Absorption kinetics are a key factor regulating postprandial protein metabolism in response to qualitative and quantitative variations in protein intake

Hélène Fouillet,1,2 Barbara Juillet,1,2 Claire Gaudichon,1,2 François Mariotti,1,2 Daniel Tomé,1,2 and Cécile Bos1,2
1INRA, CRNH-IdF, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France; and 2AgroParisTech, CRNH-IdF, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France

Submitted 20 May 2009; accepted in final form 5 October 2009

Fouillet H, Juillet B, Gaudichon C, Mariotti F, Tomé D, Bos C. Absorption kinetics are a key factor regulating postprandial protein metabolism in response to qualitative and quantitative variations in protein intake. Am J Physiol Regul Integr Comp Physiol 297: R1691–R1705, 2009. First published October 7, 2009; doi:10.1152/ajpregu.00281.2009.—We have previously demonstrated that increasing the habitual protein intake widened the gap in nutritional quality between proteins through mechanisms that are not yet fully understood. We hypothesized that the differences in gastrointestinal kinetics between dietary proteins were an important factor affecting their differential response to an increased protein intake. To test this hypothesis, we built a 13-compartment model providing integrative insight into the sequential dynamics of meal nitrogen (Nm) absorption, splanchnic uptake, and metabolism, and subsequent peripheral transfer and disposition. The model was developed from data on postprandial Nm kinetics in certain accessible pools, obtained from subjects having ingested a 15N-labeled milk or soy protein meal, after adaptation to normal (NP) or high (HP) protein diets. The faster absorption of Nm after soy vs. milk caused its earlier and stronger splanchnic delivery, which favored its local catabolic utilization (up to +30%) and limited its peripheral accretion (down to −20%). Nm absorption was also accelerated after HP vs. NP adaptation, and this kinetic effect accounted for most of the HP-induced increase (up to +20%) in splanchnic Nm catabolic use, and the decrease (down to −25%) in peripheral Nm anabolic utilization. The HP-induced acceleration in Nm absorption was more pronounced with soy than with milk, as were the HP effects on Nm regional metabolism. Our integrative approach identified Nm absorption kinetics, which exert a direct and lasting impact on Nm splanchnic catabolic use and peripheral delivery, as being critical in adaptation to both qualitative and quantitative changes in protein intake.

interorgan amino acid metabolism; milk protein; soy protein; high-protein diets; compartmental model

THE POSTPRANDIAL ASSIMILATION of dietary protein ensures the anabolic repletion of protein-depleted stores during the postabsorptive period (40, 76, 79). Both qualitative (14, 15, 36, 54) and quantitative (58, 60, 62) characteristics of protein intake are potent modulators of amino acid (AA) and nitrogen (N) metabolism during the postprandial phase. However, the influence of these factors, possible interactions between them, and the mechanisms underlying their combined action and impacts on interorgan N metabolism are not yet fully understood in humans.

Regarding the acute, qualitative effect of protein intake, a large body of evidence shows that the dietary protein source affects the regional metabolism of dietary AA during the postprandial phase (15, 35, 36, 59). For instance, in the splanchnic bed, AA from soy are more markedly catabolized and transferred to urea than AA from milk, partly because their more massive and rapid appearance from the gut induces earlier and stronger splanchnic catabolism (15, 36), a difference that is ascribed to variations in gastric emptying and/or absorption rates between proteins (14, 21). The peripheral metabolism of dietary AA is also impacted by the protein source: compared with milk, the greater splanchnic catabolic use of dietary AA from soy results in their lower peripheral delivery and incorporation into proteins in both animals (60) and humans (36).

Considerable attention has been paid to the effects on N metabolism of chronic, quantitative changes in dietary protein levels. Increasing the protein level in the diet primarily enhances AA catabolism, as well as increasing the daily cycling of whole-body protein gains and losses (30, 41, 62, 63, 65). However, few data are available regarding the influence of the prevailing protein intake on the postprandial interorgan assimilation and specific metabolic utilization of dietary proteins in humans. In a previous study, we showed that the habitual protein intake modified the effect of protein quality, inasmuch as the postprandial whole-body retention of soy protein was more markedly affected by prior adaptation to an increased protein intake than that of milk protein (59). Consistent with this, in elderly women switching from a moderate to a high-protein diet, the N balance was favorably improved by a diet rich in animal protein compared with a diet predominantly rich in vegetable protein (64). However, the reasons explaining such a differential response between protein sources to an increased protein intake have not been elucidated.

We hypothesized that in the context of activated catabolic activity because of chronic habituation to a high-protein diet, the increase in the gap between soy and milk nutritional values (59) might arise from the interaction between differences in absorption kinetics and the catabolic pathways activated. We also assumed that overall, these phenomena would impact the further regional utilization of Nm in the splanchnic and peripheral areas. Because both gastrointestinal kinetics and regional metabolic utilization are particularly difficult to assess directly and in vivo in humans, we resorted to the compartmental modeling of clinical data, this being an appropriate approach to follow specifically the metabolic fate of Nm through digestive, splanchnic, and peripheral areas in the postprandial non-steady
Protein Source and Level Effect on Dietary N Metabolism

MATERIALS AND METHODS

Collection of Experimental Data

Experimental data were collected as described previously (59). The protocol was approved by the Ethical Committee for Saint-Germain-en-Laye Hospital, and informed consent was obtained from each subject. Briefly, 20 healthy subjects (10 male and 10 female) aged 30 ± 6 yr with a mean BMI of 21.7 ± 2.4 kg/m² were first of all subjected to dietary standardization for 7 days (week 1) with a normal protein (NP: 1 g·kg⁻¹·day⁻¹) intake. At the end of this adaptation period, they attended the hospital for a postprandial metabolic test: after an overnight fast, NP-adapted subjects ingested a single mixed meal containing either milk (NP-milk group; n = 12) or soy (NP-soy group; n = 8) proteins. The subjects were then adapted for the next 7 days (week 2) to a high-protein (HP: 2 g·kg⁻¹·day⁻¹) diet. At the end of this second adaptation period, they repeated the postprandial metabolic test by ingesting the same test meal as before: after an overnight fast, HP-adapted subjects thus ingested either the mixed milk (HP-milk group: n = 12, mean body weight: 64.4 ± 8.5 kg) or soy (HP-soy group: n = 8, mean body weight: 60.8 ± 10.4 kg) protein meals. The protein level in the diet was thus doubled when switching from the normal protein (NP) to the high protein (HP) diet, and a fixed dose of protein (0.41 g/kg of milk or soy proteins) was given in the test meal before the 8-h-postprandial metabolic experiments conducted at the end of each adaptation period.

As previously detailed (59), the adaptation diets (NP and HP) were designed to be isoenergetic (138 kJ·kg⁻¹·day⁻¹) and supply the same amount of carbohydrate (4.5 g·kg⁻¹·day⁻¹), with an isoenergetic exchange between protein (NP: 1 g·kg⁻¹·day⁻¹ and HP: 2 g·kg⁻¹·day⁻¹) and fat (NP: 1.2 g·kg⁻¹·day⁻¹ and HP: 0.8 g·kg⁻¹·day⁻¹). The test meals (milk or soy) ingested by subjects during postprandial metabolic experiments were semisynthetic, liquid, mixed meals providing 46 kJ/kg, i.e., one-third of the previous daily intake. The composition of these standard meals corresponded to dietary allowances of 15% of total energy as protein, 55% as carbohydrate, and 30% as fat. The test-meal loads were adjusted for body weight and provided the same amount of N (~0.68 mg/kg, i.e., protein ~0.41 g/kg), 1.51 g/kg of carbohydrate (99 and 90 g in the milk and soy groups, respectively), and 0.38 g/kg of fat (24 and 22 g in the milk and soy groups, respectively). Proteins were intrinsically and uniformly ¹⁵N-labeled and provided in the form of either total cow’s milk protein or a soy protein isolate, as previously used and described (15, 53). AA concentrations in proteins and individual enrichment are depicted in Ref. 15.

During the postprandial metabolic tests, blood samples were drawn hourly from a forearm vein and urine collected every 2 h over an 8-h period. The Nm content in these blood and urine samples was determined by measuring ¹⁵N enrichment in plasma-free AA, proteins and urea, and urinary urea and ammonia by isotope ratio mass spectrometry. The collection of experimental data, analytic methods, and calculations have been described in full detail elsewhere (59). Briefly, Nm pools were quantified in serum after separation of protein (acid precipitation), urea (hydrolysis and fixation of ammonia on cation-exchange resins), and AA (remaining in supernatant after extraction of protein and urea), as well as in urine (fixation of ammonia and of urea-derived ammonia on cation-exchange resins). Nm pools were then quantified using isotope-dilution equations as the product of the Nm concentration in the corresponding fraction and its volume of distribution, as estimated as follows: the volume of distribution was the serum volume (estimated at 5% of body weight) for serum-free AA and protein, the total body water [determined using multiple-frequency Bio-Impedance Analysis (Analytcor 5W, Spengler, Cachan, France)] corrected by a multiplying factor of 0.92 (representing the water content of serum) for body urea, and the volume of urine excreted for urinary urea and ammonia (59). Serum AA concentrations were measured by HPLC combined with a postcolumn ninhydrin derivatization (Bistek Instruments, St. Quentin en Yvelines, France).

The Nm content of each sampled pool (plasma free AA, plasma proteins, body urea, urinary urea, and urinary ammonia) was determined kinetically for each subject. Because Nm kinetics were not determined in ileal effluents during this experiment, we used analogous results from a previously reported study conducted in our laboratory, where 13 healthy subjects equipped with an ileal tube had ingested mixed ¹⁵N milk or soy protein meals (42). During this previous study, meals were made up of protein and sucrose, and their composition was very similar to that of our current test meals as far as the protein and energy contents were concerned. Because Nm ileal digestibility is barely modified when meals have the same energy content, the same protein content and nature, the mean data for cumulated Nm recovered in ileal effluents 8 h after each meal (5% and 9% of the Nm dose for milk and soy, respectively) were used to complete individual data during the current experiment. All data (Nm recovered from cumulated ileal effluents at 8 h, postprandial kinetics of Nm appearance in plasma-free AA, plasma proteins and body urea, postprandial kinetics of Nm accumulation in urinary urea and urinary ammonia) were converted into percents of the Nm dose. Urinary data were interpolated to obtain the same 1-h data step size for each data set.

Structural Modeling and Theoretical Identifiability of the Model

The model development was conducted according to gold-standard methods in compartmental analysis, by ensuring achievement and validation of each of the structural (model selection and theoretical identifiability) and numerical (model calibration and numerical identifiability) steps of model identification (18, 20, 48). In the iterative process of model development, the first objective was to propose and identify an adequate model structure for the compartmental analysis of our experimental data. The model was designed to describe the postprandial utilization of Nm in the body, i.e., the cascade of its ingestion, transfer through the gastrointestinal tract, absorption, elimination in the urine, and distribution in different regional metabolic pools. The model had thus to integrate the six sampled compartments and other nonsampled compartments, including the main N pools of the digestive, splanchnic, and peripheral areas. The model compartments represented kinetically homogeneous amounts of Nm present at a particular site in the body (e.g., in the intestinal lumen), or in a particular metabolic form (e.g., in body urea), or a combination of the two (e.g., in a free AA form in the splanchnic bed). The model thus consisted of several compartments representing distinct amounts of Nm, and different pathways of exchange between these compartments, each characterized by a constant diffusion coefficient kij representing the fraction of Nm in compartment j transferred to compartment i per unit time. As previously explained (48), the
mechanistic, dynamic model was governed by a set of ordinary differential equations containing the unknown parameters \( k_{ij} \) and describing the temporal evolution of each compartment’s size according to the instantaneous mass conservation law. The system of differential equations was solved using numerical integration for parameter estimation, by adjusting \( k_{ij} \) values until the model predictions fitted the data in all the sampled compartments.

According to the principle of parsimony, the model structure was selected from different candidates structures with a firm physiological basis, as the minimum structure necessary to fit all sampled compartments simultaneously (32). We tested the goodness of fit of different candidate structures of increasing order, including a basic model previously developed using similar experimental data (32). This basic model was confronted with the mean experimental data obtained for each group (NP-milk, NP-soy, HP-milk, and HP-soy), so as to obtain an unique or different (four at most) new—either reduced or expanded—model(s) that would adequately fit the data for each group. We retained the simplest structure that adequately fitted the data vs. higher-order models, which did not significantly improve the fit (20, 34). To achieve this, candidate models were all confronted with the mean of individual experimental data for each group using SIMUSOLV Software (26) and then discriminated using the Akaike and the Schwarz criteria (20, 49) and the likelihood ratio test (70).

Moreover, to validate this first stage in model development, i.e., structural modeling, it was necessary to test the a priori identifiability of the selected model(s) (18, 20) by evaluating the theoretical number of solutions for each model parameter \( k_{ij} \) in the ideal context of noise-free and continuous time measurements (4, 5, 7). This identifiability test was necessary to avoid the choice of a structure that would allow several or an infinite number of parameter sets to reproduce the data. Testing the a priori identifiability of our candidate models was made possible using a new algorithm, recently developed by Saccomani and colleagues (7, 67) for both linear and nonlinear models described using polynomial or rational functions. We used this new, differential algebra algorithm to assess the theoretical identifiability of each candidate model, before to turn to the problem of its numerical identification, using noisy and discrete experimental data to estimate its parameter values.

Parameter Estimation and Numerical Identifiability of the Model

Each candidate model was confronted with the mean experimental data for each group (NP-milk, NP-soy, HP-milk, and HP-soy), and once determined, the selected model was confronted with both mean and individual experimental data for each group. Model predictions concerning the Nm postprandial fate for each group were deduced from the parameter estimation process carried out on the mean of individual data, whereas individual fits were used to assess statistical differences between groups and the discriminatory capacity of the model. SIMUSOLV Software was used systematically to estimate the parameter values that would produce the closest model predictions, by adjusting rate constant values until the model predictions fitted the data for all sampled compartments simultaneously. The objective function iteratively maximized during the parameter estimation process under SIMUSOLV was the logarithm of the Likelihood Function (26, 34). The fitting process was first conducted on experimental data of the NP-milk group, by exploring a broad range of variations for all parameters, allowing values to change individually or in various combinations to fit the data of this reference group. The fitting process was then conducted on data of the three other groups by making minor adjustments to the smallest set of parameters (i.e., compared with the reference group, changing only the parameter values that were both necessary and sufficient to explain the observed differences in kinetics), according to the Berman’s minimal change postulate (9). In the context of our study, this implies that we could characterize the changes ensuing from our experimental perturbation (nature of the protein in the meal and level of the habitual protein intake) by exploring the minimal change to the “reference” model (NP-milk group), thus bringing the predictions into line with the different data sets. These parameters, in which changes were identified as making the greatest contribution to the separation of groups, were considered as regulatory steps involved in the metabolic response to variations in meal protein source and diet protein level. Moreover, different values for initial parameter estimates were tested to reduce the probability of falling into a local optimum if the starting point were not in the neighborhood of the global optimum. Final parameter estimates were verified as providing the best possible fit, and not a local optimum.

Parameter estimation was also validated by checking the a posteriori or numerical identifiability of the model, so as to be confident in its results and ensuing predictions. The numerical identifiability of the model was tested as previously detailed and documented (32, 34, 48); this included visual inspection of the goodness of fit (model-simulated curves vs. experimental data), analysis of the percentage variations explained for each sampled compartment (\( R^2 > 95\% \) considered as satisfactory), further analysis of the standardized residuals to detect any systematic deviations between data and predictions (tests of normality and randomness of the standardized residuals), and assessment of the reliability of parameter estimates (parameter values with a coefficient of variation <50% being considered as estimated with sufficient precision).

External Validation, Discriminatory Capacity, and Sensitivity Analysis of the Model

Model validation was completed by an external validation that consisted in testing the physiological plausibility of the model predictions with respect to our current knowledge of the system under study.

The discriminatory capacity of the model was tested by discriminatory analysis of the 40 individual sets of model parameters (12 from the NP-milk group, 12 from the HP-milk group, 8 from the NP-soy group, and 8 from the HP-soy group), performed using the SYSTAT statistical package (SYSTAT, Evanston, IL). This discriminatory analysis optimally separates all individual sets of \( k_{ij} \) into four groups of closer characteristics, on the basis of linear combinations of the parameters. We could thus verify that subjects in the same group were a posteriori correctly grouped together, and the percentage of subjects who were correctly replaced determined the discriminatory capacity of the model.

Sensitivity analysis of the model was performed on the parameter estimate values obtained after optimization using the mean of individual data for each group, by evaluating their influence on the model responses for each compartment. Sensitivity analysis was performed by evaluating the effect of a 1% change in parameter value on the prediction of a variable response, i.e., by calculating a sensitivity coefficient for each pair: \( \delta(\text{model response})/\delta(\text{model parameters}) \). However, to eliminate the bias caused by the magnitude in parameter values, sensitivity coefficients were log-normalized and calculated using the direct decoupled method under SIMUSOLV (26).

Model Predictions for Different Indices of Dietary Protein Metabolism and Quality

Model predictions permitted the assessment of various new indices of dietary protein quality at regional levels, by enabling predictions of Nm transfer and incorporation into regional AA and protein pools. Compartmental analysis produced predictions of the following variables and parameters: compartment size \( q_{ij}(t) \); the Nm content of compartment i at time \( t \), as a % of the Nm dose; transfer rates \( k_{ij} \); the fraction of Nm transferred from compartment j to compartment i per unit of time, in min\(^{-1}\); instantaneous fluxes, \( \text{flux}_{ij}(t) \) [calculated as \( k_{ij} \times q_{ij}(t) \)]; the quantity of Nm transferred from compartment j to compartment i per unit of time at time \( t \), as a % of the Nm dose per hour; and cumulated fluxes, \( \int_0^t \text{flux}_{ij}(t) \) dt [calculated as \( k_{ij} \times \int_0^t q_{ij}(t) \) dt]; the total amount of Nm transferred from compartment j to compartment i at time \( t \), as a % of the Nm dose.
The sizes of compartments were used (see Fig. 1 for a description of the compartment names) to calculate 8-h postprandial deamination losses of Nm [BU(8h) + UU(8h)] and the 8-h postprandial regional accretion of Nm, representing its net postprandial incorporation into regional protein [SCP(8h) + SEP(8h) in the splanchic bed and PP(8h) in the peripheral area]. These indices of regional accretion reflected the percentage of the Nm dose finally deposited in proteins in each area at the end of the postprandial period.

In addition, cumulated fluxes were used to calculate the half-times of Nm gastric-emptying and intestinal absorption {[calculated as the times necessary to achieve 50% of $\int_{0}^{t} k_{2,i} + GI(t) \cdot dt$ and 50% of $\int_{0}^{t} k_{5,i} \cdot IL(t) \cdot dt$, respectively] as well as 8-h postprandial Nm splanchic catabolic use [$\int_{0}^{t} k_{11} \cdot SA(t) \cdot dt$], Nm splanchic anabolic use [$\int_{0}^{t} (k_{8.5} + k_{9.5}) \cdot SA(t) \cdot dt$], Nm splanchic escape or peripheral delivery [$\int_{0}^{t} k_{6.0} \cdot SA(t) \cdot dt$] and Nm peripheral anabolic use [$\int_{0}^{t} k_{10,7} \cdot PA(t) \cdot dt$]. Because of the relations between compartment sizes and cumulated fluxes, Nm deamination losses were equal to the amounts of urea produced [$\int_{0}^{t} k_{1.5} \cdot SA(t) \cdot dt$] minus those salvaged by enterohepatic recycling [$\int_{0}^{t} k_{5.1} \cdot BU(t) \cdot dt$], while Nm regional accretions were equal to the amounts of protein synthesized minus those degraded by proteolysis.

Statistical Analysis

Predicted data were expressed as mean ± SD. Differences with $P < 0.05$ were considered as significant. Simple correlation analyses were performed between the different model parameters (i.e., transfer rates $k_{i,j}$). Differences between groups regarding the predicted postprandial kinetics of Nm (i.e., predicted Nm kinetics of appearance in sampled and nonsampled compartments and instantaneous and cumulated fluxes of Nm transfer between compartments) were analyzed using the SAS statistical software package (SAS/STAT Version 9.1.3, SAS Institute, Cary, NC). In the 20 subjects studied during the postmeal session, comparisons between dietary adaptations (diet = NP or HP) and protein sources (protein = milk or soy) were evaluated using mixed-models for repeated-measures analysis (PROC MIXED procedure of SAS) with time and diet as repeated factors and protein as a between-group factor. Interactions between diet, protein, and time were also tested. In case of significant interaction, post hoc contrasts were conducted using the Bonferroni adjustment with corrections to control for multiple comparisons.

Moreover, the relations between the absorption half-time of Nm and its further splanchic catabolic use, splanchic anabolic use, and splanchic escape at the end of the 8-h postprandial period were investigated in all individuals. Pearson correlation coefficients ($r$) were calculated (PROC CORR procedure of SAS) to assess the relation between pairs of variables. In addition, multiple linear regression analyses were used (PROC REG procedure of SAS) to assess the relation between all the variables taken together. Variables correlated with the dependent variable were included, and stepwise regression was used to select the minimum number of variables explaining the variability in the dependent variable. Cumulative $R^2$ indicated how much of the total variation in the dependent variable could be accounted for by the function.

RESULTS

Experimental Data

The mean experimental data (i.e., the sizes of each sampled compartment) for each group (NP-milk, NP-soy, HP-milk, and HP-soy) are depicted in Supplemental Fig. 2 (in the online version of this article): Nm content in ileal effluents (E, Supplemental Fig. 2A), plasma free AA (PL, Supplemental Fig. 2B), plasma proteins (SEP, Supplemental Fig. 2C), body urea (BU, Supplemental Fig. 2D), urinary urea (UU, Supplemental Fig. 2E), and urinary ammonia (UA, Supplemental Fig. 2F). Additional data collected concomitantly (like detailed AA plasma concentrations and ileal losses, net postprandial whole-body protein utilization, etc.) were previously reported elsewhere (42, 59).

Structural Modeling and Theoretical Identifiability

The structural modeling process led to the selection of a unique model as the minimum structure able to fit adequately the data of each group. The model thus selected (Fig. 1) consisted of 13 compartments representing distinct amounts of Nm and 21 different pathways of exchange between these compartments, each characterized by a constant diffusion coefficient $k_{i,j}$, representing the fraction of Nm in compartment $j$ transferred to compartment $i$ per minute. The chosen model was linear, i.e., characterized by first-order kinetics of Nm transfer between compartments, while certain metabolic phenomena of the modeled system were most probably nonlinear. Although nonlinear transfer laws (e.g., saturable laws) may have been physiologically relevant when considering certain metabolic processes, it was not necessary—on parsimonious grounds—to make the model and corresponding equations nonlinear and more complex, because the modeling results were judged a posteriori satisfactory (19). The selected model integrated the six sampled compartments, as well as seven other compartments that were not monitored experimentally, including Nm in the gastric lumen (G), Nm in the lumen of the proximal (ILP), and distal (ILD) small intestine, Nm in the splanchic (SA) and peripheral (PA) free AA, and Nm in the splanchic (SP) and peripheral (PP) proteins. The specific physiological entity of each nonsampled compartment was assigned with confidence on the basis of both structural (particular location and existing pathways of exchange with neighborhood and sampled compartments) and quantitative (Nm sizes and Nm fluxes from and toward nonsampled compartments) considerations. Taken together, these structural and quantitative characteristics signed the physiological meaning of each unsampled compartment.

This model was selected from the different candidates depicted in Supplemental Fig. 1 (in the online version of this article). Whatever the group, Akaike and Schwarz criteria and ratio tests all led to selection of the chosen model, as is shown in Supplemental Table 1 (in the online version of this article). In addition, using the recently developed differential algebra algorithm (7, 67), the selected model was shown to be uniquely identifiable (i.e., all of its parameters had a unique solution in a theoretically ideal context of noise-free and continuous time measurements), on condition that the transfer rates from SA to ILP and SA to ILD were equal. An equality constraint ($k_{2.5} = k_{3.5}$, see Fig. 1) was thus imposed to ensure an a priori identifiability of the model, this condition being consistent with our current knowledge of the physiological system.

Parameter Estimation and Numerical Validation

For each group (NP-milk, NP-soy, HP-milk, and HP-soy), the model was numerically identified using parameter estimation, and its numerical identifiability was tested successively. The optimization criteria are shown in the supplemental information (see Supplemental Table 2 online), and parameter estimate values are given in Table 1. Whatever the group and sampled compartment, goodness-of-fit appeared to be very...
acceptable from visual inspection of a plot of model predictions vs. experimental data (see Supplemental Fig. 2 online), the residuals were generally consistent with the hypothesis of normality and randomness (data not shown), and the percentage variations explained ($R^2$) were always superior to 95%, which indicates that the model predictions were highly satisfactory (see Supplemental Table 2 online). In addition, as shown in Table 1, the parameter values were estimated with good precision, since most coefficients of variation were less than 50%, the mean coefficient of variation varying between than 50%, the mean coefficient of variation varying between 15% and 20%, depending on the group.

**Model Predictions: Nm Fluxes of Intestinal Absorption, Splanchnic Extraction, and Peripheral Delivery**

As represented in Fig. 2, the model enabled the simulation of successive transfers of Nm between compartments, and in particular, the Nm fluxes of intestinal absorption ($\text{flux}_{S2} + \text{flux}_{S3} = k_{S2} \times \text{ILP} + k_{S3} \times \text{ILD}$, Fig. 2A), splanchnic anabolic use ($\text{flux}_{S8} + \text{flux}_{S9} = k_{S8} \times \text{SA} + k_{S9} \times \text{SA}$, Fig. 2B), splanchnic catabolic use ($\text{flux}_{S11} = k_{S11} \times \text{SA}$, Fig. 2C), and peripheral delivery ($\text{flux}_{S12} = k_{S12} \times \text{SA}$, Fig. 2D). The predicted Nm fluxes of intestinal absorption, splanchnic anabolic and catabolic use, and peripheral delivery, were all modulated by both the protein level in the habitual diet (NP or HP) and the protein source in the test meal (milk or soy): they were slightly accelerated after prior HP vs. NP adaptation and more markedly accelerated after soy vs. milk protein ingestion, by extents and mechanisms that are detailed below.

**Gastrointestinal kinetics.** After prior HP vs. NP adaptation, and whatever the protein source in the meal, the gastric emptying of Nm was slowed down (half-time delayed by 10–15 min, Table 2), although its further absorption from the intestinal lumen was, nevertheless, accelerated (half-time accelerated by ~7–14 min, Table 2). Concurrently, the contribution of the proximal intestine to total intestinal absorption was
higher after prior HP (~85%) than NP (~75%) adaptation (Table 2). In addition, whatever the adaptation diet, the kinetics of gastric emptying and intestinal transit and absorption at both the proximal and distal levels were all more rapid after the ingestion of soy vs. milk proteins (half-time of gastric emptying and intestinal absorption accelerated by ~20 and 40 min, respectively, Table 2). In consequence, and as represented in Fig. 2A, the splanchnic influx from intestinal absorption into the splanchnic free AA (SA) compartment (calculated as the sum of SA influxes from both proximal and distal absorption) was slightly accelerated after prior HP vs. NP adaptation, with a peak that was ~20 min earlier whatever the test meal, and was also more markedly accelerated after soy vs. milk protein ingestion, with a peak ~30 min earlier, whatever the adaptation diets. In addition, the peak value was also higher after soy vs. milk protein ingestion (~25% vs. 20% of the Nm dose per hour), since the Nm splanchnic influx occurred more suddenly after soy protein ingestion with a more dramatic but shorter peak that was more marked, with a greater and more prolonged increase after soy vs. milk protein ingestion whatever the adaptation level. In particular, the splanchnic efflux for urea production (Fig. 2C) was very similar to the splanchnic influx from intestinal absorption (Fig. 2A), both of them exhibiting a significant, but only transient (between 0.5 h and 2.5 h postmeal), increase after soy vs. milk protein ingestion. In contrast, the splanchnic efflux for urea production (Fig. 2C) exhibited significant differences between groups that were much more marked, with a greater and more prolonged increase after soy vs. milk protein ingestion but also after prior HP vs. NP adaptation.

Transfer to peripheral tissues. The kinetics of Nm release to peripheral tissues resulted from both the previous Nm gastrointestinal kinetics and the splanchnic handling of this Nm influx. In consequence, the flux of Nm peripheral delivery (i.e., the flux of splanchnic escape), which is represented in Fig. 2D, was accelerated after soy vs. milk protein ingestion (peak ~35 min earlier) and, to a lesser extent, after prior HP vs. NP adaptation (peak ~25 min earlier). In addition, this peripheral delivery of Nm was also lower after prior HP vs. NP adaptation (between 1.5 h and 7.5 h postmeal), this lowering effect being more important after soy than after milk protein ingestion.

### Table 1. Parameter estimates and their precision

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NP-milk</th>
<th>HP-milk</th>
<th>NP-soy</th>
<th>HP-soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{2,1}$</td>
<td>7.82E-03 (-)</td>
<td>6.64E-03 (-)</td>
<td>1.00E-02 (-)</td>
<td>8.76E-03 (-)</td>
</tr>
<tr>
<td>$k_{2,5}$</td>
<td>1.70E-03 (-)</td>
<td>1.07E-03 (-)</td>
<td>1.70E-03 (-)</td>
<td>1.70E-03 (-)</td>
</tr>
<tr>
<td>$k_{2,12}$</td>
<td>3.64E-03 (-)</td>
<td>3.60E-03 (-)</td>
<td>5.45E-03 (-)</td>
<td>6.14E-03 (-)</td>
</tr>
<tr>
<td>$k_{3,5}$</td>
<td>1.70E-03 (-)</td>
<td>1.70E-03 (-)</td>
<td>1.70E-03 (-)</td>
<td>1.70E-03 (-)</td>
</tr>
<tr>
<td>$k_{4,3}$</td>
<td>3.32E-03 (-)</td>
<td>3.66E-03 (-)</td>
<td>5.33E-03 (-)</td>
<td>5.71E-03 (-)</td>
</tr>
<tr>
<td>$k_{5,2}$</td>
<td>8.86E-03 (-)</td>
<td>1.43E-02 (-)</td>
<td>1.24E-02 (-)</td>
<td>2.16E-02 (-)</td>
</tr>
<tr>
<td>$k_{5,3}$</td>
<td>1.42E-02 (-)</td>
<td>9.29E-03 (-)</td>
<td>1.27E-02 (-)</td>
<td>8.09E-03 (-)</td>
</tr>
<tr>
<td>$k_{6,6}$</td>
<td>4.50E-04 (-)</td>
<td>4.50E-04 (-)</td>
<td>4.50E-04 (-)</td>
<td>4.50E-04 (-)</td>
</tr>
<tr>
<td>$k_{8,8}$</td>
<td>1.40E-04 (-)</td>
<td>1.40E-04 (-)</td>
<td>1.40E-04 (-)</td>
<td>1.40E-04 (-)</td>
</tr>
<tr>
<td>$k_{11,6}$</td>
<td>3.17E-04 (-)</td>
<td>3.11E-04 (-)</td>
<td>1.31E-03 (-)</td>
<td>6.66E-04 (-)</td>
</tr>
<tr>
<td>$k_{5,2}$</td>
<td>3.19E-02 (-)</td>
<td>3.86E-02 (-)</td>
<td>3.45E-02 (-)</td>
<td>3.70E-02 (-)</td>
</tr>
<tr>
<td>$k_{5,6}$</td>
<td>7.72E-03 (-)</td>
<td>7.72E-03 (-)</td>
<td>7.72E-03 (-)</td>
<td>7.72E-03 (-)</td>
</tr>
<tr>
<td>$k_{7,8}$</td>
<td>2.43E-01 (-)</td>
<td>2.28E-01 (-)</td>
<td>1.60E-01 (-)</td>
<td>1.71E-01 (-)</td>
</tr>
<tr>
<td>$k_{7,10}$</td>
<td>8.00E-04 (-)</td>
<td>8.00E-04 (-)</td>
<td>8.00E-04 (-)</td>
<td>8.00E-04 (-)</td>
</tr>
<tr>
<td>$k_{8,8}$</td>
<td>4.46E-02 (-)</td>
<td>5.80E-02 (-)</td>
<td>5.70E-02 (-)</td>
<td>6.80E-02 (-)</td>
</tr>
<tr>
<td>$k_{9,5}$</td>
<td>9.75E-03 (-)</td>
<td>1.23E-02 (-)</td>
<td>1.25E-02 (-)</td>
<td>1.58E-02 (-)</td>
</tr>
<tr>
<td>$k_{10,7}$</td>
<td>8.58E-01 (-)</td>
<td>1.09E-01 (-)</td>
<td>1.01E-01 (-)</td>
<td>7.89E-03 (-)</td>
</tr>
<tr>
<td>$k_{11,5}$</td>
<td>2.81E-02 (-)</td>
<td>4.45E-02 (-)</td>
<td>4.33E-02 (-)</td>
<td>6.91E-02 (-)</td>
</tr>
<tr>
<td>$k_{12,11}$</td>
<td>1.73E-03 (-)</td>
<td>1.70E-03 (-)</td>
<td>1.46E-03 (-)</td>
<td>1.59E-03 (-)</td>
</tr>
<tr>
<td>$k_{13,5}$</td>
<td>3.05E-04 (-)</td>
<td>3.86E-04 (-)</td>
<td>3.71E-04 (-)</td>
<td>7.66E-04 (-)</td>
</tr>
<tr>
<td>$k_{13,7}$</td>
<td>3.92E-05 (-)</td>
<td>1.73E-04 (-)</td>
<td>3.93E-05 (-)</td>
<td>1.13E-04 (-)</td>
</tr>
<tr>
<td>LAG</td>
<td>30.6</td>
<td>29.6</td>
<td>31.4</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Parameter estimate values: $k_{ij}$ in min$^{-1}$ and LAG (delay value) in min (SDs expressed as coefficients of variation [(SD/value)$\times$100]). Values obtained after optimization using the mean of individual data for each group. Subjects in the milk ($n = 12$) or soy ($n = 8$) groups twice ingested a mixed milk or soy protein meal providing 4.85 mmol N/kg body wt, respectively, the first time after a 7-day adaptation to a normal protein diet (NP-milk and NP-soy groups, respectively) and the second time after 7-day adaptation to a high-protein diet (HP-milk and HP-soy group, respectively). Parameters for which no CV are reported were maintained constant throughout the final step of optimization due to multicollinearity features.

regarding the kinetics of these SA effluxes reflected those in the SA influx from intestinal absorption: acute Nm orientation through both anabolic and catabolic splanchnic pathways was slightly accelerated after prior HP vs. NP adaptation whatever the test meal and was also more markedly accelerated after soy vs. milk protein ingestion whatever the adaptation level. In particular, the splanchnic efflux for protein synthesis (Fig. 2B) was very similar to the splanchnic influx from intestinal absorption (Fig. 2A), both of them exhibiting a significant, but only transient (between 0.5 h and 2.5 h postmeal), increase after soy vs. milk protein ingestion. In contrast, the splanchnic efflux for urea production (Fig. 2C) exhibited significant differences between groups that were much more marked, with a greater and more prolonged increase after soy vs. milk protein ingestion but also after prior HP vs. NP adaptation.
8 h was modulated by the protein source and level (Table 2): HP vs. NP adaptation resulted in increased urinary losses and lowered peripheral accretion of Nm that were more marked when the meal protein was soy than milk, while neither the protein source nor level significantly affected the Nm splanchnic accretion.

The model indeed enabled the simulation of Nm kinetics in nonsampled compartments, as reported in Supplemental Fig. 3 (see the online version of this article). In particular, the kinetics of Nm incorporation in splanchic protein (SP = SCP + SEP; see Supplemental Fig. 3D online) were only transiently affected by the protein source and were unaffected by the prior adaptation level. The kinetics of Nm incorporation in peripheral protein (PP; see Supplemental Fig. 3F online) exhibited a significant interaction between the protein level and source: they were significantly lowered in one of the four groups, when the protein level was HP and the protein source was soy. In addition, the kinetics of Nm incorporation in splanchic and peripheral proteins were affected differently by the protein source: after soy vs. milk ingestion, Nm incorporation in proteins was temporarily stimulated in the splanchic bed during the first postprandial hours (see Supplemental Fig. 3D online), while it was either unaffected (under NP conditions) or lowered (under HP conditions) in the peripheral area during the later postprandial period (see Supplemental Fig. 3F online).

Furthermore, some indices were calculated at the end of the postprandial period (8 h) to summarize the postprandial metabolic fate of dietary proteins and provide a concise overview of their regional anabolic and catabolic utilization in all groups (Table 2). The regional indices of Nm accretion represented the effective incorporation of Nm in the proteins of each zone after 8 h. These indices of Nm accretion were found to range from ~40% to 45% of the Nm dose in the splanchic bed and from ~15% to 25% of the Nm dose in the peripheral area (Table 2). The peripheral Nm accretion was significantly modulated in one case, when the protein level was HP and the protein source was soy (in that case, peripheral Nm accretion was 25% lower when comparing the protein levels, i.e., for HP vs. NP in the case of soy protein ingestion, and 20% lower when comparing the protein sources, i.e., for soy vs. milk in the case of prior HP adaptation). In contrast, neither the ingestion of soy vs. milk proteins nor adaptation to prior HP vs. NP significantly affected the splanchic accretion of Nm, but these two qualitative (soy vs. milk) and quantitative (HP vs. NP) nutritional modulations both increased the splanchic catabolic use of dietary AA for urea production, which over the 8-h postprandial period reached 22, 26, 28, and 34% of the Nm dose in the NP-milk, HP-milk, NP-soy, and HP-soy groups, respectively. The catabolic use of Nm was thus ~30% higher after soy vs. milk protein ingestion and was also enhanced by ~20% after

**Fig. 2.** Predicted values for the meal nitrogen (Nm) fluxes of intestinal absorption (flux5,3 + flux5,5 = k5,3 × ILP + k5,5 × ILG, A) splanchic anabolic use (flux8,5 + flux5,5 = k8,5 × SA + k5,5 × SA; B), splanchic catabolic use (flux11,5 = k11,5 × SA; C) and peripheral delivery (flux6,5 = k6,5 × SA; D) after the repeated ingestion of a mixed milk or soy protein meal (4.85 mmol N/kg) in humans, the first time after a normal protein diet adaptation (NP-milk and NP-soy groups, respectively) and the second time after high-protein diet adaptation (HP-milk and HP-soy group, respectively). Model predictions (lines: black line, NP-milk; gray line, NP-soy; dashed black line, HP-milk; dashed gray line, HP-soy) are expressed as % of the Nm dose per hour. The main effects of time, protein (P: meal protein source) and diet (D: diet protein level) and interactions between them were tested (repeated-measures mixed model). Effects of time, time × P and time × D were significant (P < 0.0001) whatever the variable, in contrast with the effects of P, D, and P × D that are reported on the corresponding panel when significant (until P < 0.1), * and #Effect of P (soy vs. milk) whatever D and the effect of D (HP vs. NP) whatever P, respectively (P < 0.05, Bonferroni post hoc tests at different time points).
Model predictions for the gastrointestinal kinetics and postprandial metabolism of meal nitrogen and comparison with findings in the literature

<table>
<thead>
<tr>
<th>Main Postprandial Characteristics in Each Model Subsystem</th>
<th>NP-Milk</th>
<th>HP-Milk</th>
<th>NP-Soy</th>
<th>HP-Soy</th>
<th>Findings in Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrointestinal kinetics of Nm in the gastrointestinal tract subsystem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-time values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric emptying, min</td>
<td>89 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69 ± 9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>79 ± 6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60–120&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intestinal absorption, min</td>
<td>180 ± 9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>173 ± 9&lt;sup&gt;o&lt;/sup&gt;</td>
<td>144 ± 7&lt;sup&gt;h&lt;/sup&gt;</td>
<td>130 ± 8</td>
<td></td>
</tr>
<tr>
<td>Postprandial (8 h) values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal absorption, % of the Nm dose</td>
<td>90 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Proximal absorption, % of the Nm dose</td>
<td>68 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75 ± 3&lt;sup&gt;h&lt;/sup&gt;</td>
<td>68 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60–70&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proximal absorption, % of total absorption</td>
<td>75 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85 ± 1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>76 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86 ± 1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>75–85&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Postprandial (8 h) orientation of Nm through urea metabolism in the deamination subsystem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea losses, % of the Nm dose</td>
<td>20 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Urea production, % of the Nm dose</td>
<td>22 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26 ± 1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>28 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Urea recycling, % of the Nm dose</td>
<td>2 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Urea recycling efficiency, %</td>
<td>8 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10–50&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Postprandial (8 h) incorporation of Nm in body proteins in the retention subsystem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splanchnic accretion, % of the Nm dose</td>
<td>41 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15–50&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peripheral accretion, % of the Nm dose</td>
<td>20 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18–38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Model predictions and findings in the literature on the gastrointestinal kinetics and postprandial metabolic use of Nm. Predicted values were obtained after optimization using the mean of individual data for each group. Subjects in the milk (n = 12) or soy (n = 8) groups had twice ingested a mixed milk or soy protein meal providing 4.85 mmol N/kg body wt, respectively, first after a 7-day adaptation to a normal protein diet (NP-milk and NP-soy groups, respectively) and then after a 7-day adaptation to a high-protein diet (HP-milk and HP-soy group, respectively). Predicted gastrointestinal kinetics of meal nitrogen (Nm): half-time values (in min) for gastric emptying and intestinal absorption [times necessary to achieve 50% of \(\int_0^t k_2 \cdot G(t) \cdot dt\) and \(\int_0^t k_3 \cdot \Delta L(t) \cdot dt + \int_0^t k_5 \cdot \Delta L(t) \cdot dt\), respectively, cumulated values at 8 h for total and proximal absorption (in % of the Nm dose), and the relative contribution of the proximal intestine to total absorption over 8 h (in %). Observed deamination losses of Nm after 8 h (body urea + urinary urea (BU + UU)) and predicted catabolic use resulting in these losses (in % of the Nm dose): dietary urea N losses are equal to the amounts of urea produced [\(\int_0^t k_1 \cdot \Delta S(t) \cdot dt\)] minus that salvaged by enterohepatic recycling [\(\int_0^t k_{11} \cdot \Delta U(t) \cdot dt\)], with urea recycling efficiency calculated as the fraction of the urea produced that had been recycled (in %). Predicted regional accretion of Nm over the postprandial period (in % of the Nm dose): incorporation in regional protein at 8 h [splanchnic constitutive protein + splanchnic-exported protein (SCP + SEP) in the splanchnic bed and peripheral proteins (PP) in the peripheral area)]. Values with different letters within rows for a specific variable are statistically significantly different from each other (for half-time values: t-tests, \(P < 0.05\); for 8-h postprandial values: mixed models for repeated-measures analysis, \(P < 0.05\)). Values with different letters (a–d) within rows for a specific variable are statistically significantly different from each other. Range of variation in literature values (min-max) for \(t\)-half-time of gastric emptying of dietary proteins after mixed meals in humans (10), \(t\)-proximal (duodeno-jejunal) absorption of protein in miniature pigs (27) and humans (44), \(t\)-contribution of the proximal intestine to the total intestinal absorption of Nm in dogs (83) and humans (44), \(t\)-urea recycling efficiency in humans (6, 30, 50, 56), \(t\)-splanchnic anabolic use of dietary amino acids or proteins in pigs or pigeons (25, 72, 73), and \(t\)-peripheral anabolic use of dietary proteins in rats (60, 61).

prior HP vs. NP adaptation. Meanwhile, the concomitant enterohepatic recycling of urea permitted reincorporation of a fraction of the urea N produced in SA, which reached 8, 5, 25, and 15% of the urea produced in the NP-milk, HP-milk, NP-soy, and HP-soy groups, respectively (Table 2): this was enhanced after soy vs. milk protein ingestion but lowered after HP vs. NP adaptation. Postprandial losses of dietary urea N, which amounted to the quantity produced minus that salvaged by enterohepatic recycling, were finally still higher with the soy vs. milk protein meal but only under HP conditions, and were enhanced by up to 40% after prior HP vs. NP adaptation for the soy test meal.

External Validation, Discriminatory Capacity, and Sensitivity Analysis of the Model

Finally, an external validation ensured the physiological relevance of the model, and the discriminatory and sensitivity analyses proved the model’s ability to localize and explain the mechanisms involved in the between-groups differences in Nm metabolic utilization.

Quantitative assessment of model validity was thus completed with an external validation of the model. Model simulations for the nonsampled compartments were strictly validated to ensure that they captured most known features of the postprandial Nm metabolism. As summarized in Table 2, the range of variation covered by the model predictions for all groups was strikingly in line with corresponding findings available in the literature regarding gastrointestinal kinetics (10, 27, 44, 83), and both the splanchnic (11, 25, 72, 73) and peripheral (17, 29, 60, 61) metabolism of Nm.

We also tested the discriminatory capacity of the model using discriminant analysis. We verified thereby that subjects in the same group were posteriori correctly grouped together, the percentage of subjects correctly replaced determining the discriminatory capacity of the model. Whatever the comparison (i.e., pairwise comparisons or comparing the four groups together), 100% of subjects were correctly replaced in their original group. This discriminant analysis further indicated that the parameters accounting for most of the difference between meals were the following: \(k_{2.1}\) and \(k_{5.2}\), the rates of Nm gastric emptying and proximal intestinal absorption, respectively, \(k_{5.5}\), and \(k_{1.1}\), the rates of Nm incorporation in splanchnic-exported proteins and utilization for urea production in the splanchnic bed, respectively, and \(k_{6.5}\) and \(k_{7.6}\), the rates of splanchnic escape of Nm and its following uptake by peripheral tissues, respectively. The analysis showed that groups were fully discriminated on the basis of their model parameter values for the rates of proximal intestinal absorption (\(k_{5.2}\), urea production
(k_{11,5}), and peripheral uptake (k_{7,0}), as shown in Supplemental Fig. 4 (in the online version of this article). The model thus constituted an explanatory tool that was capable of localizing the processes involved in the differential metabolic utilization of Nm between the four groups.

Furthermore, the results of the sensitivity analysis (data not shown), which enabled identification of those parameters with the greatest influence on the system, agreed with both the results of the discriminant analysis and our knowledge of the system behavior. Whatever the compartment, the gastric emptying rate of Nm (k_{2,1}), and to a lesser extent, its rate of absorption in the proximal intestine (k_{5,2}), exhibited a considerable initial influence, which then slowly declined, while the rate of Nm absorption in the distal intestine (k_{5,3}) exhibited a weaker influence, although increasing over time and finally reaching that of the proximal absorption rate (k_{5,2}). Moreover, with respect to the influence of parameters on regional protein pools (i.e., SCP, SEP, and PP), the rates of Nm incorporation in splanchnic constitutive (k_{8,5}), splanchnic export (k_{9,5}), and peripheral (k_{10,7}) proteins, as well as the rates of Nm delivery to the periphery (k_{6,5}) and transfer to body urea (k_{1,1,5}), were also identified as important governing parameters. Splanchnic proteins were positively sensitive to variations in their protein synthesis rates (k_{6,5} and k_{9,5} for SCP and SEP, respectively), while they were negatively sensitive to variations in urea production (k_{11,5}) and peripheral delivery (k_{6,5}) in descending order. Moreover, peripheral proteins were positively sensitive to variations in rates of both peripheral protein delivery (k_{6,5}) and synthesis (k_{10,7}), in descending order, and negatively to those affecting urea production (k_{11,5}) and splanchnic constitutive protein synthesis (k_{8,5}), in descending order.

Identification of Factors Regulating Postprandial Nm Metabolism

When analyzing the relationships between the absorption kinetics of Nm and its further regional metabolic fate, it emerged that a faster Nm absorption was associated with its higher catabolic use in the splanchnic bed and its lesser peripheral delivery. As depicted in Fig. 3, the acute, dynamic effects of the absorption kinetics of Nm on its further splanchnic metabolism were investigated in all individuals by examining the relationships between the individual rates of Nm absorption in the proximal intestine (k_{5,2}), splanchnic use for urea production (k_{11,5}), and splanchnic use for protein synthesis (k_{8,5} + k_{9,5}). A highly positive linear relationship was found between the rates of absorption and catabolic use (r = 0.94; P < 0.01), while the rate of anabolic use increased linearly with that of catabolic use (r = 0.87; P < 0.05). The global effects of these acute modulations of Nm absorption kinetics occurring during the first postmeal hours on the balance of Nm splanchnic utilization at the end of the postprandial phase (8 h splanchnic catabolic use, anabolic use and escape) were investigated in all individuals using a correlation and a regression analysis (Table 3). There was a strong negative association between 8-h splanchnic catabolic use and the absorption halftime, and a weaker but reasonable positive correlation between 8-h splanchnic escape and the absorption halftime. This indicated that a rapid absorption of dietary protein was associated with its higher catabolic use in the splanchnic bed and its lesser peripheral delivery over the postprandial period. When investigating relations between all of these variables taken together (multiple linear regression analyses), the overall R^2 was 0.96 and 0.87 for 8-h splanchnic catabolic use and splanchnic escape, with two statistically significant parameters remaining in the model, including the absorption halftime. As indicated by the partial R^2, 77% of the total variation in splanchnic catabolic use could be accounted for by variations in absorption kinetics, while, in turn, 74% of the total variation in peripheral delivery could be accounted for by variations in splanchnic catabolic use. By contrast, final 8-h splanchnic anabolic use was not significantly correlated with any of these variables, despite the above-mentioned correlation between the
Overall, the plausibility and physiological relevance of the model were strongly supported by the consistency of its predictions with respect to our current knowledge of the system (external validation, Table 2). For instance, whatever the nutritional conditions, around 80% of Nm absorption, was predicted to occur in the upper part of the gastrointestinal tract, in line with the idea that Nm is principally absorbed in the proximal part of the small intestine (43, 44, 83). The model also predicted that meal ingestion gave rise to a strong splanchnic anabolic response, since ~70% of the total 8-h postprandial accretion of Nm concerned splanchnic proteins, which is consistent with our current knowledge of the system (1, 17, 71) and previous findings of compartmental modeling of Nm postprandial interorgan metabolism (32, 33, 36). This result is in agreement with the idea that the acute anabolic effect of a mixed meal occurs primarily in the splanchnic area (24, 60, 72, 77), while muscle protein synthesis only makes a minor contribution to the whole-body postprandial anabolic response, despite the large mass of muscle (57, 68, 80). In addition, the model predicted a recycling efficiency of dietary urea N ranging from 5% to 25% at the end of the postprandial period, in line with previous findings in the literature (6, 30, 50, 56).

**DISCUSSION**

During this study, we investigated the main factors that could explain the combined effects of the protein source in the meal and the prevailing protein level in the diet on postprandial interorgan Nm metabolism in humans. Using an integrative modeling approach, gastrointestinal kinetics emerged as the key regulating factor in response to both qualitative and quantitative variations in protein intake. Gastrointestinal kinetics impacted the ensuing splanchnic and peripheral metabolism of dietary AA and explained most of the single and combined effects of qualitative and quantitative variations in the protein intake on these variables.

**Physiological Relevance of Model Predictions on Nm Absorption and Metabolism**

### Table 3. Correlation analysis and multiple linear regression models for relations between the absorption kinetics of meal nitrogen (Nm) and its further postprandial metabolic balance over 8 postprandial hours

<table>
<thead>
<tr>
<th>Variable</th>
<th>Splanchnic Catabolic Use</th>
<th>Splanchnic Anabolic Use</th>
<th>Splanchnic Escape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption half-time</td>
<td>$r = -0.88^* (1st)$ R² = 0.77*</td>
<td>$r = -0.27$ nil</td>
<td>$r = 0.57^* (2nd)$ R² = 0.13*</td>
</tr>
<tr>
<td>Splanchnic catabolic use</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Splanchnic anabolic use</td>
<td>$r = -0.05$ nil</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>Splanchnic escape</td>
<td>$r = -0.86^* (2nd)$ R² = 0.19*</td>
<td>0.20</td>
<td>1</td>
</tr>
</tbody>
</table>

Cumulative R² = 0.96* cumulative R² = 0.87*

Relations between the absorption half-time of Nm (4.85 mmol N/kg body wt) and its further splanchnic catabolic use, splanchnic anabolic use, and splanchnic escape at the end of the postprandial period (8 h) in all the individuals. For each individual in each group, the absorption half-time of Nm was calculated as the time necessary to achieve 50% of $\int_0^t k_{11.5}\cdot SA(t)\cdot dt + \int_0^t k_{3.5}\cdot IL(t)\cdot dt$, and the postprandial splanchnic catabolic use, anabolic use, and escape of Nm were calculated as $\int_0^t k_{11.5}\cdot SA(t)\cdot dt$, $\int_0^t (k_{3.5}+k_{0.5})\cdot SA(t)\cdot dt$ and $\int_0^t k_{0.5}\cdot SA(t)\cdot dt$, respectively. Pearson correlation coefficients ($r$) were calculated (PROC CORR procedure of SAS) to assess the relation between pairs of variables, and multiple linear regression analyses were used (PROC REG procedure of SAS) to assess the relation between all the variables taken together. For multiple linear regression, variables correlated with the dependent variable were included, and stepwise regression was used to select the minimum number of variables explaining the variability in the dependent variable. Cumulative R² (coefficient of variation) indicated how much of the total variation in the dependent variable could be accounted for by the function. The overall R² was 0.96 and 0.87 for splanchnic catabolic use and escape, with two statistically significant parameters remaining in the model, including the absorption half-time. In contrast, splanchnic anabolic use was not significantly correlated with any of these variables. ni, not included in the final regression model (for reasons of either insufficient correlation or multicollinearity). *P < 0.001.

**Kinetics of Absorption: Modulation and Impact on Ensuing Splanchnic Metabolism**

Gastrointestinal kinetics were predicted to be an important factor of regulation that would modulate the wave of entry of Nm into the body as a function of meal protein source and diet protein level. After soy vs. milk protein ingestion, the kinetics of Nm gastric emptying, intestinal transit, and absorption were all more rapid, in line with previous results (15, 36). After HP vs. NP adaptation, the gastric emptying of Nm was slowed down, while its further absorption was facilitated and accelerated in the proximal intestine. As a result of these complex, acute, and chronic modulations of gastrointestinal kinetics, the resulting Nm splanchnic influx was markedly affected by the meal protein source, while it was more moderately affected by the diet protein level: the splanchnic influx occurred more abruptly and displayed a more dramatic, but shorter, increase after soy vs. milk protein ingestion, while it was slightly accelerated after HP vs. NP adaptation (Fig. 2A).

When examining splanchnic handling of the Nm influx, the differences between groups regarding the kinetics of both the anabolic (Fig. 2B) and catabolic (Fig. 2C) acute utilization of Nm reflected those of the influx availability of Nm from intestinal absorption (Fig. 2A). In particular, the splanchnic efflux for protein synthesis (Fig. 2B) was very similar to the splanchnic influx from intestinal absorption (Fig. 2A) and was similarly impacted by the protein source and level. By contrast, the splanchnic efflux for urea production (Fig. 2C) differed somewhat because a minor but significant part of the total variation in urea production could not be imputed to differences in absorption kinetics (namely, 30% and 20% of the stimulation occurring after soy vs. milk and HP vs. NP, respectively, as assessed when comparing the peak values of intestinal absorption and urea absorption half-time).
production fluxes among groups). These results clearly showed that the higher splanchnic catabolism of dietary AA from soy vs. milk proteins (15, 36, 38) had to be ascribed, in addition to differences in absorption kinetics, to certain differences in the AA composition of dietary proteins. The higher splanchnic catabolism after HP vs. NP was also in line with the role of the splanchnic zone in preventing sharp increases in blood ammonia and AA levels in the event of a high protein intake, by means of stimulating dietary AA catabolism (23, 46, 59).

Beyond these acute (soy vs. milk) and chronic (HP vs. NP) stimulations of splanchnic Nm catabolism, we showed that differences in Nm absorption kinetics between groups were further involved in differences in Nm splanchnic metabolic use and escape. First, sensitivity analysis indicated that the rates of Nm gastric emptying (k5,2) and intestinal absorption (k5,2 and k5,3) exerted a markedly positive initial influence on its splanchnic catabolism and accretion. Furthermore, from a dynamic point of view, a more rapid intestinal absorption of Nm induced greater rates of Nm both catabolic and anabolic use in the splanchnic bed (Fig. 3). From a static point of view at the end of the postprandial phase, the acute, dynamic effect of gastrointestinal kinetics could always be observed regarding splanchnic Nm catabolic use, but not splanchnic Nm anabolic use after 8 postmeal hours (Table 3). Finally, the rapid absorption of a dietary protein stimulated Nm catabolic use in the splanchnic bed and limited Nm peripheral delivery over the postprandial period, absorption kinetics being responsible for 77% of between-group variations in splanchnic catabolism, while, in turn, splanchnic catabolism was responsible for 74% of between-group variations in peripheral delivery (Table 3). Thus, the kinetics of intestinal absorption of a dietary protein exerted a durable impact on its splanchnic catabolic use and peripheral delivery, but its effects on splanchnic anabolic use and accretion were only moderate and transient. The importance of the absorption kinetics of dietary proteins on postprandial N metabolism has already been highlighted with respect to the concept of slow and fast proteins (14, 21). Rapidly absorbed AA arrive early and massively in the splanchnic zone after ingestion, which favors their local utilization for both anabolic and catabolic purposes, and reduces their peripheral delivery (15, 36, 38). The results of the present study are in line with these data but further identify splanchnic catabolism and escape, but not splanchnic anabolism, as the main variables that are steadily affected over the entire 8-h postprandial period by modulations of Nm absorption kinetics in response to acute qualitative changes in protein intake. Furthermore, our results reveal that modulations of Nm absorption kinetics are also involved in longer-term adaptation to chronic quantitative changes in protein intake.

Modulation of Urea Production and Enterohepatic Recycling by Protein Source and Level

After 8 postprandial hours, the splanchnic catabolic use of Nm was ~30% higher after soy vs. milk protein ingestion and was also enhanced by ~20% after prior HP vs. NP adaptation (Table 2). Meanwhile, the concomitant enterohepatic recycling of urea, which allowed reincorporation in splanchnic free AA of a fraction of the dietary urea N produced, was enhanced (by 250–400%) after soy vs. milk protein ingestion but reduced (by 30–50%) after HP vs. NP adaptation. All of these results are strictly in line with our recent findings using a different modeling approach for both total and dietary N metabolism (35), as well as previous classical tracer studies showing that both the production and recycling of urea increase after the ingestion of soy vs. milk proteins (25, 52) and showing that urea production increases (30, 81) but urea recycling decreases (30, 50, 66) when the protein level in the diet increases. Our results show that enterohepatic recycling contributed to alleviating the higher deamination of soy vs. milk proteins but also to increasing the difference in deamination after prior HP vs. NP adaptation. Thus, when the dietary protein supply was moderate, this N-sparing mechanism was efficient enough to almost totally counterbalance the higher level of dietary urea N production after the ingestion of soy vs. milk proteins, but this was no longer the case after switching to the HP diet.

Modulation of Peripheral Nm Metabolism

In peripheral tissues, the effects of protein quality and quantity on Nm delivery kinetics (Fig. 2D) were similar (albeit weakened) to those previously found for the Nm splanchnic influx (Fig. 2A). Furthermore, whatever the nutritional conditions, the same proportion of dietary AA that had been delivered to the periphery was incorporated in proteins after eight postprandial hours. Therefore, the peripheral anabolic use of Nm was mainly driven by the amount of dietary AA escaping splanchnic extraction and made available to peripheral tissues. Consistent with our model predictions, it has been reported elsewhere that peripheral AA availability is one of the principal determinants of muscle protein synthesis, due to both an increase in substrate availability and a stimulation of the signaling pathways regulating protein synthesis (3, 8, 12, 13, 39, 75, 78, 82). Moreover, our results are in line with the very slight overall influence of a high-protein intake on muscle protein synthesis (2, 37, 51, 55, 60, 74), despite some discrepancies in the literature (37, 45, 51, 74).

Impact of Protein Source and Level on the Regional Anabolic Use of Dietary Protein

Nm regional accretion, calculated as the final incorporation of Nm in proteins at each regional level after 8 h (Table 2), ranged from ~40 to 45% of the Nm dose in the splanchnic bed and from ~15 to 20% of the Nm dose in the peripheral area (Table 2). Despite being transiently modulated during the first postmeal hours, the splanchnic accretion of Nm at the end of the postprandial period was unaffected by either of the two qualitative (soy vs. milk) or quantitative (HP vs. NP) nutritional modulations studied. In contrast, the 8-h-postprandial accretion of Nm was more variable in the peripheral area: it was 20% lower after soy vs. milk protein ingestion in the case of prior HP adaptation and 25% lower after prior HP vs. NP adaptation in the case of soy protein ingestion. To further explore the reliability of our conclusions based on the nutritional conditions studied here, Fig. 4 summarizes the 8-h balance of postprandial metabolic Nm utilization in the four groups of this study (NP-milk, NP-soy, HP-milk and HP-soy) alongside corresponding values recently obtained in two additional groups (NP-wheat and HP-wheat). These latter values
arose from a comparable compartmental analysis of experimental data collected using the same experimental design but with a test meal containing wheat protein (47). All of these results together confirmed that the 8-h splanchnic anabolic use of Nm remained remarkably stable whatever the dietary protein source and adaptation level, contrary to 8-h splanchnic catabolic use and 8-h peripheral anabolic use that varied in contrasting ways. Compared with animal protein, vegetable proteins were more catabolized in the splanchnic bed and less incorporated in peripheral protein, markedly so for wheat and to a lesser extent for soy (which is of better nutritional quality). Therefore, differences in peripheral anabolic Nm utilization explained most of the HP-induced gap between the nutritional values of dietary proteins. Lastly, the fact that splanchnic Nm anabolic use and accretion was modulated only transiently, with ultimately no change at the end of the postprandial period, constitutes a novel finding that opens new perspectives regarding our understanding of the function of the splanchnic organs within the interregional, homeodynamic, nutritional system. This point certainly deserves further examination.

Conclusions

The present findings provide evidence that absorption kinetics are a key factor in the regulation of postprandial protein metabolism in response to both qualitative and quantitative variations in protein intake. While confirming that the postprandial utilization of dietary protein is mainly under the control of kinetic factors, our results identify the regional components of postprandial protein metabolism that are strongly and durably impacted by absorption kinetics, namely, splanchnic catabolic use and peripheral delivery of Nm, but not its splanchnic anabolic use. The lesser sensitivity of splanchnic Nm anabolic than catabolic use to modulations in Nm absorption kinetics probably relates to the more limited capacities of splanchnic proteosynthesis than ureogenesis to deal with an AA excess, inasmuch as the protein intake satisfies or is above the requirements (16, 28). As a consequence, acceleration in Nm absorption, resulting in a sharp and massive Nm rise in the splanchnic free AA precursor pool, could lead to the saturation of splanchnic synthesis capacities and expose splanchnic AA to oxidation (69, 79), thus limiting their ensuing peripheral availability. This plausible mechanism explains well our predicted modulations in Nm regional metabolism in response to variations in Nm absorption kinetics. When compared with slowly absorbed milk protein, rapidly absorbed soy protein indeed stimulated the splanchnic extraction of Nm by markedly increasing its catabolic use but only transiently its anabolic use, resulting in its decreased peripheral availability and anabolic use. This phenomenon was also exacerbated after HP vs. NP adaptation, because of both the amplified acceleration of intestinal absorption and the amplified increase in the splanchnic catabolism of Nm from soy vs. milk protein. Finally, under HP conditions, this resulted in a 25% lower peripheral anabolic use of Nm from soy vs. milk proteins. We conclude that increasing the habitual protein intake further amplifies the differences in protein sources regarding their peripheral delivery and anabolic utilization.

![Fig. 4. Model-predicted postprandial (8 h) utilization of meal nitrogen (Nm) and its modulation by both the protein source in the meal and the habitual protein intake. Subjects in the milk, soy, and wheat groups twice ingested a mixed milk, soy, or wheat protein meal providing 4.85 mmol N/kg body wt, respectively, the first time after a normal protein diet adaptation (NP-milk, NP-soy, and NP-wheat groups, respectively) and the second time after high-protein diet adaptation (HP-milk, HP-soy, and HP-wheat groups, respectively). Values for the milk and soy groups were obtained during this study and compared with values for the wheat groups obtained during a previous study (47). Open bars denote postprandial utilization of Nm for splanchnic catabolic, splanchnic anabolic, and peripheral anabolic purposes at 8 h. Hatched bars denote Nm enterohepatic recycling into the metabolic N pool through urea hydrolysis at 8 h. Values were calculated as the integral over 8 h of the fluxes of Nm utilization for urea production [$\int_{0}^{8} k_{1,1} \cdot Bu(t) \cdot dt$], dietary urea N reincorporation in splanchnic free amino acids through urea N enterohepatic recycling [$\int_{0}^{8} k_{1,1} \cdot Bu(t) \cdot dt$], Nm utilization for the synthesis of splanchnic constitutive and exported proteins [$\int_{0}^{8} (k_{0,5} + k_{0,5}) \cdot S(a) \cdot dt$], and Nm utilization for the synthesis of peripheral proteins [$\int_{0}^{8} k_{0,5} \cdot PA(t) \cdot dt$]. For a description of the model, see Fig. 1. Model predictions for each group are mean of individual predictions ± SD, expressed as a percentage of the Nm dose. The meal protein source (P) and diet protein level (D) had significant effects (P < 0.01) on all variables except for splanchnic anabolic use (mixed models with diet as repeated factor). The P × D interaction had a significant effect on splanchnic urea recycling (P < 0.01) and peripheral anabolic use (P < 0.05). * and #Effect of P (soy vs. milk or wheat vs. both milk and soy) for a given D (NP or HP) and the effect of D (HP vs. NP) for a given P (milk or soy or wheat), respectively (P < 0.05, Tukey post hoc tests).
Perspectives and Significance

This was observed in normal subjects with sufficient protein intake, in which the homeodynamic control of postprandial Nm excursions was achieved through the preferential orientation of massive Nm influxes from intestinal absorption toward oxidative than anabolic pathways at the splanchnic branchpoint. Our conclusions would have probably been different under some other nutritional or physiopathological situations (protein-energy deficiency, catch-up growth, inflammation, sepsis, etc.), where there could be a preferential orientation of Nm toward anabolic rather than catabolic pathways, to spare more Nm to satisfy the increased metabolic demand. Likewise, the way and extent to which absorption kinetics impact the postprandial anabolism may vary during aging: a better anabolism is achieved with rapidly absorbed proteins in young people, in line with our results, but with slowly absorbed proteins in the elderly (22). Nevertheless, we believe that the key role played by the kinetics of dietary AA delivery from the gut in governing the impact of quantitative and qualitative variations in protein intake constitutes an important finding. Because absorption kinetics are located upstream and are regulated in an integrative manner by both acute and chronic factors, they may be the central effectors of the fine tuning of protein nutrition homeodynamics.

GRANTS

This work was supported by Danone (Paris, France).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


