Urea-nitrogen production and salvage are modulated by protein intake in fed humans: results of an oral stable-isotope-tracer protocol and compartmental modeling\textsuperscript{1–4}

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ABSTRACT

Background: The influence of protein source on postprandial urea kinetics is poorly understood, despite its nutritional significance with respect to nitrogen homeostasis. Furthermore, traditional tracer infusion studies underestimate acute postprandial change in urea kinetics.

Objective: We investigated postprandial, non–steady state urea kinetics and their modulation by qualitative and quantitative factors of protein intake by the combined use of robust clinical data on nitrogen postprandial distribution and mathematical modeling.

Design: In healthy subjects standardized to a normal protein intake for 7 d, dietary and total nitrogen kinetics were measured for 8 h in plasma proteins, body, and urinary urea after the ingestion of a \textsuperscript{15}N-labeled milk (n = 8), soy (n = 8), or wheat (n = 8) protein meal. In subjects who received the soy protein meal, these postprandial measurements were repeated after a further 7-d adaptation to a high protein intake. A 4-compartment model was developed to calculate from these data the postprandial kinetics of production, urinary excretion, and intestinal hydrolysis of urea nitrogen from both dietary and endogenous sources.

Results: Urinary urea excretion was not influenced by the protein source in the meal but was influenced by the protein level in the diet. By contrast, urea production and hydrolysis were higher when ingesting plant versus animal protein, together with a higher efficiency of urea hydrolysis (50–60% versus 25% of the urea produced being hydrolyzed, respectively).

Conclusions: We conclude that urea hydrolysis is an acute nitrogen-sparing mechanism that can counterbalance a postprandial higher urea production, and the efficiency of this recycling is higher when the usual protein intake is lower. Am J Clin Nutr 2008;87:1702–14.

INTRODUCTION

Urea, the primary end product of nitrogen and amino acid metabolism, is produced by the liver in larger amounts than is eliminated in the urine (1–3). Indeed, in normal conditions, \approx 20–30% of the urea synthesized is hydrolyzed by the action of bacterial urease in the gastrointestinal tract (1–11), leading to the production of ammonia that can be used as a nitrogen source for microbial protein synthesis or can be reabsorbed and made available for subsequent catabolic or anabolic purposes in the body (2, 12–14). Because this process provides substantial amounts of ammonia nitrogen available for amino acid biosynthesis (15, 16), urea hydrolysis may be a key step for the salvage of urea nitrogen, and it may play a central role in nitrogen homeostasis (4, 9, 13, 17, 18). The kinetics of urea production, excretion, and hydrolysis have been widely investigated in humans, as has their modulation by different physiopathologic and dietary factors, including physical activity (19, 20), pregnancy (5, 21), malnutrition and recovery (4, 22–24), and the prevailing protein intake (7, 18, 20, 25–27). However, it remains controversial whether control of the body nitrogen balance is achieved via changes in urea production in parallel with protein intake (25) or via a regulation of urea hydrolysis (27). In addition, few data are available regarding the influence of the dietary protein source on urea kinetics (28, 29), despite their importance for nitrogen homeostasis (14, 17). These knowledge gaps result from the lack of an accurate method for assessing acute changes in urea kinetics in the postprandial (non-steady) state.

In all the studies referred to above, urea kinetics were investigated using different tracer methods at steady state (1, 3, 10–12, 30). Early tracer studies used the single bolus method (1, 2), wherein a single dose of urea tracer is administered intravenously and the urea production rate is calculated by measuring the urea tracer disappearance from plasma and its cumulative appearance in the urine over a long-term period (commonly 24 or 48 h). In an attempt to rapidly attain an isotopic equilibrium in plasma, later investigations used a primed, constant intravenous tracer infusion (3, 6, 10, 11, 19, 23, 30–33). Other protocols, wherein labeled urea is given orally and urea kinetics are calculated from the plateau enrichment in urinary urea, have also been described (4, 12, 22, 34). However, it was shown several years ago that, although the primed, continuous tracer urea infusion method provides a valid measure of the urea production rate under basal steady state conditions, this method greatly underestimates...
short-term variations in urea production, even when Steele’s non–steady state equations were applied (8). Thus, in the scarce studies in which urea kinetics were investigated in the fed state (3, 6), the rate of urea nitrogen production might have been greatly underestimated, which would explain why these studies obtained such low (almost null) values when quantifying urea nitrogen hydrolysis during the feeding period (6).

In the present work, we developed a new method coupling a stable nitrogen isotope-tracer protocol with kinetic modeling to overcome these limitations. This approach accounts for short-term changes in urea kinetics during the normal postprandial situation. It distinguishes the postprandial kinetics of urea production, excretion, and hydrolysis, i.e., the proportion of urea produced that is not excreted in urine and can be recycled in the body. We have used this approach to assess the effects of varying the protein source in the meal and increasing its prevailing level in the diet on the urea kinetics in fed humans.

SUBJECTS AND METHODS

Our method consisted of 1) collecting experimental data in healthy adults subjected for 8 h to a postmeal investigation with measurement of nitrogen kinetics in certain accessible pools of blood and urine (plasma proteins, body urea, and urinary urea) after the ingestion of a mixed meal containing 15N-labeled protein, and 2) determining postprandial urea kinetics by further confronting the experimental data thus obtained with a mathematical model based on non–steady state equations. This experimental method using a 15N tracer has already been shown on many occasions to enable a determination of the postprandial kinetics of dietary and total (dietary + endogenous) nitrogen appearance in these nitrogen pools of blood and urine (35–38).

During this study, postmeal investigations were conducted in 3 groups receiving either milk (n = 8), soy (n = 8), or wheat (n = 8) proteins after prior 7-d adaptation to a normal intake of protein (1 g · kg−1 · d−1) and were moreover repeated after subsequent 7-d adaptation to a 2-fold higher protein intake (2 g · kg−1 · d−1) in subjects receiving the soy protein meal (n = 8).

Human subjects and protocol

Subjects and study design

Experimental data for this study were drawn from 2 investigations conducted in our laboratory over the past 4 y (35, 39). Briefly, healthy subjects (n = 24: 11 women, 13 men) aged 28 ± 5 y with a mean body mass index (in kg/m²) of 21.5 ± 2.5 first underwent dietary standardization for 7 d with a normal protein source in the test meal, which contained either milk (assigned to 1 of the 3 following groups according to the protein source in the test meal, which contained either milk (assigned to 1 of the 3 following groups according to the protein source in the test meal, which contained either milk (assigned to 1 of the 3 following groups according to the protein source in the test meal, which contained either milk (assigned to 1 of the 3 following groups according to the protein source). Subjects were studied for 8 postmeal hours. The subjects were randomly assigned to 1 of the 3 following groups according to the protein source in the test meal, which contained either milk (n = 8), soy (n = 8), or wheat (n = 8) protein (study 1, n = 24). Subjects having received the soy protein meal were then adapted for the next 7 d to a high-protein diet (HP: 2 g · kg−1 · d−1) and repeated the postprandial metabolic test at the end of this second adaptation period (day 16) by receiving the same test meal with soy protein (study 2, n = 8). The design and aims of the study were fully explained to each subject, and written informed consent was obtained. All procedures during the study were approved by the Institutional Review Board for St-Germain-en-Laye Hospital, France.

Adaptation diets

Both the NP and the HP adaptation diets were adjusted for body weight and were designed to be isocaloric (138 kJ · kg−1 · d−1). They supplied the same amount of carbohydrate (4.5 g · kg−1 · d−1), whereas an isoenergetic exchange occurred between protein (NP: 1 g · kg−1 · d−1; HP: 2 g · kg−1 · d−1) and fat (NP: 1.2 g · kg−1 · d−1; HP: 0.8 g · kg−1 · d−1). Total energy was distributed into 12% protein, 55% carbohydrate, and 33% fat for the NP diet compared with 24% protein, 54% carbohydrate, and 22% fat for the HP diet.

Test meals

The test meals were semisynthetic, liquid (milk or soy) or solid (wheat) mixed meals. They were adjusted for body weight and were isoenergetic, providing 46 kJ/kg body wt (ie, one-third of the previous daily energy intake). The composition of the test meals corresponded with dietary allowances: 15% of total energy as protein, 55% as carbohydrate (one-quarter in the form of sucrose and three-quarters in the form of maltodextrins), and 30% as fat (sunflower oil). The test meals provided 0.41 g/kg of protein (purified proteins, containing <0.5% of nonprotein nitrogen), 1.51 g/kg of carbohydrate, and 0.38 g/kg of fat. The mean amount of ingested nitrogen was 67.9 mg/kg. Proteins were uniformly and intrinsically 15N-labeled, as previously described (36–38).

Postprandial metabolic test

The subjects were admitted to the hospital on the morning of days 8 (all subjects, study 1) and 16 (8 of the subjects, study 2) after they had fasted overnight. A catheter was inserted into their superficial forearm vein for blood sampling. At time zero, after a baseline blood sample and urine collection, the test meal containing milk, soy, or wheat protein was ingested. Blood was sampled every 30 min for 3 h and then hourly for 5 h. Total urine was collected every 2 h throughout the 8-h postprandial period.

Analytic methods

Urea concentrations were determined in both serum and urine samples by using an enzymatic method on a Dimension automat (du Pont de Nemours, Les Ulis, France). 15N enrichment in serum urea and proteins, and in urine samples, was measured by isotope ratio mass spectrometry (Optima; Fisons Instruments, Manchester, United Kingdom), coupled to an elemental nitrogen analyzer (NA 1500 series 2, Fisons Instruments), as detailed elsewhere (37).

Kinetic data preparation

Dietary nitrogen incorporation (Ndiet) into each sampled pool (serum proteins, body urea, and urinary urea) was evaluated at each sampling time for each subject, as follows:

\[ N_{\text{diet}}(t) = APE(t) \times N_{\text{pool}}(t)/APE_{\text{meal}} \]  

where \( N_{\text{pool}}(t) \) is the total nitrogen content (in mmol N) of the considered sampled pool at sampling time \( t \), \( APE(t) \) its 15N enrichment above baseline at time \( t \), and \( APE_{\text{meal}} \) the 15N enrichment of the meal. The total nitrogen content in serum proteins
was calculated from the nitrogen concentration measured in this fraction and the plasma volume, estimated at 5% of body weight (40). The total nitrogen content in body urea was calculated knowing the plasma urea nitrogen concentration and its volume of distribution (total body water), corrected by the multiplying factor 0.92 representing the water content in blood. The total nitrogen content in urinary urea was determined from the volume of urine and the urea nitrogen concentration. Each data set was fitted to smoothing functions to both unify the data step size and minimize the effects of experimental noise. Data were fitted to sums of exponentials, which are solutions of multicompartmental models, with the number of exponential terms being suitably selected on the basis of statistical criteria (41, 42). This allowed us to obtain the same 1-h data step size for all sampled compartments (by accurate interpolation of the urinary data) and to reduce the level of noise contained in the experimental data (under the assumption of a normal distribution of the errors). Smoothing the data before analyzing them by compartmental modeling minimizes the error made on estimated values of the model parameters and therefore on the model predictions for postprandial urea kinetics. For each subject, the postprandial kinetics of dietary and total nitrogen into plasma proteins, body urea, and urinary urea were fitted by using SIGMAPLOT software (version 6.00; SPSS Inc, Chicago, IL) to the sum of 3 exponential terms; the need for a fourth or a fifth exponential term was obviated by appeal to Akaike and Schwarz information criteria.

**Mathematical modeling of postprandial urea kinetics**

**Model structure and assumptions**

The model shown in Figure 1 was developed to describe the postprandial kinetics of the production, urinary excretion, and intestinal hydrolysis of urea nitrogen from both dietary and endogenous sources by using our experimental data on both dietary and total nitrogen postprandial kinetics in body urea, urinary urea, and plasma proteins. The same 4-compartment, 4-parameter model was used to describe the postprandial kinetics of both total and dietary urea nitrogen. It integrated the 3 sampled compartments, where BU (BU*) represented the amount of total (dietary) nitrogen in body urea, UU (UU*) the amount of total (dietary) nitrogen in urinary urea, and SEP (SEP*) the amount of total (dietary) nitrogen in splanchnic-expressed proteins. The model also included an additional unsampled compartment SA (SA*), which represented the amount of total (dietary) nitrogen in splanchnic free amino acids. The model was constructed to depict the following processes. Urea production takes place in the liver, concomitantly with the synthesis of plasma exported proteins. The urea thus produced mixes with the general BU compartment, and a proportion of the urea synthesized is eliminated in the urine through urea excretion and fills up the BU compartment. The model is based on the following assumptions: 1) dietary and total nitrogen fluxes are governed by the law of mass action, 2) SA is the main precursor pool for both urea production and the synthesis of splanchnic-exported proteins, 3) urea nitrogen leaving the BU compartment is directed toward urinary excretion or intestinal hydrolysis, and 4) the rates of dietary and total urea nitrogen hydrolysis are supposed to be equal (ie, $k_{UH} = k_{UH}$), because the urea molecules synthesized in the liver from either dietary or endogenous origin are rapidly mixed within the general BU compartment and then become indistinguishable in terms of their subsequent metabolism.

**Model notations and equations**

In the text that follows, variables and parameters relative to dietary nitrogen kinetics will be marked with an asterisk, by contrast with those relative to total nitrogen kinetics, which will not be marked. The postprandial nitrogen kinetics were represented in the model by a system of linear ordinary differential equations describing the instantaneous evolution of the compartmental sizes of BU (BU*), UU (UU*), and SEP (SEP*) for total (dietary) nitrogen. The evolution of BU was described by the following non–steady state equation, according to the mass conservation principle:

$$dBU(t)/dt = up(t) - ue(t) - uh(t)$$  \hspace{1cm} (2)

where $up(t)$, $ue(t)$, and $uh(t)$ denote the instantaneous fluxes of total urea nitrogen production, excretion, and hydrolysis at each
time $t$. In addition, these fluxes were modeled by standard mass action kinetics, with $up(t) = k_{up} \times SA(t)$, $ue(t) = k_{ue} \times BU(t)$, and $uh(t) = k_{uh} \times BU(t)$, where $k_{up}$, $k_{ue}$, and $k_{uh}$ were the rates of total urea nitrogen production, excretion, and hydrolysis, respectively. A similar equation applied for $BU^*$, and similar notations and equations applied for dietary nitrogen fluxes $[up^*(t), ue^*(t), and uh^*(t)]$, with parameters $k_{up^*}$, $k_{ue^*}$, and $k_{uh^*}$.

In addition, we have similarly, according to the mass conservation principle:

$$dUU(t)/dt = ue(t)$$

a similar equation applying for $UU^*$.

Concomitantly, a fraction of splanchnic free amino acids was used for the synthesis of splanchnic-exported proteins, and we denoted $s_{SEP}$ the instantaneous flux of splanchnic-exported proteins synthesis and $d_{SEP}$ the instantaneous flux of their disappearance from the intravascular pool through protein degradation or distribution within the total exchangeable intra- and extravascular protein pools. According to the mass conservation principle, the evolution of $SEP$ was described by the following non–steady state equation:

$$dSEP(t)/dt = s_{SEP}(t) - d_{SEP}(t)$$

where $s_{SEP}(t) = k_{sep} \times SA(t)$ and $d_{SEP}(t) = k_{dsep} \times SEP(t)$, with $k_{sep}$ and $k_{dsep}$ the rates of total nitrogen incorporation into splanchnic-exported proteins and its subsequent disappearance from the intravascular pool, respectively. Similar notations and equations applied for $SEP^*$ and the dietary nitrogen fluxes $s_{SEP^*}$ and $d_{SEP^*}$ (with parameters $k_{sep^*}$ and $k_{dsep^*}$).

Finally, the cumulated fluxes over the $t$-period of total urea nitrogen production, excretion, and hydrolysis were denoted as $UP(t)$, $UE(t)$, and $UH(t)$, respectively, where

$$UP(t) = \int_0^t up(v)dv,$$

$$UE(t) = \int_0^t ue(v)dv,$$

and

$$UH(t) = \int_0^t uh(v)dv$$

Similar notations and equations applied for the cumulated fluxes of dietary urea nitrogen production, excretion, and hydrolysis ($UP^*$, $UE^*$, and $UH^*$, respectively).

**Model solving and outputs**

As shown in Appendix A, Equations A1 to A3 (Eq $AI^*$ to $A3^*$) can be combined with the total (dietary) nitrogen data obtained in blood and urine to calculate the following parameters and variables: 1) the constant transfer rates $k_{ue}$ ($k_{ue^*}$) and $k_{uh}$ ($k_{uh^*}$), 2) the $k_{up^*}$:$k_{sep^*}$ ratio, 3) the instantaneous fluxes $ue^*$ and $uh^*$, and 4) the cumulated fluxes $UP^*$, $UE^*$, and $UH^*$.

The parameters $k_{ue^*}$, $k_{uh^*}$, and $k_{up^*}$:$k_{sep^*}$ are the solutions of systems of linear equations, where the number of equations is higher than the number of unknowns (Appendix A). Because most of our systems of linear equations were badly conditioned (large condition numbers), ie, they were highly sensitive to experimental error in the data, we could not use the classic method of resolution, requiring inversion of the system matrix. However, the resolution of such systems could come down to minimization problems (Appendix A), where the estimated parameters were those that best accounted for the observed data. Each minimization problem was solved by using a simplex, derivative-free algorithm, which has been presented in full detail elsewhere (43, 44). The resolution method was implemented under MATLAB 6.1 (The Mathworks Inc, Natick, MA).

**Statistical analysis**

Results are presented as means ± SDs. Statistical analyses were performed by using the SAS statistical software package (SAS/STAT version 9.1.3; SAS Institute, Cary, NC). For analysis of serial data, mixed models analysis of variance for repeated-measures was performed, with fixed factors being the dietary condition, time, and their interaction. In study 1, time was a repeated factor and the dietary condition (ie, the protein source in the meal: milk, soy, or wheat) was a between-group factor, whereas in study 2 both time and the dietary condition (ie, the protein level in the diet: NP or HP) were repeated factors. For measurements in which there was a significant interaction, the post hoc testing of differences between protein sources at each time point (study 1) or between diet type at each time point (study 2) was performed by using Tukey’s test (SAS/STAT). Differences were considered as significant if $P < 0.05$.

**RESULTS**

**Postprandial kinetics of total and dietary nitrogen in sampled pools**

The results regarding the effects of the protein source in the meal and the habitual protein intake in the diet on the postprandial kinetics of total and dietary nitrogen in the sampled pools (serum proteins, body, and urinary urea) have already been detailed (35, 39). Briefly, according to the data obtained on the acute effect of the dietary protein source in the meal (study 1: comparison of data obtained when ingesting milk, soy, or wheat proteins) after NP adaptation, the protein source produced no change in the postprandial kinetics of total nitrogen in the sampled pools but greatly modulated those of the appearance of dietary nitrogen in serum protein ($SEP^*$), body urea ($BU^*$), and urinary urea ($UU^*$). Moreover, according to the data obtained on the chronic effect of the 2 prior adaptation levels of protein intake (NP and HP) in the diet when the protein source in the meal was soy (study 2), the amounts of total and dietary nitrogen recovered in body urea (BU and BU*, respectively) and urinary urea (UU and UU*, respectively) after 8 h were significantly higher after HP than after NP adaptation.

**Postprandial kinetics of urea production, excretion, and hydrolysis**

The experimental data obtained during the 2 studies for the postprandial kinetics of both dietary and total nitrogen ($SEP^*$, $BU^*$, and $UU^*$ and $SEP$, $BU$, and $UU$, respectively) were further analyzed by compartmental modeling (model in Figure 1). By
doing this, we determined some parameters and variables regarding (1) total urea nitrogen production and its part from dietary origin (UP and UP*, respectively), 2) total urea nitrogen excretion and its part from dietary origin (UE and UE*, respectively), and 3) total urea nitrogen hydrolysis and its part from dietary origin (UH and UH*, respectively). Model predictions for the parameter values of $k_{\text{UR}^*}$ ($k_{\text{US}^*}$) and $k_{\text{UH}^*}$ ($k_{\text{US}^*}$), representing the rates of total (dietary) nitrogen urinary excretion and intestinal hydrolysis, respectively, are given in Table 1, together with the ratio $k_{\text{UR}^*}:k_{\text{US}^*}$, representing the relative capacity of dietary nitrogen splanchnic utilization for urea production versus the synthesis of plasma exported proteins.

Model predictions for the postprandial time courses of UP (UP* and UH (UH*), representing the cumulated fluxes over time of total (dietary) urea nitrogen production and hydrolysis, respectively, are depicted in Figure 2. The final postprandial values of UP*, UH*, and UE* at 8 h are shown in Table 2, and the final 8-h postprandial values of UP, UH, and UE are shown in Table 3. Finally, the relative contribution of dietary nitrogen to the total urea nitrogen kinetics of production, hydrolysis, and excretion (UP*:UP, UH*:UH, and UE*:UE, respectively) are represented in Table 4.

Urea nitrogen kinetics of dietary origin

The protein source in the meal exerted a significant effect ($P < 0.0001$) on the postprandial kinetics of both UP* and UH* (Figure 2 and Table 2, analysis of data from study 1). Regarding UP*, its 8-h values were markedly higher after wheat than after milk or soy protein ingestion ($P < 0.03$, Table 2), and differences between protein sources occurred as early as 4 h, because greater amounts of dietary nitrogen were deaminated after the ingestion of wheat versus milk ($P < 0.0001$) or wheat versus soy ($P < 0.05$) proteins as from this time point (Figure 2). Further, the $k_{\text{UR}^*}$: $k_{\text{US}^*}$ ratio, which represented the relative capacity of dietary nitrogen utilization for urea production versus that for the synthesis of splanchnic-exported proteins, was increased by $\approx 60\%$ ($P < 0.0001$) after the ingestion of wheat versus milk or soy protein (Table 1), indicating that UP* was markedly stimulated after the ingestion of wheat protein (Table 2). Regarding UH*, it was also significantly increased, as early as the 4th postmeal hour ($P < 0.001$, Figure 2), after the ingestion of wheat versus milk protein, but also, as early as the 6th postmeal hour, after the ingestion of wheat versus soy ($P < 0.001$) and soy versus milk ($P < 0.01$) proteins. Moreover, when the final postprandial value of UH* was expressed as a percentage of UP*, the proportion was higher in the wheat versus milk ($P < 0.0001$), the wheat versus soy ($P < 0.03$), and the soy versus milk ($P < 0.0001$) protein groups (Table 2). These increases in UH* and UH* relative to UP* (UH*:UP*) resulted from the increase in both UP* and the transfer rate $k_{\text{UH}^*}$ (Table 1). In contrast, the $k_{\text{UR}^*}$ rate did not differ significantly between groups (Table 1), leading to smaller differences between dietary proteins in terms of the final 8-h values for UE*. However, UE* was still higher at 8 h after the ingestion of wheat versus milk or soy protein ($P < 0.02$), because of the higher UP* value (Table 2).

Moreover, the prevailing protein level in the diet also exerted a significant effect ($P < 0.01$) on both UP* and UE* (Table 2, analysis of data from study 2): when switching from the NP to the HP diet, UP* and UE* were thus significantly increased ($P < 0.01$) at 8 h after the ingestion of the same soy protein load. In addition, a doubling of the habitual protein intake was followed by a $\approx 50\%$ decrease ($P < 0.01$) in the transfer rate $k_{\text{UH}^*}$ (Table 1). However, although the final 8-h value of UH* was not significantly affected by the prior protein level, when UH* was expressed as a percentage of UP*, it fell significantly ($P < 0.01$) at the end of the postprandial period in HP- versus NP-adapted subjects (Table 2).

The relative contributions of dietary nitrogen to total urea nitrogen kinetics are represented in Table 4. Whatever the meal, dietary nitrogen contributed more to urea production than to urea hydrolysis and excretion: dietary nitrogen accounted for $\approx 20\%$ of the total urea nitrogen production (UP*:UP = 21.0 $\pm$ 3.1%, mean value for all subjects), but only for $\approx 10\%$ of urea hydrolysis (UH*:UH = 9.8 $\pm$ 2.2%) and excretion (UE*:UE = 11.3 $\pm$ 2.8%). The contribution of dietary nitrogen to total urea nitrogen kinetics was not affected by the prevailing protein level in the diet.

### Table 1

<table>
<thead>
<tr>
<th>Protein source in the meal and level in the diet</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk after NP</td>
<td>Soy after NP</td>
</tr>
<tr>
<td>($n = 8$)</td>
<td>($n = 8$)</td>
<td>($n = 8$)</td>
</tr>
<tr>
<td>$k_{\text{UR}^*}$ (%/h)</td>
<td>9.6 $\pm$ 1.1</td>
<td>9.3 $\pm$ 4.0</td>
</tr>
<tr>
<td>$k_{\text{UR}}$ (%/h)</td>
<td>8.3 $\pm$ 0.8</td>
<td>7.9 $\pm$ 3.0</td>
</tr>
<tr>
<td>$k_{\text{UH}} = k_{\text{UR}^*}$ (%/h)</td>
<td>2.5 $\pm$ 0.6</td>
<td>7.6 $\pm$ 1.5</td>
</tr>
<tr>
<td>$k_{\text{UR}^<em>} k_{\text{US}^</em>}$ (%)</td>
<td>3.4 $\pm$ 0.6</td>
<td>3.4 $\pm$ 0.6</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x} \pm$ SD and were calculated by using the model presented in Figure 1. $k_{\text{UR}^*}$ is the rate of total (dietary) urea N excretion. $k_{\text{UR}^*} k_{\text{US}^*}$ is the rate of total (dietary) urea N hydrolysis (modeling hypothesis: $k_{\text{UR}^*} = k_{\text{US}^*}$). $k_{\text{UR}^*}$ and $k_{\text{US}^*}$ are the rates of dietary urea N production and dietary N incorporation into splanchnic-exported proteins, respectively, with $k_{\text{UR}^*} k_{\text{US}^*}$ representing the relative capacity of dietary N utilization for urea production vs the synthesis of splanchnic-exported proteins (see Appendix A). In study 1, subjects ingested a single meal with either milk ($n = 8$), soy ($n = 8$), or wheat ($n = 8$) protein after their 7-d standardization to an NP intake (1 g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2.5 g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$) before ingesting the same soy protein meal ($n = 8$). Mixed-model with protein as between-group factor: data from study 1 showed that the protein source exerted a significant effect on $k_{\text{US}^*}$ and $k_{\text{US}^*}$ ($P < 0.0001$) and on $k_{\text{UR}^*} k_{\text{US}^*}$ ($P < 0.05$), and data from study 2 showed that the protein level exerted a significant effect on $k_{\text{US}^*}$ and $k_{\text{US}^*}$ ($P < 0.02$).

2 Significantly different from the milk protein group, $P < 0.001$ (post hoc Tukey’s test).

3 Significantly different from the soy protein group, $P < 0.001$ (post hoc Tukey’s test).

4 Significantly different from the (soy) NP group, $P < 0.02$ (post hoc Tukey’s test).
but was affected by the protein source in the meal: the contribution of dietary nitrogen to urea nitrogen hydrolysis (UH*/UH) and excretion (UE*/UE), but not production (UP*/UP), were higher after wheat than after milk or soy proteins.

**Total urea nitrogen kinetics**

Postprandial UE was not significantly affected by the protein source in the meal (Table 3), and the $k_{UE}$ rate was quite similar in the different protein groups, with $\approx 10\%$ of the total urea nitrogen present in body urea being eliminated each hour in urine (Table 1). By contrast, the protein source in the meal exerted a significant effect ($P < 0.0001$) on the postprandial kinetics of both UP and UH (Figure 2 and Table 3, analysis of data from study 1). Regarding UP, its 8-h value was markedly higher after the ingestion of wheat versus milk protein (from $72 \pm 9$ to $134 \pm 24$ mg N/kg; $P < 0.0001$) and, to a lesser extent, after the ingestion of wheat versus soy protein (from $98 \pm 24$ to $134 \pm 24$ mg N/kg; $P < 0.01$). Similarly, UP was higher after soy versus milk protein ingestion at 8 h ($P < 0.05$). Differences between protein sources tended to appear less early and more progressively for UP than for UP*; for example, the ingestion of wheat versus soy protein increased UP only after 6 h instead of after 4 h for UP* (Figure 2). UH values were also significantly increased at 8 h after the ingestion of wheat versus milk ($P < 0.0001$) or soy ($P < 0.02$) proteins (Table 3), and also after the ingestion of soy versus milk protein ($P < 0.01$). The final 8-h value of UH thus reached $17 \pm 4$, $52 \pm 20$, and $80 \pm 25$ mg N/kg after the ingestion of milk, soy, and wheat protein, respectively. Differences between protein sources also tended to appear more progressively for UH than for UP* (Figure 2): UH was significantly higher after the ingestion of wheat versus milk and soy versus milk proteins as early as the 4th ($P < 0.001$) and 6th ($P < 0.01$) postmeal hours, respectively, but it was only significantly higher after the ingestion of wheat versus soy after 8 postmeal hours ($P < 0.02$). Moreover, when UH was expressed relative to UP, the percentage calculated at 8 h was modulated by the protein source ($P < 0.0001$), reaching $24 \pm 5\%$, $52 \pm 13\%$, and $58 \pm 7\%$ after the ingestion of milk, soy, or wheat protein, respectively. In particular, the proportion of the urea produced that was hydrolyzed in the gastrointestinal tract at the end of the postprandial period showed a significant increase in the wheat versus milk and in the soy versus milk protein groups ($P < 0.0001$), but not in the wheat versus soy protein groups (Table 3). These increases in UH and UP relative to UP (UH:UP) resulted from the increase in both UP and the transfer rate $k_{UP}$, which was greater in both wheat versus milk, soy versus milk, and wheat versus soy protein groups (Table 1). The individual UH and UP values after the milk, soy, or wheat protein meals in subjects adapted to an NP diet (subjects of study 1) are reported in Figure 3. UH was closely (Pearson $r = 0.96$) and significantly ($P < 0.0001$) related to UP by a positive linear relation (UH = $0.93 \times$ UP + 44.69 mg·kg$^{-1}$·h$^{-1}$). In contrast, there was no such direct relation between UE and UP ($r = 0.27$, $P = 0.2$) or UH ($r = 0.13$, $P = 0.6$).

The prevailing protein level in the diet also exerted a significant effect on UE postprandial kinetics ($P < 0.01$) and, to a lesser extent, on the final 8-h value of UH:UP ($P < 0.01$), as tested when the meal protein was soy (Table 3, analysis of data from study 2). Because of changes to the body urea pool size (but no change to $k_{UE}$ rates), postprandial UE at 8 h was significantly increased from $48 \pm 11$ to $79 \pm 13$ mg N/kg ($P < 0.01$) when switching from the NP to the HP diet (Table 3). By contrast, UP and UH did not differ significantly in HP- versus NP-adapted subjects at 8 h after the ingestion of the same soy protein load (Table 3). However, the proportion of UP that was hydrolyzed at the end of the postprandial period was significantly decreased ($P < 0.01$) from $52 \pm 13\%$ to $35 \pm 5\%$ when subjects switched from the NP to the HP diet (Table 3), which mainly resulted from an $\approx 50\%$ decrease in the $k_{UE}$ rate ($P < 0.01$, Table 1).

The range of variation for all the dietary protein sources and levels covered by the model predictions for UE, UP, and UH

**FIGURE 2.** Time course of both dietary (A) and total (B) urea nitrogen (N) production (UP* and UP, respectively) and hydrolysis (UH* and UH, respectively) for 8 h after the ingestion of a mixed meal containing milk ($n = 8$), soy ($n = 8$), or wheat ($n = 8$) protein in subjects adapted to a normal-protein diet. Values are means ± SDs. Note that the scale on the y axis differs between panels A and B. The protein source (milk, soy, or wheat) exerted a significant effect on the postprandial kinetics of UP*, UP, UH*, and UH ($P < 0.0001$, mixed-models for repeated-measures analysis, with time as a repeated factor and protein as a between-group factor). UP* and UP: † the wheat protein group differed from the milk protein group at that time ($P < 0.0001$, post hoc Tukey’s test); ‡ the wheat protein group differed from the soy protein group at that time ($P < 0.05$, post hoc Tukey’s test). UH* and UH: # the soy or wheat protein groups differed from the milk protein group at that time ($P < 0.05$, post hoc Tukey’s test); ¥ the wheat protein group differed from the soy protein group at that time ($P < 0.05$, post hoc Tukey’s test).
Postprandial dietary urea nitrogen (N) kinetics in subjects adapted to normal-protein (NP) and high-protein (HP) diets after the ingestion of a mixed meal containing milk, soy, or wheat protein.

Mixed-model for repeated-measures analysis, with time and diet as repeated factors and protein as a between-group factor: data from study 1 showed that the protein source exerted a significant effect (P < 0.001) on dietary UP*, UE*, and UH* (mg/L50512 kg−1·8h−1). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2 g · kg−1·d−1). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2 g · kg−1·d−1) before ingesting the same soy protein meal (n = 8). Mixed-model for repeated-measures analysis, with time as a repeated factor and protein as a between-group factor: data from study 1 showed that the protein source exerted a significant effect (P < 0.001) on dietary UP*, UE*, and UH*·UP*.

Postprandial dietary urea nitrogen (N) kinetics in subjects adapted to normal-protein (NP) and high-protein (HP) diets after the ingestion of a mixed meal containing milk, soy, or wheat protein.

Table 2

<table>
<thead>
<tr>
<th>Protein source in the meal and level in the diet</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk after NP (n = 8)</td>
<td>Soy after NP (n = 8)</td>
<td>Wheat after NP (n = 8)</td>
</tr>
<tr>
<td>UE* (mg · kg−1·8h−1)</td>
<td>5 ± 1</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>UP* (mg · kg−1·8h−1)</td>
<td>15 ± 2</td>
<td>19 ± 4а</td>
</tr>
<tr>
<td>UH* (mg · kg−1·8h−1)</td>
<td>2 ± 1</td>
<td>5 ± 2а</td>
</tr>
<tr>
<td>UH*:UP* (%)</td>
<td>9 ± 3</td>
<td>26 ± 6а</td>
</tr>
</tbody>
</table>

All values are x ± SD for postprandial dietary N utilization for urea excretion (UE*), production (UP*), hydrolysis (UH*), and hydrolysis relative to production (UH*:UP*), calculated at 8 h after the meal by using the model presented in Figure 1. In study 1, subjects ingested a single meal with either milk (n = 8), soy (n = 8), or wheat (n = 8) proteins after their 7-d standardization to an NP intake (1 g · kg−1·d−1). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2 g · kg−1·d−1) before ingesting the same soy protein meal (n = 8). Mixed-model for repeated-measures analysis, with time as a repeated factor and protein as a between-group factor: data from study 1 showed that the protein source exerted a significant effect (P < 0.001) on dietary UP*, UE*, and UH*·UP*.

Significantly different from the milk protein group, P < 0.05 (post hoc Tukey’s test).

Significantly different from the soy protein group, P < 0.03 (post hoc Tukey’s test).

Significantly different from the soy protein group, P < 0.02 (post hoc Tukey’s test).

DISCUSSION

We investigated the short-term kinetics of urea during the postprandial non-steady state and their modulation by protein intake in humans by using a combination of clinical investigation and kinetic modeling. In subjects adapted to different protein levels in the diet, experimental data were obtained on both dietary and total nitrogen postprandial kinetics in certain accessible pools of blood and urine after a bolus mixed meal containing different 15N-labeled dietary proteins. Compartmental analysis of these data predicted the postprandial kinetics of dietary and total urea nitrogen production (UP* and UP, respectively), excretion (UE* and UE, respectively), and hydrolysis (UH* and UH, respectively). UE was not influenced by the meal protein source but was influenced by the diet protein level. By contrast, UP and UH increased after the ingestion of plant versus animal protein, as did the hydrolysis efficiency (UH:UP).

Our mathematical model for postprandial urea kinetics is based on the following assumptions: 1) nitrogen fluxes are governed by the law of mass action, a classic hypothesis when dealing with nutritional or metabolic systems (2, 42, 51–53); 2) there is a single precursor compartment for both urea production and kinetic modeling.

Table 3

<table>
<thead>
<tr>
<th>Protein source in the meal and level in the diet</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk after NP (n = 8)</td>
<td>Soy after NP (n = 8)</td>
<td>Wheat after NP (n = 8)</td>
</tr>
<tr>
<td>UE (mg · kg−1·8h−1)</td>
<td>55 ± 7</td>
<td>48 ± 11</td>
</tr>
<tr>
<td>UP (mg · kg−1·8h−1)</td>
<td>72 ± 9</td>
<td>98 ± 24а</td>
</tr>
<tr>
<td>UH (mg · kg−1·8h−1)</td>
<td>17 ± 4</td>
<td>52 ± 20а</td>
</tr>
<tr>
<td>UH:UP (%)</td>
<td>24 ± 5</td>
<td>52 ± 13а</td>
</tr>
</tbody>
</table>

All values are x ± SD for postprandial total urea N excretion (UE), production (UP), hydrolysis (UH), and hydrolysis relative to production (UH:UP), calculated at 8 h after the meal by using the model presented in Figure 1. In study 1, the subjects ingested a single meal with either milk (n = 8), soy (n = 8), or wheat (n = 8) proteins after their 7-d standardization to an NP intake (1 g · kg−1·d−1). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2 g · kg−1·d−1) before ingesting the same soy protein meal (n = 8). Mixed-model for repeated-measures analysis, with time as a repeated factor and protein as a between-group factor: data from study 1 showed that the protein source exerted a significant effect (P < 0.0001) on UP and UH and also on hydrolysis relative to production (UH:UP). Mixed-model for repeated-measures analysis, with time and diet as repeated factors: data from study 2 showed that the protein level exerted a significant effect (P < 0.05) on UE and UH:UP.

Significantly different from the milk protein group, P < 0.05 (post hoc Tukey’s test).

Significantly different from the soy protein group, P < 0.02 (post hoc Tukey’s test).

Significantly different from the (soy) NP group, P < 0.01 (post hoc Tukey’s test).
the synthesis of plasma exported proteins, in accordance with previous modeling of these metabolic processes (51, 54); 3) urea leaving the body urea compartment is directed toward either urinary excretion or intestinal hydrolysis, because sweat nitrogen losses are negligible under our experimental conditions; and 4) the rates of dietary and total urea nitrogen hydrolysis are equal (ie, $k_{\text{UE}} = k_{\text{UE}}^*$), because urea synthesized from either dietary or endogenous origin is undistinguishable in terms of its further intestinal or renal metabolism. This latter option was supported by the fact that dietary and total urea nitrogen urinary excretion rates were similar (ie, $k_{\text{UE}} = k_{\text{UE}}^*$). Table 1) when calculated directly from the experimental data. By contrast, dietary and endogenous nitrogen sources were not supposed to be used at similar rates for urea production (ie, $k_{\text{UP}} \neq k_{\text{UP}}^*$, not estimated here) because of the functional and metabolic zonation of the liver (55–58). In line with this, dietary nitrogen was predicted to contribute more preferentially to urea production than to urea excretion or hydrolysis (Table 4). In addition, the model plausibility was supported by the consistency of its predictions with respect to our current knowledge (49). As reported in Table 5, the model estimated postprandial UP and UE values within the range of previously reported values in the literature (3, 6, 18, 19, 26, 33, 45). Postprandial UP also compared well with previous estimates in postabsorptive humans (3, 6, 18, 26, 33, 45), but exceeded the scarce results obtained in the fed state (6). However, this latter study had reported peculiarly low values for UE (almost null), related to the use of a primed-continuous tracer infusion method (3), which has since been shown to underestimate short-term variations in UP (8). Last, the hydrolysis efficiency (ie, UE/UP) during the postprandial phase also compared well with previous findings over 24 h (4, 7, 18, 24, 26).

Whereas postprandial UE was unaffected by the protein source in the meal, it rose when switching from the NP to the HP diet (Table 3), in line with our previous observations in humans (35, 38, 39, 59, 60) and classic studies that reported increasing daily UE in line with protein intake (7, 18, 24). Postprandial UE was confirmed to be sensitive to chronic, quantitative variations in protein intake, but not to those of an acute, qualitative nature, which agrees well with the use of 24-h urine nitrogen as a biomarker of the protein level in the diet (61). This would mean that qualitative changes to protein intake may only affect UE on a chronic basis, as was shown in animals (daily UE increased after chronic adaptation to a protein source of lower quality: 62–64). By contrast, adaptive changes affected postprandial UP in response to acute, qualitative variations in protein intake, but not those of a chronic, quantitative nature. Both UP and UP* varied as a function of the protein source in the meal, but when doubling the prevailing protein intake, UP did not vary significantly, despite increased UP* values (Tables 2 and 3). This lack of postprandial UP stimulation under HP versus NP could be clearly explained by our particular experimental design, in which the same protein load (0.41 g/kg) was ingested on the day of the postprandial test in both HP- and NP-adapted subjects. Nevertheless, some controversy persists concerning whether UP values change (7, 25) or not (9) in parallel with the protein intake.

In line with the prevailing concept (25), because UP* increased after HP versus NP adaptation, UP would probably have also increased if a protein load proportional to the habitual protein level in the diet (ie, 2-fold higher) had been given in the test meal. In addition, UP responded rapidly to acute, qualitative

---

**TABLE 4**

Participation of dietary to total urea nitrogen (N) kinetics in subjects adapted to normal-protein (NP) and high-protein (HP) diets after the ingestion of a mixed meal containing milk, soy, or wheat protein.

<table>
<thead>
<tr>
<th>Protein source in the meal and level in the diet</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk after NP ($n = 8$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UE* : UE (%)</td>
<td>9.2 ± 2.2</td>
<td>14.4 ± 2.9$^*$</td>
</tr>
<tr>
<td>UP* : UP (%)</td>
<td>20.5 ± 2.5</td>
<td>21.4 ± 3.8</td>
</tr>
<tr>
<td>UH* : UE (%)</td>
<td>7.9 ± 1.7</td>
<td>12.0 ± 2.5$^*$</td>
</tr>
<tr>
<td>Soy after NP ($n = 8$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UE* : UE (%)</td>
<td>11.4 ± 1.8</td>
<td>10.4 ± 0.7</td>
</tr>
<tr>
<td>UP* : UP (%)</td>
<td>19.9 ± 3.0</td>
<td>22.4 ± 3.1</td>
</tr>
<tr>
<td>UH* : UE (%)</td>
<td>9.7 ± 1.0</td>
<td>9.3 ± 1.0</td>
</tr>
<tr>
<td>Wheat after NP ($n = 8$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UE* : UE (%)</td>
<td>14.4 ± 2.9$^*$</td>
<td></td>
</tr>
<tr>
<td>UP* : UP (%)</td>
<td>21.4 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>UH* : UE (%)</td>
<td>12.0 ± 2.5$^*$</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x} \pm SD$ for dietary participation to total urea N excretion (UE* : UE), production (UP* : UP), and hydrolysis (UH* : UEH), calculated at 8 h by using the model presented in Figure 1. In study 1, subjects ingested a single meal with either milk ($n = 8$), soy ($n = 8$), or wheat ($n = 8$) proteins after their 7-d standardization to an NP intake (1 g·kg$^{-1}$·d$^{-1}$). In study 2, subjects previously assigned to soy were adapted for the next 7 d to an HP intake (2 g·kg$^{-1}$·d$^{-1}$) before ingesting the same soy protein meal ($n = 8$). Mixed-model for repeated-measures analysis, with time as a repeated factor and protein as a between-group factor: data from study 1 showed that the protein source exerted a significant effect ($P < 0.0001$) on total UE* : UE and UH* : UEH. Mixed-model for repeated-measures analysis, with time and diet as repeated factors: data from study 2 showed no significant differences between diets.

$^2$ Significantly different from the milk protein group, $P < 0.001$ (post hoc Tukey’s test).

$^3$ Significantly different from the soy protein group, $P < 0.05$ (post hoc Tukey’s test).
variations in the protein intake. UP increased after soy versus milk ($P < 0.05$) and even more after wheat versus milk ($P < 0.0001$). At first sight, a higher UP after plant versus animal protein might be not considered a new finding, inasmuch as indirect evidence can be traced back to the pioneering work by Mitchell (65), but this is indeed unclear. Actually, we previously found no difference in postprandial UP between soy and milk (35, 66) by using a nonisotopic method (measurement of urea excreted in urine plus the net amount accumulated in body water) that evaluates apparent UP (ie, UP minus UH). Only scarce studies have reported true UP data after plant versus animal protein by using tracer-based methods, and UP has been shown to be stimulated after soy versus milk proteins in pigs (67) and humans (29). However, during these studies, the meal was administered by continuous enteral feeding, which is little relevant to the physiologic dynamic postprandial challenge that stimulates UP, so that it led to an unexpected decrease in UP during the fasted-fed transition (29).

Our data are therefore the first to measure UP in the postprandial non–steady state in humans, and they reveal an acute increase after the ingestion of plant versus animal protein. This effect may be due to differences in digestive kinetics (66, 68–70) or the amino acid composition of dietary proteins (38, 66, 70). Indeed, amino acids derived from soy proteins are more rapidly available to the body than are those derived from milk proteins, leading to an earlier and more dramatic influx of dietary amino acids that stimulates the splanchnic catabolic pathways, as previously shown by us (66, 70), in line with the “slow and fast protein” concept (68, 69). In addition, the slightly poorer, more imbalanced amino acid pattern in plant proteins may also contribute to the higher UP values after soy versus milk and is likely to be the rationale for the peculiar increase in UP after wheat versus milk and soy. Such an acute, qualitative effect of protein amino acid composition had previously been discussed by us (38, 70), and it may also operate on a chronic basis, because urea synthesis has been found to be enhanced in the context of unbalanced amino acid patterns in the diet (71).

Postprandial UH and UH* also responded rapidly to acute qualitative variations in the protein intake. Their values increased markedly after soy versus milk and even more after wheat versus milk. UP and UH* thus changed in parallel with postprandial UP and UP*, respectively, when comparing the different protein sources (Tables 2 and 3). One major finding was evidence (for the first time) that both UP and UH values were higher after the ingestion of plant versus animal protein, which explains why apparent UP (ie, UP minus UH) was found to be quite similar between protein sources when measured by use of nonisotopic methods (35, 66). When examining individual 8-h values (Figure 3), whereas there was no direct relation between UE and UP, UH was closely related to UP with a highly

### Table 5

| Table 5: External validation: comparison of findings in the literature and model predictions for urea kinetics

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Finding in literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urea N excretion (UE, mg kg⁻¹ h⁻¹)</td>
<td>6–10</td>
</tr>
<tr>
<td>Total urea N production (UP, mg kg⁻¹ h⁻¹)</td>
<td>9–17</td>
</tr>
<tr>
<td>Total urea N hydrolysis (UH, mg kg⁻¹ h⁻¹)</td>
<td>1–10</td>
</tr>
<tr>
<td>Total hydrolysis efficiency (UH:UP, %)</td>
<td>25–60</td>
</tr>
<tr>
<td>Contribution of dietary N to total UP (UP*:UP, %)</td>
<td>20–23</td>
</tr>
</tbody>
</table>

1. Model predictions and literature findings in healthy humans adapted to protein intakes from normal to high (≈ 0.9 to 3 g kg⁻¹ d⁻¹). Predicted and observed values for total urea nitrogen (N) excretion (UE), production (UP), and hydrolysis (UH) are given in mg kg⁻¹ h⁻¹. Predicted and observed values for participation of dietary N to total urea N production (UP*:UP) are given in %. The range of variation in model predictions for all dietary protein sources and levels is given as min-max of the mean values over 8 h between groups.

2. Range of variation in literature values (min-max) for UE (3, 6, 18, 19, 26, 45).

3. Range of variation in literature values (min-max) for UP (3, 6, 18, 19, 26, 33, 45).

4. Range of variation in literature values (min-max) for UH:UP when estimated over 24 h with 12-h fasted and 12-h fed (4, 7, 18, 24, 26, 45).

5. Range of variation in literature values (min-max) for UH in postabsorptive studies (3, 6, 18, 24, 26, 45).

6. Range of variation in literature values (min-max) for UP:UP when estimated over 24 h with 12-h fasted and 12-h fed (4, 7, 18, 24, 26).

7. Range of variation in literature values (min-max) for UP*:UP using nonisotopic (35, 47) and urea tracer methods (48).

---

**FIGURE 4.** Postprandial urea nitrogen (N) kinetics and salvage in subjects ingesting different protein sources in the meal after adaptation to different protein levels in the diet. Values are expressed in g N over the 8-h postprandial period: values are means ± SDs over 4 groups of subjects receiving milk ($n = 8$), soy ($n = 8$), or wheat ($n = 8$) protein in the meal after prior adaptation to a normal or high protein intake (4 groups in studies 1 and 2; see Subjects and Methods for explanations). Urea N salvage is estimated by assuming that it is superior to 80% of urea nitrogen hydrolysis, because it has been reported that >80% of the urea N hydrolyzed does not return directly to urea formation but is reincorporated into metabolic N (9, 33, 50). Total postprandial urea nitrogen hydrolysis averaged 2.7 g N at 8 h in the different groups, so that urea salvage should provide at least 2.2 g N over 8 h, ie, one-half of the initial N load of 4.3 g N given in the meal.
positive linear relation, in line with previous reports (45, 72). The extent to which UP might drive UH or UH might drive UP is an important question that has already been raised when studying UP and UH on a 24-h basis (27, 45). In our work, we focused on the acute UP and UH kinetics during the first postmeal hours, which were linked together in a homeodynamic sequence. Because of this postprandial setting, UP was affected first, being mechanistically stimulated by the amino acid inflow consequent to the meal. UP then drove UH, with time courses of increase in UH lagging slightly behind those of UP. Therefore, reverse causality (ie, UH driving UP) must be rejected in our postmeal context. Last, the hydrolysis efficiency (UH:UP) was higher after the ingestion of plant versus animal protein, indicating that it was stimulated in response to an imbalanced amino acid intake, which is an important new finding. It was also higher when the habitual protein intake was lower, as previously described in healthy adults (7, 18) and children recovering from malnutrition (4, 24).

This study has some limitations. Focusing on the postprandial period allowed us to disclose an acute dynamic variation in urea metabolism that may have a large effect on nitrogen metabolism. However, possible further adaptation in the postabsorptive state was out of the scope of the study. In addition, whereas UH is a critical step, it is higher than the true salvage that the model could not estimate. We could therefore not directly conclude on the extent of the final nitrogen sparing effect induced by the herein shown modulation. However, because postprandial changes in UH counterbalanced those in UP, with an efficiency that was even more important when the usual protein intake was lower, we propose that postprandial UH is an acute nitrogen-sparing mechanism, which is tightly regulated. These results emphasize the potentially important role played by urea metabolism in regulating body nitrogen homeostasis (4, 17, 18). Given that UH averaged 2.7 g N at 8 h between groups, and taking account that <20% of hydrolyzed urea nitrogen returns directly to urea formation (9, 33, 50), urea salvage should provide at least 2.2 g N over 8 h, ie, one-half of the initial nitrogen load in the meal (Figure 4).

In conclusion, we have developed a novel method based on the use of 15N-labeled proteins and the mathematical analysis of kinetic data in serum proteins, body, and urinary urea to assess urea kinetics in the postprandial non–steady state. We show that UH accounts for a substantial proportion of UP in the fed state, and that postprandial urea kinetics are modulated by different factors of the protein intake. Whereas UH is mostly a function of the level of dietary protein, carbohydrate and fat on urea kinetics in young children during rapid catch-up weight gain. Br J Nutr 1990;64: 371–85.


Torrallardona D, Harris CI, Coates ME, Fuller MF. Microbial amino acid synthesis and utilization in rats: incorporation of 15N from 15NH4Cl into lysine in the tissues of germ-free and conventional rats. Br J Nutr 1996;76:689–700.


42. Cobelli C, Caumo A. Using what is accessible to measure that which is not: necessity of model of system. Metabolism 1998;47:1009–35.
parameters relative to dietary N kinetics will be marked with an asterisk, in contrast with those relative to total N kinetics, which will not be marked. Eq A1 to A3 (Eq A1* to A3*) are the following:

\[
\frac{dBU}{dt}(t) = up(t) - ue(t) - uh(t) \quad (A1)
\]
and

\[
\frac{dBU^*}{dt}(t) = up^*(t) - ue^*(t) - uh^*(t) \quad (A1^*)
\]

\[
\frac{dUU}{dt}(t) = ue(t) \quad (A2)
\]
and

\[
\frac{dUU^*}{dt}(t) = ue^*(t) \quad (A2^*)
\]

\[
\frac{dSEP}{dt}(t) = sSEP(t) - dSEP(t) \quad (A3)
\]
and

\[
\frac{dSEP^*}{dt}(t) = sSEP^*(t) - dSEP^*(t) \quad (A3^*)
\]

with

\[
up(t) = k_{UP} \times SA(t) \quad (A4)
\]
and

\[
up^*(t) = k_{UP^*} \times SA^*(t), \quad (A4^*)
\]

\[
ue(t) = k_{UE} \times BU(t) \quad (A5)
\]
and

\[
ue^*(t) = k_{UE^*} \times BU^*(t), \quad (A5^*)
\]

\[
uh(t) = k_{ Uh} \times BU(t) \quad (A6)
\]
and

\[
uh^*(t) = k_{ Uh^*} \times BU^*(t), \quad (A6^*)
\]

and

\[
s_{SEP}(t) = k_{SEP} \times SA(t) \quad (A7)
\]
and

\[
s_{SEP^*}(t) = k_{SEP^*} \times SA^*(t) \quad (A7^*)
\]

\[
d_{SEP}(t) = k_{dSEP} \times SEP(t) \quad (A8)
\]
and

\[
d_{SEP^*}(t) = k_{dSEP^*} \times SEP^*(t) \quad (A8^*)
\]

where BU (BU*), UU (UU*), SA (SA*), and SEP (SEP*) are the amounts of total (dietary) N in body urea, urinary urea, splanchnic free amino acids, and splanchnic-exported proteins, respectively; up (up*), ue (ue*), and uh (uh*) are the fluxes of total (dietary) urea N production, excretion, and hydrolysis, respectively; s_{SEP} (s_{SEP}*) and d_{SEP} (d_{SEP}*) are the fluxes of total (dietary) N incorporation into splanchnic-exported proteins and its subsequent disappearance from the intravascular pool (by protein degradation or distribution within the total exchangeable intra- and extracellular protein pools), respectively; k_{UP} (k_{UP}^*), k_{UE} (k_{UE}^*), and k_{Uh} (k_{Uh}^*) are the rates of total (dietary) urea N production, excretion, and hydrolysis, respectively; k_{SEP} (k_{SEP}^*) and k_{dSEP} (k_{dSEP}^*) are the rates of total (dietary) N incorporation into splanchnic-exported proteins and its subsequent disappearance from the intravascular pool, respectively. Let \( f(t) = f(t) - f(0) \). Then, by using Eq A4 to A8 (Eqs A4* to A8*), Eqs A1 to A3 (Eq A1* to A3*) turn into:

\[
k_{UP} \times \frac{\Delta SEP(t) + k_{dSEP} \times \int_0^t SEP(v) dv}{k_{SEP}} - \frac{k_{UE} \times \Delta UU(t)}{k_{UE}} = \Delta BU(t) + \Delta UU(t), \quad (A9)
\]

\[
k_{UP^*} \times \frac{\Delta SEP^*(t) + k_{dSEP^*} \times \int_0^t SEP^*(v) dv}{k_{SEP^*}} - \frac{k_{UE^*} \times \Delta UU^*(t)}{k_{UE^*}} = \Delta BU^*(t) + \Delta UU^*(t) \quad (A9^*)
\]

By integrating Eq A5 and A5*, one can isolate the parameters \( k_{UE} \) and \( k_{UE^*} \):

\[
k_{UE} = \frac{\Delta UU(t)}{\int_0^t BU(v) dv} \quad (A10)
\]
and

\[
k_{UE^*} = \frac{\Delta UU^*(t)}{\int_0^t BU^*(v) dv} \quad (A10^*)
\]

All of the equations presented above can be written at each sampling time. This implies that the parameters involved in these equations can be calculated by solving systems of linear equations, where the number of equations is higher than the number of unknowns. For instance, Eq A9* can be solved 8 times (each h for 8 h) by using data on SEP, BU, and UU, which is enough to determine the 3 unknowns in this equation (\( k_{UP}^*/k_{dSEP}^*, k_{dSEP^*}, \) and \( k_{UE}^*/k_{UE^*}^* \)). Because most of these systems of linear equations were badly conditioned (i.e., they had large condition numbers, a commonly encountered problem), we did not use the classic method of resolution, requiring inversion of the system matrix. Instead, because resolution of the following system (corresponding to the resolution of each equation at each sampling time \( i = 1 \) to \( n \))

\[
\left\{ \begin{array}{l}
ft(x_1, \ldots, x_p) = 0 \\
\vdots \\
f_n(x_1, \ldots, x_p) = 0 
\end{array} \right.
\]

can be down to a search for the minimum of the following function:

\[
h(x_1, \ldots, x_p) = f_1^2(x_1, \ldots, x_p) + \ldots + f_n^2(x_1, \ldots, x_p)
\]

we estimated the parameters involved in Eqs A9 and A10 (Eqs A9* and A10*) by solving minimization problems with a classic resolution method implemented under Matlab. We thus calculated the parameters \( k_{UP}^*/k_{dSEP}^*, k_{dSEP^*}, \) and \( k_{UE}^*/k_{UE^*}^* \) from Eq A9* and A10*.

Knowing the values of \( k_{UE}^* \) and \( k_{UE^*}^* \) and using the dietary N data obtained for compartment BU, one can determine the instantaneous fluxes of dietary urea N excretion (ue*) and hydrolysis (uh*) from Eq A5* and A6*, respectively. The cumulated flux of dietary urea N excretion UE* (t) can be determined over the 8-h postprandial period from the dietary N data obtained for compartment BU, as UE* (t) = UE* (t). Knowing the values of \( k_{UE}^* \) and \( k_{UE^*}^* \), the cumulated flux of dietary N hydrolysis Uh (t) can then be calculated over the 8-h postprandial period, as Uh (t) = (k_{Uh}^*/k_{UE^*}^*) x UE* (t). By
integrating Eqs A1*, one can finally calculate the cumulated flux of dietary urea N production (UP*) over the 8-h postprandial period, as
\[ \text{UP}^*(t) = \Delta \text{BU}^*(t) + \text{UE}^*(t) + \text{UH}^*(t). \]

Because the urea molecules synthesized in the liver from either dietary or endogenous origin are rapidly mixed within the general BU pool and then become indistinguishable in terms of their subsequent intestinal metabolism, we assumed that:
\[ k_{\text{UH}} = k_{\text{UH}^*} \quad (A11) \]

As detailed above for dietary urea N kinetics, the cumulated fluxes of total urea N excretion (UE), hydrolysis (UH), and production (UP) can be estimated over the 8-h postprandial period, as follows:
\[ \text{UE}(t) = \text{UU}(t), \quad (A12) \]
\[ \text{UH}(t) = (k_{\text{UH}}/k_{\text{UH}^*}) \times \text{UE}(t), \quad (A13) \]
and
\[ \text{UP}(t) = \Delta \text{BU}(t) + \text{UE}(t) + \text{UH}(t) \quad (A14) \]