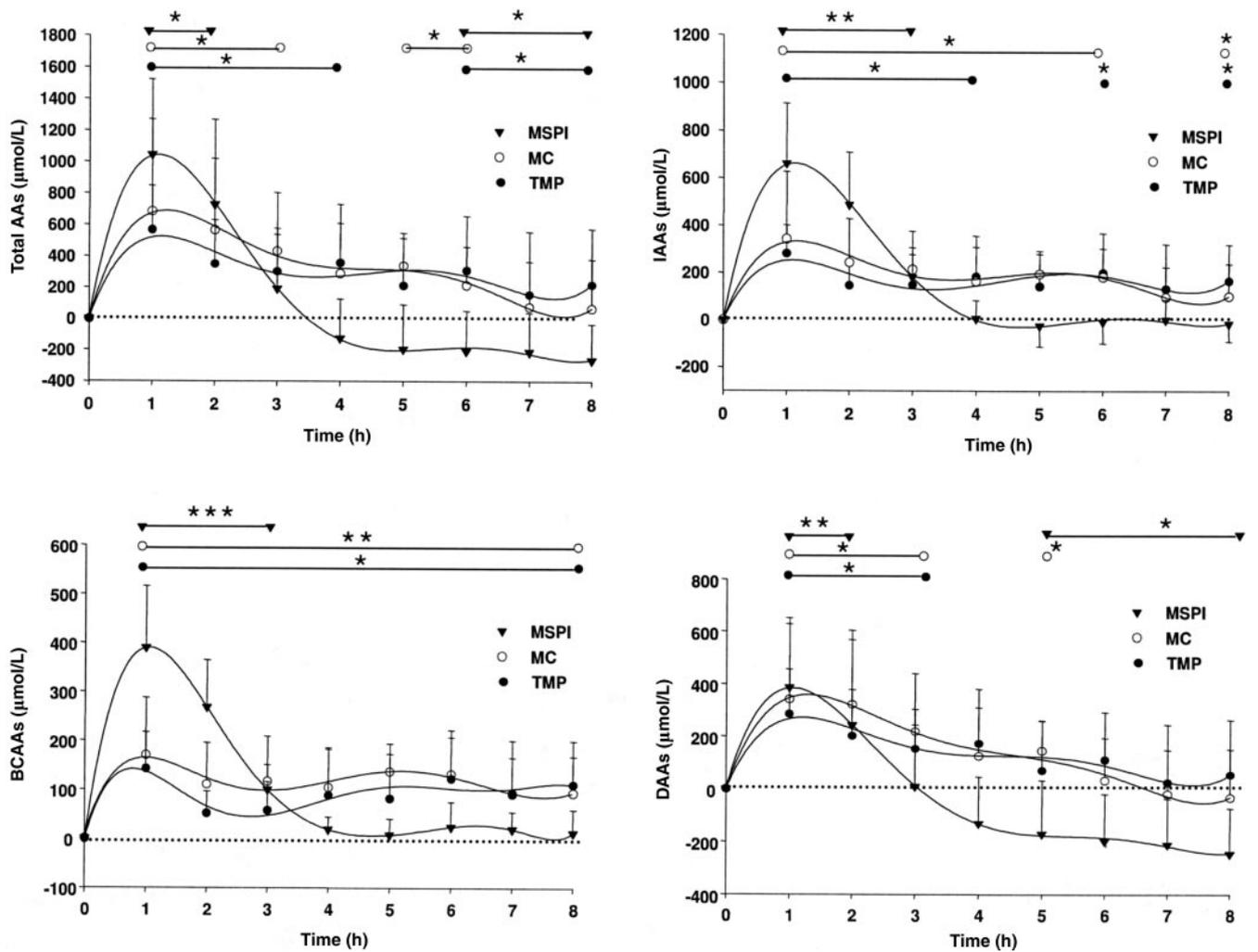
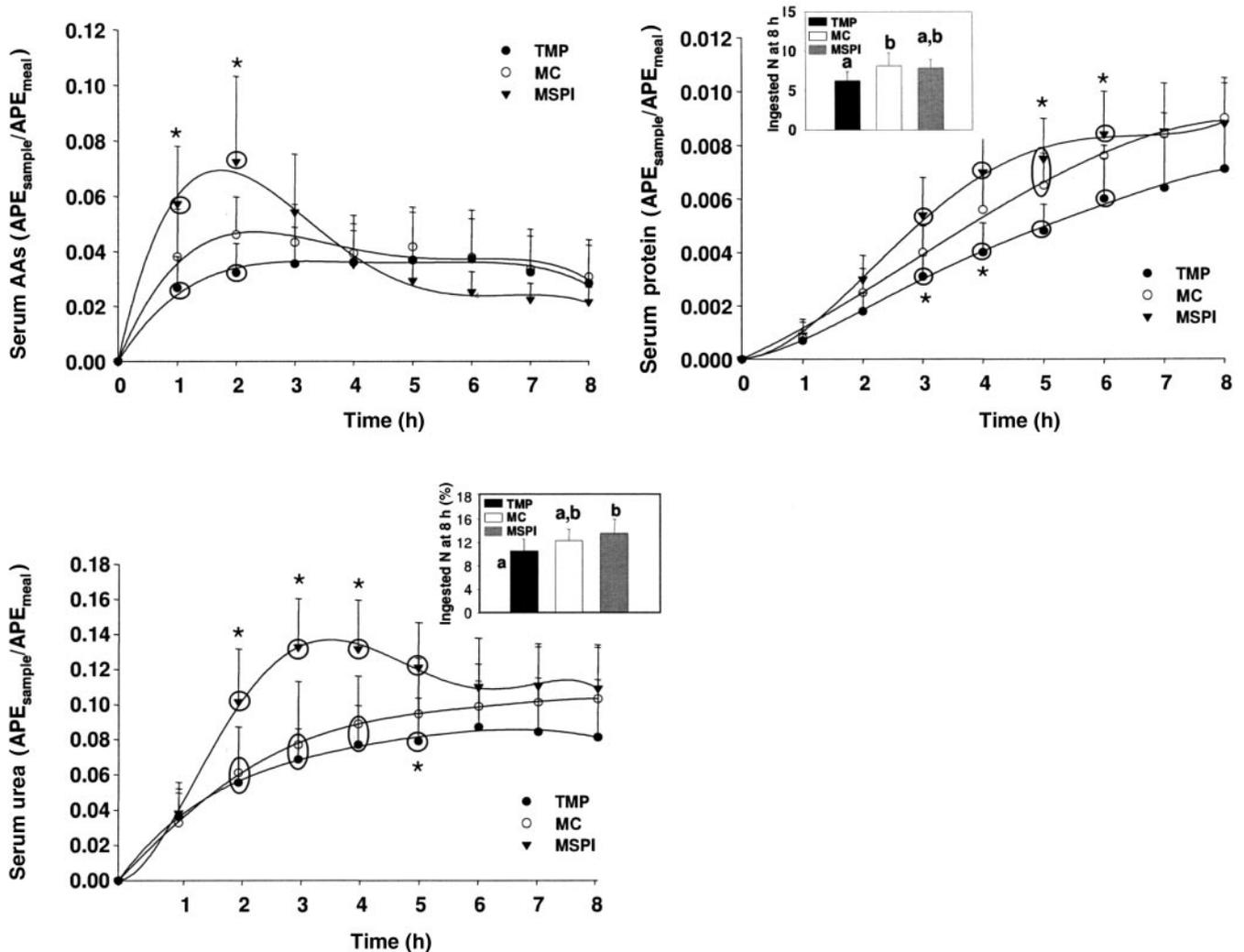


FIGURE 3. Mean ( $\pm$ SD) changes from baseline in serum total amino acid (AA), indispensable AA (IAA), branched-chain AA (BCAA), and dispensable AA (DAA) concentrations in the subjects after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). A significant effect of time ( $P < 0.0001$ ) and a significant meal-by-time interaction ( $P < 0.01$ ) were observed for all variables as tested on the crude values with a mixed-model ANOVA with time as a repeated measure [ARMA (1,1) matrix]. \*, \*\*, \*\*\*Significantly different from baseline: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$ .

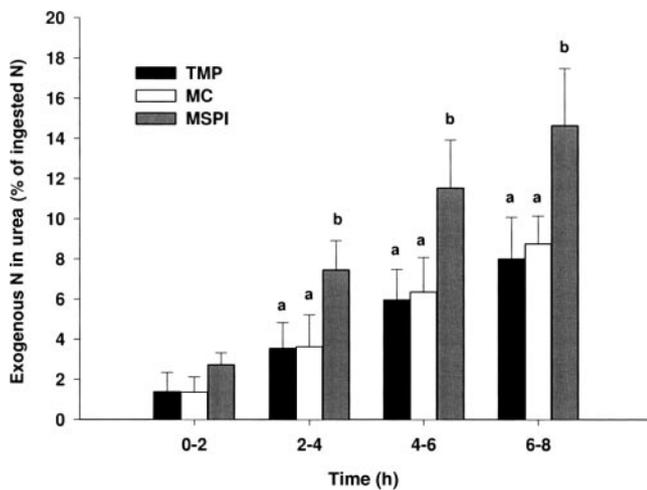


**FIGURE 4.** Mean ( $\pm$ SD) [ $^{15}\text{N}$ ] enrichment [expressed as atom percent excess (APE) in the sample/APE in the meal ( $\text{APE}_{\text{sample}}/\text{APE}_{\text{meal}}$ )] in the serum amino acid (AA) pool, serum protein pool, and the serum urea pool in the subjects after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). A significant effect of the meal on serum protein and urea ( $P < 0.01$ ) and both a significant effect of time ( $P < 0.0001$ ) and a significant meal-by-time interaction ( $P < 0.0001$ ) were observed for serum AAs, protein, and urea as tested with a mixed-model ANOVA with time as a repeated measure (CS matrix). \*Significant differences between groups,  $P < 0.05$  (Bonferroni post hoc test). The circled values are significantly different from each other at each time point. The inlay represent the percentage of nitrogen ingested incorporated in serum proteins or body urea 8 h after the meal; bars with different lowercase letters are significantly different,  $P < 0.05$  (one-way ANOVA).

from 2 to 6 h after the meal and thereafter reached a plateau; the APE ratio increased throughout the 8 postprandial hours in the TMP and MC groups. Eight hours after ingestion of the experimental meal, the amounts of dietary nitrogen incorporated into serum proteins were  $6.3 \pm 1.2\%$ ,  $8.1 \pm 1.7\%$ , and  $7.8 \pm 1.1\%$  of ingested nitrogen in the TMP, MC, and MSPI groups respectively, with a significantly higher amount of exogenous nitrogen transferred to serum proteins in the MC group than in the TMP group ( $P = 0.03$ ). The APE ratio in body urea (Figure 4) dramatically increased in the MSPI group between 2 and 6 h and was significantly different between the MC and TMP groups. At 8 h, the [ $^{15}\text{N}$ ] enrichment of urea did not differ significantly between the 3 groups. The amounts of dietary nitrogen still sequestered in the body urea pool 8 h after the meal were  $10.5 \pm 2.0\%$ ,  $12.3 \pm 2.1\%$ , and  $13.5 \pm 2.5\%$  of ingested nitrogen in the TMP, MC, and MSPI groups, respectively ( $P < 0.05$  between the MSPI and MC groups, Bonferroni post hoc test).

#### Urea production from exogenous and endogenous amino acids

The cumulative amount of exogenous nitrogen excreted in urinary urea was significantly higher in the MSPI group than in the TMP and MC groups (Figure 5). At the end of the clinical procedure, exogenous urea nitrogen represented  $8.0 \pm 2.1\%$  and  $8.8 \pm 1.4\%$  of ingested nitrogen in the TMP and MC groups, respectively, and was almost double in the MSPI group ( $14.6 \pm 2.8\%$  of ingested nitrogen). Finally, total deamination, which represents the sum of exogenous nitrogen still present 8 h after the meal in body urea and that excreted in urinary urea, was significantly different between groups (Table 2):  $28.2 \pm 2.9\%$ ,  $18.5 \pm 3.0\%$ , and  $21.1 \pm 2.8\%$  of ingested nitrogen were irreversibly transferred to urea in the MSPI, TMP, and MC groups, respectively ( $P < 0.05$  between MSPI and the 2 other groups, Bonferroni post hoc test).



**FIGURE 5.** Mean ( $\pm$ SD) cumulative excretion of dietary nitrogen in urinary urea in humans after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). A significant ( $P < 0.05$ ) effect of meal, time, and their interaction was observed as tested with a mixed-model ANOVA with time as a repeated measure [ARMA (1,1) matrix]. Bars with different lowercase letters are significantly different,  $P < 0.05$  (Bonferroni post hoc test).

Urea production rates of exogenous and endogenous origin are depicted in **Figure 6**. Exogenous urea production was the highest during the first 2-h period after meal ingestion, at which time it represented 33%, 38%, and 42% of total urea production in the TMP, MC, and MSPI groups, respectively. Overall, the transfer of exogenous nitrogen to urea was significantly influenced by the protein source and was significantly higher in the MSPI group than in the MC and TMP groups, especially during the first 2 h. Considering exogenous nitrogen transfers into nitrogen body pools, there was a systematic and significant meal-by-time interaction ( $P < 0.0001$ ) and a significant meal effect regarding the transfer of exogenous nitrogen into urea and serum proteins as well as the production of exogenous urea ( $P \leq 0.01$ ).

### [ $^{13}$ C]Phenylalanine oxidation

The oxidation kinetics of [ $^{13}$ C]phenylalanine as an extrinsic marker of AA deamination are shown in **Figure 7**. Peak oxidation in the MSPI group was observed 2 h after the meal, whereas it was observed 30 min earlier in the MC and TMP groups. However, no interaction between time and meal [ARMA (1,1)

**TABLE 2**

Dietary nitrogen irreversibly transferred to body and urinary urea (as % of ingested nitrogen) in humans 8 h after the ingestion of total milk protein (TMP), micellar casein (MC), or milk soluble protein isolate (MSPI)<sup>1</sup>

	Group		
	TMP ( $n = 8$ )	MC ( $n = 8$ )	MSPI ( $n = 7$ )
	% of ingested N		
Body urea	10.53 $\pm$ 2.02 <sup>a</sup>	12.31 $\pm$ 1.95 <sup>a,b</sup>	13.52 $\pm$ 2.46 <sup>b</sup>
Urinary urea	8.00 $\pm$ 2.08 <sup>a</sup>	8.76 $\pm$ 1.38 <sup>a</sup>	14.65 $\pm$ 2.84 <sup>b</sup>
Total	18.52 $\pm$ 2.95 <sup>a</sup>	21.07 $\pm$ 2.82 <sup>a</sup>	28.16 $\pm$ 2.88 <sup>b</sup>

<sup>1</sup> All values are  $\bar{x} \pm$  SD. Values in a row with different superscript letters are significantly different,  $P < 0.05$  (one-way ANOVA, Bonferroni post hoc test).

matrix] was observed. After 8 h,  $\approx 25$ –26% of the phenylalanine dose had been oxidized, and no significant difference was observed between the groups.

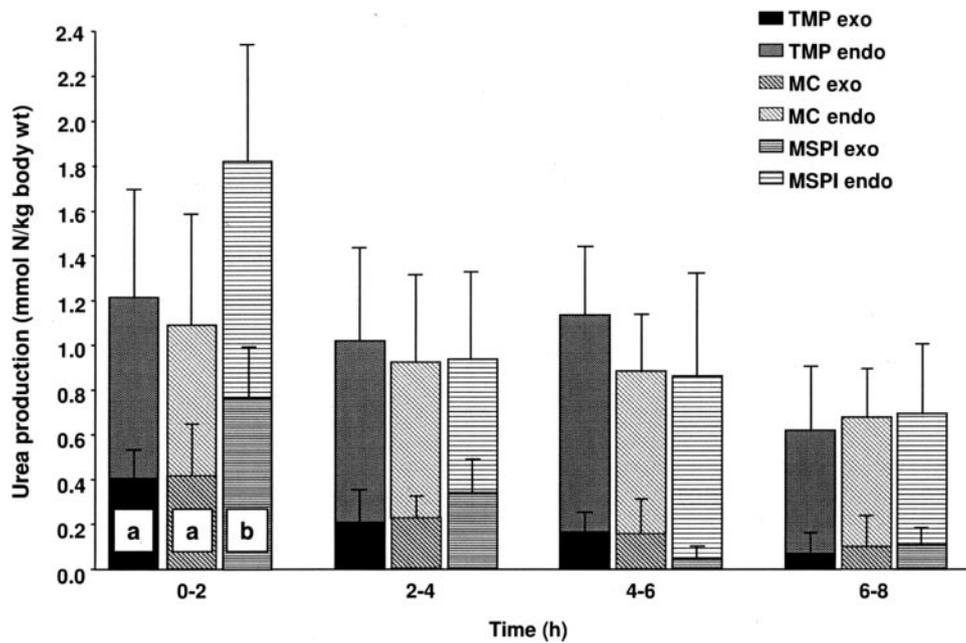
### DISCUSSION

The aim of this study was to investigate in humans the postprandial kinetics of dietary nitrogen after the ingestion of different milk protein fractions prepared from the same milk pool. The kinetics of milk soluble proteins and micellar caseins were compared with those of total milk proteins in which both fractions are combined. Our data showed significant differences in both postprandial aminoacidemia and the time course of dietary nitrogen appearance in the body nitrogen pools monitored, systematically characterized by the earlier appearance of higher amounts of dietary nitrogen after the MSPI meal than after the MC or TMP meal. These distinct metabolic behaviors translated into a higher deamination rate with MSPI than with MC and TMP.

The first difference evidenced during this study concerned serum AA kinetics after the different protein meals. Our results are in line with the findings of Hall et al (17), who showed that whey protein ingestion mediated larger increases in postmeal aminoacidemia than did casein ingestion. These differences were due to both different gastric emptying rates and the different AA composition of protein sources. Gastric emptying has proved to be a major factor controlling the kinetics of milk nitrogen absorption (18). Several experiments have estimated the half time of gastric emptying after the ingestion of whey-, casein-, or milk-based meals, with some conflicting results. Calbet and Holst (19) reported no differences in the gastric emptying rates, whereas Mahé et al (11) evidenced that the intestinal kinetics of dietary nitrogen delivery were markedly slowed after casein ingestion when compared with  $\beta$ -lactoglobulin ingestion. Some studies in infants have also shown that gastric emptying differed noticeably as a function of milk type (20–22). Consequently, caseins were considered to be slow-digested proteins and whey proteins as fast-digested proteins and were shown to exert a different effect on whole-body postprandial protein and AA metabolism (12, 23).

We also confirmed that the type of the protein can specifically influence postmeal aminoacidemia (7, 12, 19). The chemical composition of MSPI is characterized by high leucine and isoleucine contents, and its ingestion is followed by a striking peripheral elevation of these AAs, which are known to be poorly oxidized in the liver (24–26). Moreover, the higher plasma proline concentration we observed after the ingestion of casein was due to the higher proline content of this fraction. No other differences were found between plasma AAs.

The modulation of AA kinetics markedly influenced the metabolic fate of dietary nitrogen. The high MSPI content in IAAs was counterbalanced by high rates of AA deamination, thus hindering its potentially elevated nutritional value. The role of the liver in preventing an excessive increase in plasma AA concentrations was previously shown after variations in both protein quantity (9) and quality (7). In both cases, a sharp rise in aminoacidemia induced greater deamination rates. This generally occurred during the 2 first postprandial hours. In our trial, urea production of dietary origin in the subjects who ingested MSPI was twice that in the other groups. Finally, the 8-h postprandial deamination of MSPI reached 28% of ingested nitrogen compared with 21% with casein, or a difference of 7%, which was in



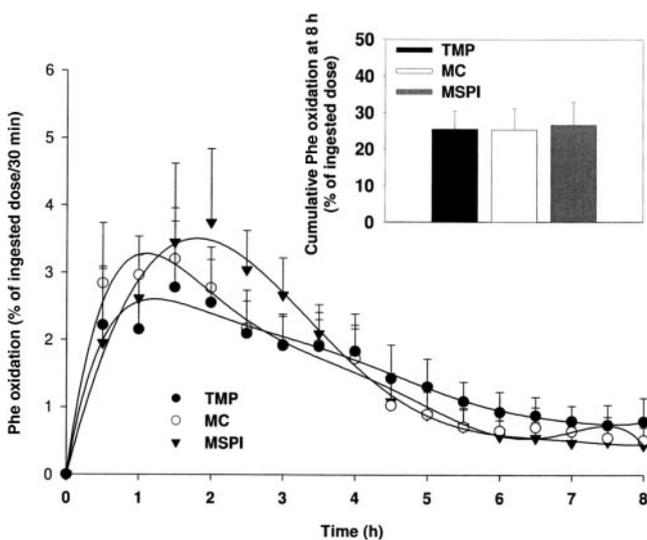
**FIGURE 6.** Mean ( $\pm$ SD) urea production from exogenous (exo; bottom portion of bars) or endogenous (endo; upper portion of bars) origin in the subjects after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). A significant effect of meal, time, and their interaction was observed for exogenous (Toeplitz matrix) but not for endogenous (CS matrix) urea production as tested with a mixed-model ANOVA with time as a repeated measure. A significant time effect ( $P < 0.0001$ ) was observed for the total urea production rate (Toeplitz matrix). Bars with different lowercase letters are significantly different,  $P < 0.05$  (Bonferroni post hoc test).

line with that already predicted on the basis of dietary leucine oxidation (31% for whey protein and 24% for casein) (12). Interestingly, the effect of kinetics, independent of AA composition, had previously been found to account for a difference of nearly 9% in postprandial AA oxidation (23). Previous results also strongly suggest that the lower postprandial retention of soy protein than of milk protein tended to be due to the more rapid appearance of dietary AAs in the blood rather than to differences in AA composition (7). Because the kinetics of MC and TMP were very similar, they showed a nonsignificant difference of 3%

in postprandial deamination. In contrast with these results, data obtained from [ $^{13}$ C]phenylalanine oxidation displayed no difference in total final oxidation, as previously reported for extrinsic oxidation marker (27). The delayed appearance of the tracer may have arisen from a higher hydroxylation rate in the MSPI group given the lower tyrosine content of this meal than of the TMP and MC meals (28).

Previous results showed a close association between a high and rapid appearance of dietary AAs in the serum and a more rapid transfer of dietary nitrogen into both serum proteins and urea pool, compared with the slowly released AAs from total milk protein. Interestingly, when compared with MSPI and TMP meals, dietary nitrogen arising from the MC meal was intermediate, with a slower rate of dietary nitrogen incorporation into liver exported proteins than that after the MSPI meal but with a trend toward a higher transfer than after the TMP meal. Plasma protein anabolism is sensitive to variations in dietary intake (29, 30) and is partly representative of hepatic protein anabolism. The contribution of exported plasma proteins to the total splanchnic retention of dietary nitrogen has been assessed to be 19% (31). Thus, we can assume that MSPI ingestion was followed by an overall higher splanchnic retention of dietary nitrogen than was TMP ingestion. By contrast, because MC ingestion and particularly TMP ingestion modified serum AA concentrations to a significantly lesser extent, we hypothesized that the TMP and MC meals did not induce a similar stimulation of protein synthesis in this area. Accordingly, the rapid incorporation of dietary nitrogen into the splanchnic free AA pool after a soy-based meal induced both an enhanced incorporation of dietary nitrogen into splanchnic proteins and deamination when compared with a milk-based meal (32).

Peripheral metabolism depends on AA delivery and availability, which results from the rates of digestion and splanchnic



**FIGURE 7.** Mean ( $\pm$ SD) [ $^{13}$ C]phenylalanine oxidation in the subjects after the ingestion of total milk protein (TMP;  $n = 8$ ), micellar casein (MC;  $n = 8$ ), or milk soluble protein isolate (MSPI;  $n = 7$ ). The inlay represents the percentage of ingested [ $^{13}$ C]phenylalanine oxidized 8 h after the meal.

extraction. Leucine, which represents  $\approx 12\%$  of AAs in MSPI, has proved to specifically stimulate muscle protein synthesis (33–35). We could thus hypothesize that high peripheral concentrations of BCAAs should stimulate muscle protein synthesis. However, we observed that 4 h after MSPI ingestion, peripheral BCAA concentrations had returned to basal concentrations in contrast with the sustained concentration observed after ingestion of the MC and TMP meals. Together with the decrease in other serum AA concentrations to below baseline values, the stimulation of peripheral anabolism under these conditions is unlikely to occur after MSPI ingestion, despite its high leucine content. This is consistent with finding that no difference in muscle protein synthesis was observed between subjects who ingested casein or whey drinks after exercise (36).

Finally, the last result of this study was the possible synergistic effect between casein and milk soluble protein in terms of metabolic utilization. Indeed, whatever the parameter measured, dietary nitrogen utilization in the TMP group was always better than, or at least identical to, that in the MC and MSPI groups; it was expected that dietary nitrogen utilization in the TMP group would be intermediate to that in the MC and MSPI groups. This lower, even if nonsignificant, exogenous nitrogen utilization during the postprandial period after the ingestion of milk protein fractions rather than total milk protein, may have been due to disruption of the native structure of milk, in which the networking of caseins and whey proteins in a 20:80 ratio is likely to produce a synergistic effect. It is therefore possible that the slightly higher dietary nitrogen retention observed after the TMP meal resulted from both an early metabolic and hormonal stimulation by the milk soluble protein fraction and a sustained delivery of AAs from caseins. Additional analyses, perhaps with the use of compartmental modeling, are required to support this hypothesis.

In conclusion, we showed that a slowly digested protein induced better postprandial utilization of dietary nitrogen than did a rapidly digested protein, despite the high chemical score of MSPI. This result, together with the hypoaminoacidemia observed 4 h after the ingestion of MSPI, strongly suggests that a too-rapid dietary AA delivery cannot support the anabolic requirement throughout the postprandial period. Additional studies are needed to assess the extent to which the kinetics of dietary AA delivery from different dietary protein fractions can specifically influence postprandial splanchnic and peripheral anabolic responses. 

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ML collected and analyzed the data and wrote the manuscript. CB and CG participated in the data collection and analysis and helped write the manuscript. CL and SD helped with the biochemical analyses. DT, HF, and JL helped with the study design. DT contributed to the manuscript. GA and RB were responsible for the clinical management of the subjects. JL and JF produced the milk fractions. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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