

# Postprandial metabolic utilization of wheat protein in humans<sup>1-3</sup>

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## ABSTRACT

**Background:** The quality of cereal protein has been little studied in humans despite its quantitative importance in the diet, particularly in developing countries.

**Objective:** The objective of this study was to determine the nutritional value of wheat protein in humans as assessed by the measurement of their real ileal digestibility and postprandial retention.

**Design:** Healthy young adults ( $n = 14$ ) were fitted with an intestinal tube to allow the collection of intestinal fluid in the duodenum or terminal ileum. Subjects received a mixed meal of 136 g wheat toast that contained 24.6 g uniformly and intrinsically [<sup>15</sup>N]-labeled wheat protein. Intestinal fluid, blood, and urine were collected for 8 h postprandially.

**Results:** The real ileal digestibility of dietary wheat nitrogen amounted to  $90.3 \pm 4.3\%$ . The cumulative amount of dietary nitrogen transferred to the deamination pools reached a plateau at 8 h of  $24.7 \pm 6.8\%$  of the amount ingested. The urinary excretion of dietary nitrogen in ammonia was high ( $0.8 \pm 0.3\%$  of ingested dose). The incorporation of dietary nitrogen into serum protein reached  $7.0 \pm 1.9\%$  of the meal. Postprandial wheat protein retention was  $66.1 \pm 5.8\%$ .

**Conclusions:** Our results show that wheat proteins had the same true ileal digestibility as did most of the plant proteins already studied in humans, but also that they had a lower postprandial nitrogen retention value. However, this low value was higher than that predicted from the calculation of indispensable amino acid scores, ie, 89% rather than 30–40% of the nutritional value of milk proteins. *Am J Clin Nutr* 2005;81:87–94.

**KEY WORDS** Nitrogen metabolism, dietary nitrogen, wheat protein, nonsteady state, urea production, protein quality, humans

## INTRODUCTION

Cereals are an important dietary protein source throughout the world, because they constitute the primary protein and energy supply in most developing countries. Cereals also make a significant contribution (of nearly 20%) to the daily protein intake in developed countries, mainly in the form of bread, pasta, rice, and breakfast cereals (1).

Wheat contains moderate amounts of protein (8–12% on a weight basis) mainly composed of storage proteins or gluten proteins (80–85% of total wheat protein), classified as gliadins and glutenins. These are also referred to as “prolamins” because of their high content in glutamine and proline, which occurs at the expense of indispensable amino acids (AAs); particularly lysine, and to a lesser extent threonine. This lysine deficiency is a common feature of all cereal proteins, with values of 22–35 mg/g protein in

wheat, 30–36 mg/g protein in rice, and 28–42 mg/g protein in maize. Although the lysine intake is evaluated at  $\sim 100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  in a typical Western diet, this intake only attains  $44 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  in British vegetarians and is likely to be much lower in countries with a cereal-based diet (2). In well-nourished young Indians, a habitual daily lysine intake of  $53 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  was recorded, but this intake falls to  $39 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  when measured in chronically undernourished Indian men (3, 4). Depending on the method used, lysine requirements in adults range from 12 to  $45 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (3–9). Thus, because of their low protein and lysine content, covering protein and lysine requirements of subjects eating predominantly cereal-based diets is an important public health issue.

The capacity of wheat protein to fulfill the protein requirements of humans was traditionally determined by using nitrogen balance methods in both humans and animals (10, 11). Although a valid method, it is not the most sensitive in assessing protein nutritional quality. In the late 1980s, the FAO/WHO proposed the limiting indispensable AA scoring method for protein quality evaluation (12). On the basis of current data, the lysine content of dietary proteins which covers the lysine requirements at a safe level of  $0.8 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  is within the range of 15–58 mg lysine/g protein, and under these conditions the quality indexes of gluten and whole wheat protein are 26–40%, respectively (13). More recently, a [<sup>13</sup>C]leucine balance protocol was applied to comparisons of wheat gluten and milk protein and showed that the wheat protein score was 61% that of milk proteins. However, these results were based on several assumptions, including the estimated AA availability of the 2 dietary proteins, and were derived from an indirect method because the nitrogen balances were extrapolated from leucine metabolism (9, 14).

To date, the ileal bioavailability and postprandial utilization of wheat protein were not addressed in humans, and the nutritional

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**TABLE 1**  
Subject characteristics<sup>1</sup>

	Whole group (n = 14)	Ileal group (n = 9)
Sex (F/M)	6/8	3/6
Age (y)	25 ± 5 <sup>2</sup>	26 ± 6
Body weight (kg)	65.1 ± 12.4	64.9 ± 13.5
Height (m)	1.74 ± 0.10	1.75 ± 0.09
BMI (kg/m <sup>2</sup> )	21.3 ± 2.4	21.1 ± 2.8
Fat-free mass (kg)	54.1 ± 12.4	53.1 ± 12.6
Percentage of fat mass (%)	19.7 ± 8.3	18.4 ± 8.4
Total body water (L)	37.3 ± 9.9	37.3 ± 9.5

<sup>1</sup> Body composition was assessed by bioelectrical impedance analysis.

<sup>2</sup>  $\bar{x} \pm$  SD (all such values).

value of wheat protein is still not clearly and directly determined, so that current opinions diverge, depending on the method used (9, 12, 13). For these reasons, during this study, we determined the quality of wheat protein by assessing its true ileal digestibility in healthy human subjects and its subsequent postprandial metabolism and retention by using intrinsically [<sup>15</sup>N]-labeled wheat protein given in a single meal to healthy young adults equipped with an ileal tube.

## SUBJECTS AND METHODS

### Subjects

Fourteen subjects (6 women, 8 men) were enrolled in the study after a thorough medical examination and routine blood tests. Their mean age was 25 y and their body mass index (BMI; in kg/m<sup>2</sup>) was 21.3 ± 2.4 (Table 1). All subjects received detailed information on the protocol and gave written informed consent to their participation to the study. The protocol was approved by the Institutional Review Board for St-Germain-en-Laye Hospital, France.

### Meals

The test meals comprised 136 g wheat toasts (in the form of biscottes, a typical French breakfast ingredient resembling toast) that provide 24.6 g protein (298 mmol N), uniformly and intrinsically labeled with [<sup>15</sup>N] (enrichment: 0.6485 atom %). The [<sup>15</sup>N]-labeled wheat was prepared by ARVALIS, the Plant Institute (Institut du Végétal, Paris, France). [<sup>15</sup>N]-wheat flour was enriched with [<sup>15</sup>N]-gluten to produce wheat toast with the use of a standard recipe. Wheat biscuits were prepared by Danone, Centre Jean Thèves (Athis-Mons, France). The profile of protein fractions was determined by using the Profilblé method (size exclusion-HPLC) as follows: 16% high-molecular-weight glutenin subunits; 24% low-molecular-weight glutenin subunits; 8%  $\omega$ -gliadins; 40%  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins; and 12% albumins and globulins. The energy content of the meal was 2448 kJ of which 54.7% was carbohydrate (starch), 16.8% protein, and 28.5% fat.

### Protocol

The subjects arrived at the hospital at 0900 the day before the experiment, in a fasted state (day 1). An intestinal tube was passed through the nose under local anesthesia and was allowed to progress through the digestive tract for the next 24 h by gravity

and peristaltic contractions, as described previously (15). The subjects were given meals at 1200 and 1900 and then fasted overnight. Body composition was measured by using bioelectrical impedance analysis (Anlycor 5w; Eugedia, Spengler, Cachan, France) in subjects lying at rest for >20 min. On the experimental day (day 2), the position of the tube in the intestine was checked under X-ray. In 9 subjects, the tube had correctly reached the terminal ileum (mean tube length from the nose: 186 ± 21 cm). In 5 subjects, the tube was stopped at the duodenum level (86 ± 5 cm from the nose) to assess the stomach delivery kinetics of the meal nitrogen. The protocol started at 1000, when a saline solution (130 mmol NaCl/L, 5 mmol KCl/L, 30 mmol D-mannitol/L) containing 400 mg phenol red/L (or phenolsulfonphthalein, PSP) was infused continuously through one lumen of the tube at a constant rate of 1 mL/min. The collection of a 30-min baseline sample of intestinal fluid started as soon as the marker (PSP) appeared in the second (collection) lumen (*t*<sub>30</sub>). At time zero, after a baseline blood sample and urine collection, the test meal was offered to the subjects. The meal was ingested within 30 min and was accompanied by 300 mL water. Intestinal fluid, blood, and urine sampling lasted for 8 h in the subjects with the ileal tube and for 6 h in the subjects with the duodenal tube. Digesta samples were collected over ice and pooled over 30-min periods. After volume measurement, samples were divided into aliquots and frozen at -20 °C. Blood was sampled every 30 min for 3 h and then every hour for the 5 remaining hours. Plasma and serum samples were obtained after centrifugation, divided into aliquots, and frozen at -20 °C. Total urine was collected every 2 h throughout the 8-h postprandial period. Urine specimens were weighed, divided into aliquots, and kept at -20 °C until analysis.

### Analytic methods

The concentration of PSP in digesta samples was determined by using a colorimetric method (16). Plasma glucose was assayed by a glucose oxidase method (Glucose GOD-DP kit; Kone, Evry, France). Urea concentrations were assayed in both serum and urine by using an enzymatic method on a Dimension automat (du Pont de Nemours, Les Ulis, France). Ammonia was measured in the urine by an enzymatic method on a Kone instrument (Kone). Amino acid concentrations were determined by HPLC with post-column ninhydrin derivatization (Biotek Instruments, St Quentin-en-Yvelines, France) in deproteinized serum samples. For isotopic determinations, urea and ammonia were isolated from urine as previously described, using a sodium and potassium form of the cation exchange resin (BioRad Dowex AG-50X8, mesh 100–200; Interchim, Montluçon, France) (17). Serum separation of nitrogen fractions (protein nitrogen, free nitrogen, and urea nitrogen) was achieved by adding 200  $\mu$ L of a 1 g 5-sulfo-salicylic acid/mL solution to 4 mL serum. After 1 h at 4 °C, the samples were centrifuged (2400g for 20 min at 4 °C), and the pellet was dried and weighed for the quantification of total nitrogen in the protein fraction. The supernatant fraction was titrated to pH 7 by the addition of 1 mol NaOH/L and 0.1 mol NaH<sub>2</sub>PO<sub>4</sub>/L and transferred on 0.5 mL cation exchange resin, and the urea was hydrolyzed with 16  $\mu$ L urease (680 U/mL; Sigma-Aldrich, Saint-Quentin Fallavier, France) for 2 h at 30 °C. The supernatant containing serum-free nitrogen was collected and dried. Before isotopic determination, ammonia and urea-derived ammonia were eluted from the resins by the addition of 2.5 mol KHSO<sub>4</sub>/L. The total nitrogen content of the digesta and

the serum protein fraction was determined by using an elemental nitrogen analyzer (NA 1500 series 2; Fisons Instruments, Manchester, United Kingdom) with atropina as standard. The isotope ratio of [<sup>15</sup>N] to [<sup>14</sup>N] was determined by isotope-ratio mass spectrometry (Optimal Fisons Instruments) in the digesta, urinary urea and ammonia, serum protein, free nitrogen, and urea. The atom percent excess (APE) of the samples was calculated by subtracting the baseline value from the atom percent determined at each time point.

### Calculations

Data are expressed as means ± SDs. The total intestinal flow rate (*F*, in mL/30 min) was derived for each 30-min period from the dilution of PSP, estimated by the following equation:

$$F = (PSP_i/PSP_s) \times Fi \times t \quad (1)$$

where *PSP<sub>i</sub>* and *PSP<sub>s</sub>* are the PSP concentrations in the infusion solution and sample, respectively, *Fi* is the infusion rate (1 mL/min), and *t* is the collection duration (30 min).

The total nitrogen content of duodenal or ileal digesta (mmol nitrogen/30 min) was derived from the formula

$$N_{tot-digesta} = (N_s \times DM_s \times F)/140 \quad (2)$$

where *N<sub>s</sub>* is the nitrogen percentage measured (g/100 g) in the freeze-dried sample, *DM<sub>s</sub>* is the dry matter of the sample (g/100 mL), and *F* is the intestinal flow rate.

The time course of dietary nitrogen incorporation (expressed as a percentage of the ingested amount) into the different body nitrogen pools monitored (digesta, serum protein and free AA, body urea, urinary urea and ammonia) was evaluated by the following equation:

$$N_{diet}(t) = N_{tot}(t) \times [APE(t)/APE_{meal}]/N_{ingested} \times 100 \quad (3)$$

where *N<sub>tot</sub>(t)* is the total nitrogen content of the pool (in mmol nitrogen) at each time point *t*, *APE(t)* is the [<sup>15</sup>N] enrichment above baseline in the nitrogen pool sampled at time *t*, *APE<sub>meal</sub>* is the [<sup>15</sup>N] enrichment of the meal, and *N<sub>ingested</sub>* is the nitrogen content of the meal (in mmol nitrogen). The total nitrogen content in urinary urea and ammonia was obtained by multiplying the volume of urine by the corresponding urea and ammonia nitrogen concentrations. The total nitrogen content in the serum protein pool was calculated as the product of the nitrogen concentration in this fraction and the plasma volume, estimated at 5% of body weight (18). The total body urea nitrogen pool was calculated as the plasma urea nitrogen concentration multiplied by its volume of distribution (total body water), corrected by a factor of 92%, which represents the water content of blood.

The cumulated recovery of dietary nitrogen in ileal samples ( $\Sigma N_{diet-ileal}$ ) served to calculate the real ileal digestibility (RID; percentage of ingested nitrogen) of wheat protein:

$$RID = (N_{ingested} - \Sigma N_{diet-ileal})/N_{ingested} \times 100 \quad (4)$$

Total urea production (mmol N · kg BW<sup>-1</sup> · 2 h<sup>-1</sup>) was evaluated for the four 2-h periods after meal ingestion by the following equation:

$$UP_{tot\ t-(t+2)} = [UU_{tot\ t-(t+2)} + (BU_{tot\ (t+2)} - BU_{tot\ t})]/BW \quad (5)$$

where *UU<sub>tot t-(t+2)</sub>* is the cumulative amount of urinary urea excreted between time *t* and *t+2* h, *BU<sub>tot t</sub>* and *BU<sub>tot (t+2)</sub>* represent the body urea pool sizes at *t* and *t+2* h, and *BW* is the body weight.

Urea production of dietary origin (*UP<sub>diet</sub>*, in mmol N · kg BW<sup>-1</sup> · 2 h<sup>-1</sup>) was calculated for each 2-h period as follows:

$$UP_{diet\ t-(t+2)} = [UU_{diet\ t-(t+2)} + (BU_{diet\ (t+2)} - BU_{diet\ t})]/BW \quad (6)$$

where *UU<sub>diet t-(t+2)</sub>* is the cumulative amount of dietary nitrogen excreted in urinary urea between time *t* and *t+2* h, and *BU<sub>diet t</sub>* and *BU<sub>diet (t+2)</sub>* represent dietary nitrogen in the body urea pool at *t* and *t+2* h.

Endogenous urea production (*UP<sub>endo</sub>*, in mmol N · 2 h<sup>-1</sup> · kg<sup>-1</sup>) was estimated for each 2-h period from the difference between total urea and dietary urea productions:

$$UP_{endo\ t-(t+2)} = UP_{tot\ t-(t+2)} - UP_{diet\ t-(t+2)} \quad (7)$$

At the end of the 8-h experimental period, the amount of dietary nitrogen retained in the body or net postprandial protein utilization (NPPU) was calculated as follows:

$$NPPU(\% \text{ of ingested nitrogen}) = (\text{Meal nitrogen intake} \times RID/100 - UP_{diet})/\text{Meal nitrogen intake} \quad (8)$$

The postprandial biological value (PBV) was calculated as the relative amount of dietary nitrogen absorbed that was not deaminated during the postprandial period:

$$PBV(\% \text{ of ingested nitrogen}) = NPPU/RID \times 100 \quad (9)$$

Because of the large volume of intestinal fluid collected in subjects with the tube positioned at the duodenal site (representing 10% of ingested nitrogen), the systemic data for these subjects were not used.

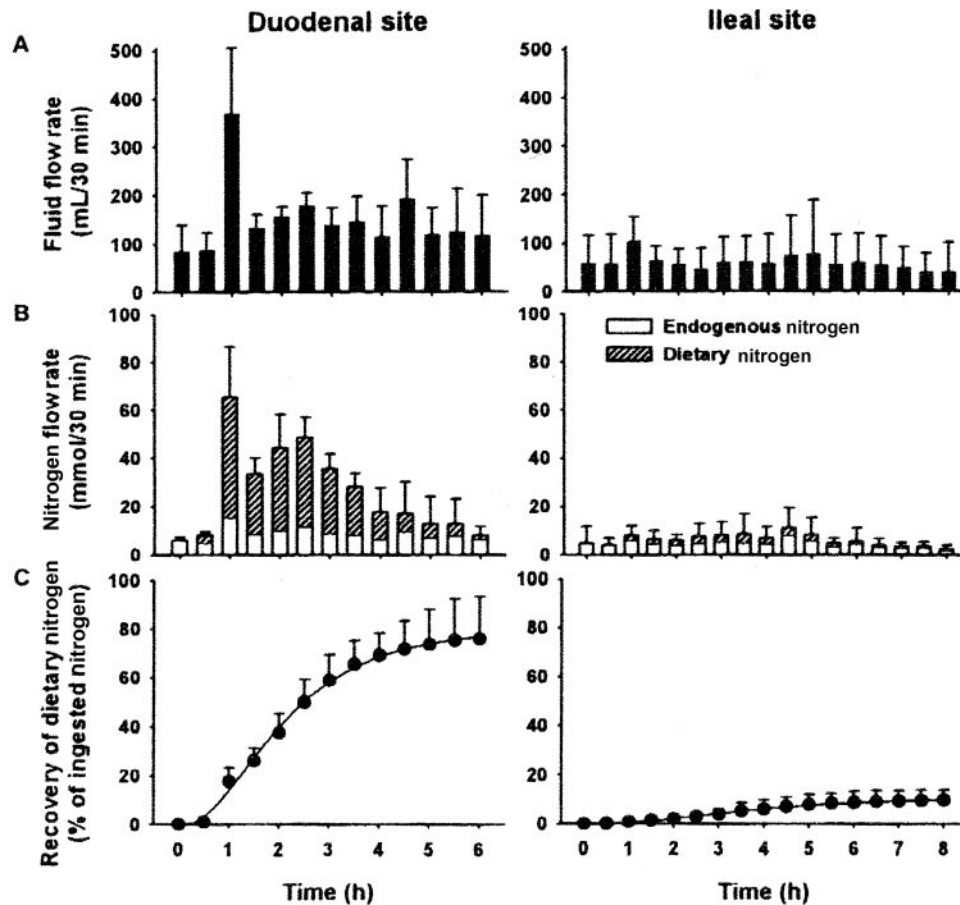
### Curve fitting

The time courses of dietary nitrogen transfer into body nitrogen pools were fitted by using equations of the  $y = a \cdot e(-1/2[\ln(t/b)/c]^2)$  type for body urea nitrogen pool and by equations of the  $y = a \cdot [1 - e(-b \cdot t)]^c$  type for serum-free nitrogen and protein-bound nitrogen, urinary urea, and urinary ammonia nitrogen (SIGMAPLOT software, version 6.00; SPSS Inc, Chicago).

### RESULTS

The intestinal liquid flow rate and nitrogen kinetics after the meal are depicted in **Figure 1**. At the duodenal site, the intestinal fluid flow rate peaked early (370 mL/30 min), 1 h after the meal, whereas in the ileum, the liquid flow rate was constant, with a mean of 60 mL/30 min. In the duodenum, dietary nitrogen peaked at 1 h and 2.5 h after the meal and was the predominant component of nitrogen transit (64–77% between 1 and 4 h). In the ileum, the dietary nitrogen flux reached its maximum 4.5 h after the meal. The proportion of dietary nitrogen to total nitrogen averaged 28%. The half-time of dietary nitrogen transit through the duodenum was 117 ± 20 min. At the end of the experimental period, the cumulated amounts of dietary nitrogen which had





**FIGURE 1.** Mean ( $\pm$  SD) intestinal liquid flow rate (A), endogenous and dietary nitrogen flow rate (B), and cumulated recovery of dietary nitrogen (C) after the ingestion of 298 mmol [ $^{15}\text{N}$ ]-labeled wheat protein in a single mixed meal in subjects with an intestinal tube, the extremity of which was located in the duodenum ( $n = 5$ ) or the ileum ( $n = 9$ ).

transited through the duodenum and ileum were  $75.8 \pm 17.4\%$  and  $9.7 \pm 4.3\%$  of ingested nitrogen (Figure 1). If the amounts were compared,  $24.2 \pm 17.4\%$  of ingested nitrogen was absorbed at the duodenal level, and the RID of wheat nitrogen was  $90.3 \pm 4.3\%$ .

Plasma glucose exhibited a two-phasic postprandial profile consistent with the intestinal nitrogen kinetics, with a first peak at 1 h and a second peak 2.5 h after the meal (Figure 2). In contrast, serum total amino acid concentration was only slightly increased 1 h after the meal and then decreased below the baseline amount. Serum lysine concentration decreased for the first 3 h after the meal from its fasting concentration ( $220 \mu\text{mol/L}$ ) to  $\approx 140 \mu\text{mol/L}$  and was stable thereafter (Figure 2).

Time courses of isotopic [ $^{15}\text{N}$ ] enrichments were measured in intestinal, systemic, and urinary nitrogen pools (Figure 3), which allowed for the quantification of dietary nitrogen in each pool. The incorporation of dietary nitrogen into the serum-free AA pool increased during the first 3 h after meal ingestion, reached a maximum of 0.56% of ingested nitrogen, and then slowly and linearly declined (Figure 4). The time course of dietary nitrogen incorporation into the serum protein pool was characterized by a sigmoid shape and a plateau that was reached after 7 h, with a value of  $7.0 \pm 1.9\%$  of the ingested amount.

Urea and ammonia pools were also monitored to measure the time course of wheat-derived AA deamination (Figure 5). Dietary nitrogen transfer into body urea increased during the first

3 h and reached a quasi-plateau from 3 to 5 h after the meal, peaking at  $16.5 \pm 4.4\%$  of the ingested nitrogen, and then declining slowly for the last 3 h to  $11.9 \pm 5.0\%$ . In parallel, the excretion of dietary nitrogen in urinary urea increased during the postprandial period and reached  $11.0 \pm 3.4\%$  at 8 h, which represented 65% of the plateau value, as indicated by curve fitting ( $y = 16.9[1 - \exp^{-0.261t}]^{3.33}$ ). Thus, the total transfer of dietary nitrogen to urea at 8 h was  $23.6 \pm 6.3\%$  of ingested nitrogen. The transfer of dietary nitrogen into urinary ammonia occurred regularly throughout the postprandial period and almost reached the plateau value at 8 h, with  $0.8 \pm 0.3\%$  of ingested nitrogen recovered in this pool (Figure 5).

The rate of both endogenous and exogenous (dietary) urea production was computed for 2-h periods after meal ingestion (Figure 6). Urea production of dietary origin reached its maximum during the first 2 h ( $0.54 \pm 0.22 \text{ mmol N} \cdot \text{kg}^{-1} \cdot 2 \text{ h}^{-1}$ ) and decreased over the next 4 h. It was null over the last 2 h of the postprandial period. Endogenous urea production was relatively constant throughout the postprandial period.

In summary, the metabolic utilization of dietary nitrogen after the ingestion of a wheat meal was characterized by losses of  $33.9 \pm 5.8\%$  (9.7% ileal losses and 24.7% deamination losses) and by a retention, or net postprandial protein utilization, of  $66.1 \pm 5.8\%$  (Table 2). Postprandial biological value reached  $72.9 \pm 6.9\%$ . Cumulated total (ie, endogenous and exogenous) nitrogen losses were  $413 \pm 118 \text{ mmol}$  8 h after the wheat meal.

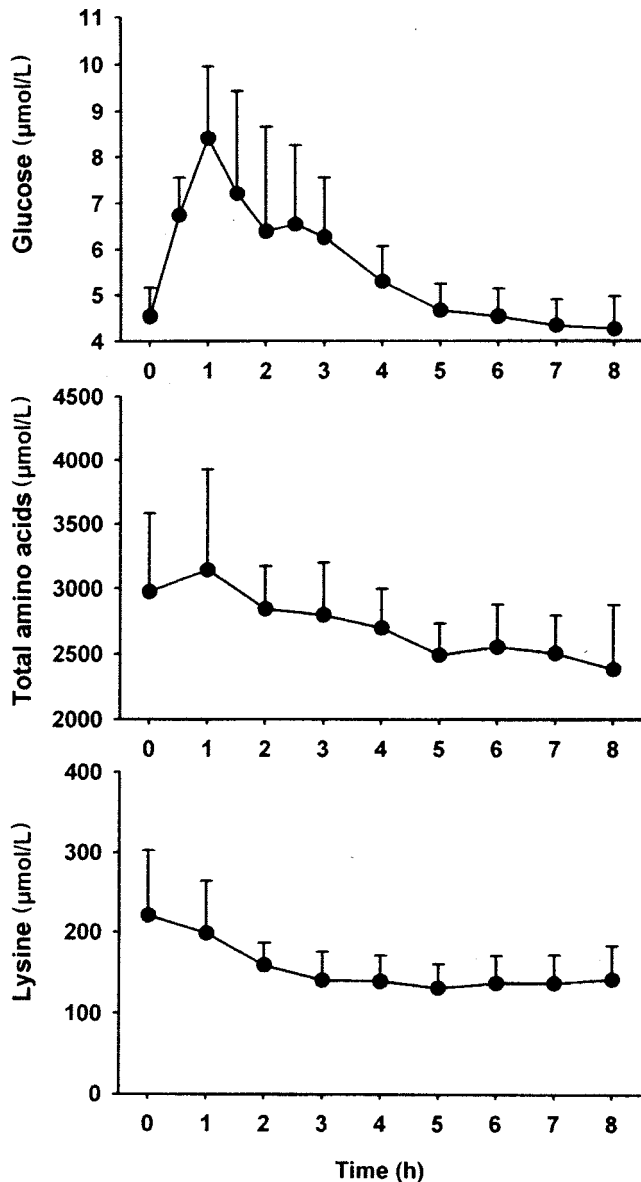


FIGURE 2. Mean ( $\pm$  SD) plasma glucose and serum total amino acid and lysine concentrations in subjects ingesting a single wheat-based meal ( $n = 9$ ).

Dietary nitrogen losses through the ileum represented 30% of total ileal losses. With respect to deamination losses, the contribution of dietary nitrogen to total losses was 22% throughout the 8 postprandial hours (Figure 7). Cumulated nitrogen losses equalled the amount provided by the meal 5 h after ingestion. Consequently, 8 h after the meal, subjects were in a negative nitrogen balance, reaching 106 mmol.

## DISCUSSION

This work constitutes the first complete and direct characterization of wheat protein bioavailability and utilization in healthy humans, as measured after a single meal containing intrinsically [ $^{15}\text{N}$ ]-labeled proteins, with sequential monitoring of dietary nitrogen transit through intestinal, serum, and urinary nitrogen

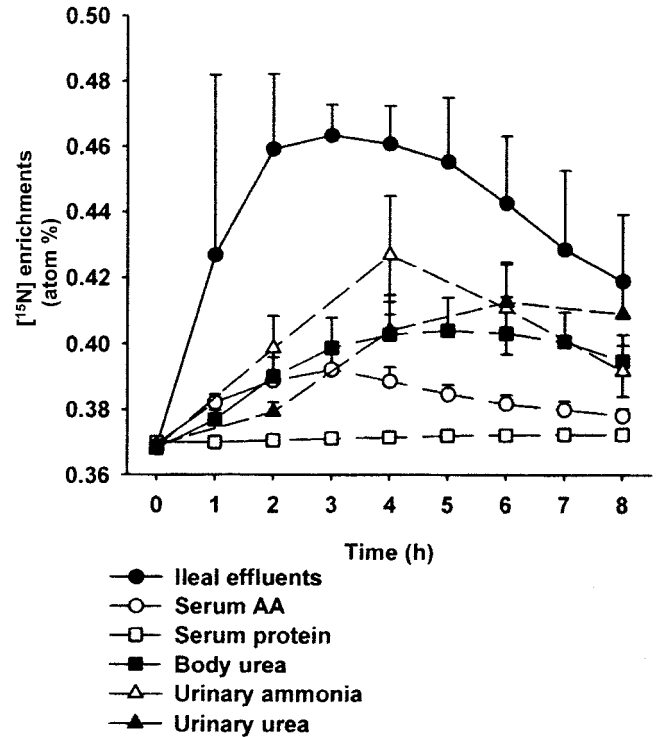


FIGURE 3. Mean ( $\pm$  SD) time courses of [ $^{15}\text{N}$ ] isotopic enrichments measured in ileal effluents, serum amino acids and protein, body urea, and urinary urea and ammonia after the ingestion of 298 mmol [ $^{15}\text{N}$ ]-labeled wheat protein in a single mixed meal in human subjects ( $n = 9$ ).

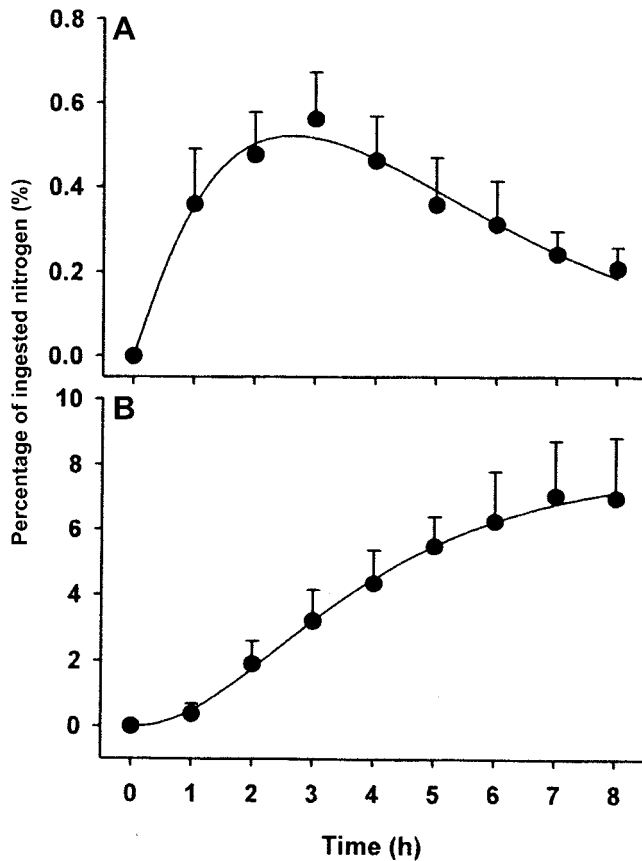
pools. The RID of wheat proteins reached 90%, which is a standard amount for the digestibility of plant proteins. The metabolic utilization of absorbed, wheat-derived AA was directed toward deamination for 25% of the amount ingested. The net postprandial retention of wheat protein was then calculated as 66%.

## Real wheat nitrogen ileal digestibility

The RID of wheat protein nitrogen was found to reach 90.3%, which is close to that of other plant proteins measured by using the same method in humans: 91.5% and 90% for soy and pea protein isolates, 89% and 91% for pea and sweet lupin flours, respectively (17, 19–21). The value obtained fell within the large data scale of true wheat ileal digestibility obtained in pigs or minipigs (83–95%) or using the rat balance method (91–93%) (13, 22–26). A critical point of our study was whether the global value for nitrogen reflected individual AA digestibilities (27). When both were measured in animals being fed wheat, it appeared that lysine was up to 14% less digestible than nitrogen (23, 25, 26). As a result, the availability of wheat protein-limiting AA may be lower than the availability of total nitrogen, with possible consequences on further metabolic utilization.

The digestibility of wheat protein nitrogen was “standard” for well-processed plant proteins, consistent with the lack of any antinutritional factors or digestion-resistant protein fractions. However, the present estimate of wheat protein digestibility was made under conditions of optimum digestion, including the refining of wheat flour, the absence of added fiber from the meal, and moderate heat treatment, the latter being likely to improve protein hydrolysis by intestinal proteases. As a result, the value



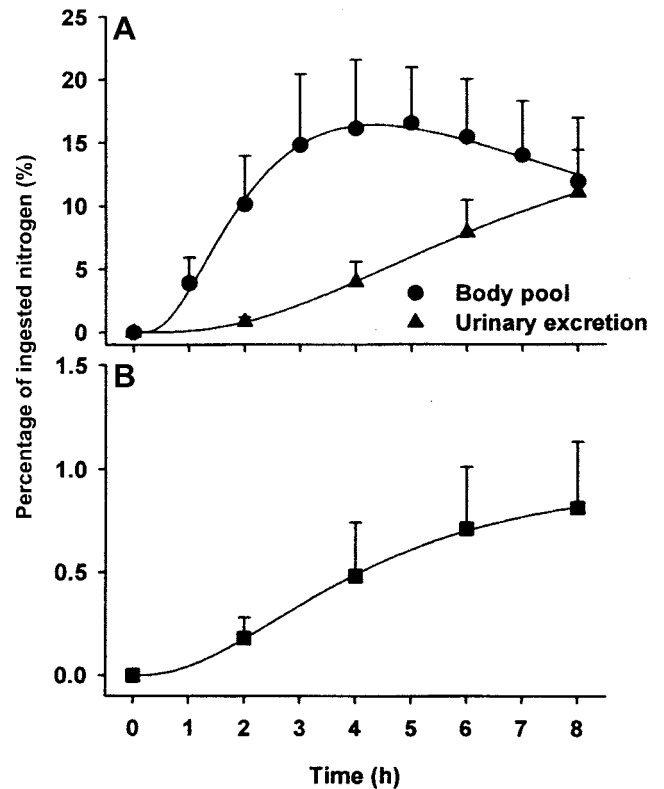


**FIGURE 4.** Mean ( $\pm$  SD) time course of dietary nitrogen incorporation into serum-free nitrogen (A) and protein-bound nitrogen pools (B) after the ingestion of 298 mmol [ $^{15}\text{N}$ ]-labeled wheat protein in a single mixed meal in human subjects ( $n = 9$ ).

found may be superior to that applicable to wheat products consumed in other parts of the world where higher contents in fibers and antinutritional factors lower the true protein digestibility values (12, 28).

#### Wheat nitrogen postprandial utilization

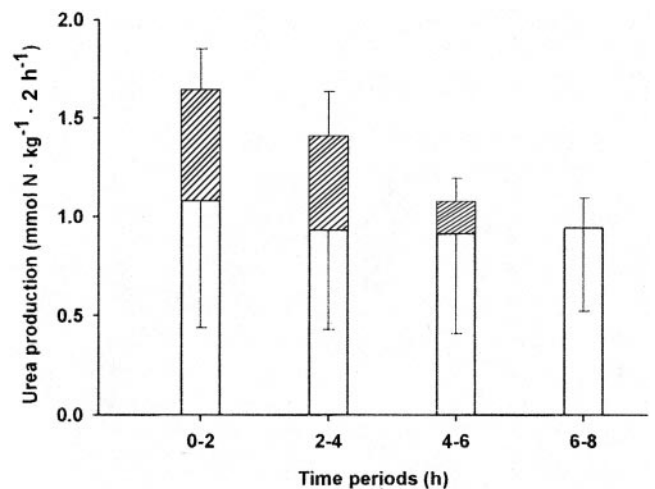
The postprandial metabolism of wheat protein was characterized by a higher deamination of wheat-derived AA (25% of ingested nitrogen over 8 h) than the amount of 16–20% measured for other protein sources including milk, soy, lupin, or pea (19–21, 29), which indicated a lower retention of wheat protein. Our results indicate that, *in vivo* and under the physiologic conditions of meal ingestion, the postprandial retention of wheat protein nitrogen was 89% that of milk protein and 94% that of soy protein (30), which is higher than the relative nutritional values of gluten and milk protein found previously (61%) (9, 14). Moreover, our results argue strongly in favor of revising the protein quality indexes on the basis of the ratio between the more limiting AA and that of an AA pattern considered as a reference (ie, protein digestibility–corrected AA score; PD-CAAS; 12). Gluten PD-CAAS values reach 25%, and those of whole-wheat protein reach 40–42%; the latter represents 45% and 34% of soy and milk protein (untruncated) PD-CAAS, respectively (13, 31). Although PD-CAAS values are likely to change with the reassessment of indispensable AA requirements, this method clearly



**FIGURE 5.** Mean ( $\pm$  SD) time course of dietary nitrogen incorporation into the body urea nitrogen pool and excretion into urinary urea nitrogen (A) and ammonia nitrogen (B) after the ingestion of 298 mmol [ $^{15}\text{N}$ ]-labeled wheat protein in a single mixed meal in human subjects ( $n = 9$ ). Note that the y-axis scales in A and B differ.

penalizes protein sources deficient in AA, as was already observed for the globulin fraction of pea protein (20).

Our findings, showing higher wheat protein retention than expected, may also suggest that well-nourished subjects have the ability to buffer an acute low dietary supply of lysine. Because the habitual occidental diet of the subjects presumably contained excess lysine and protein, and because lysine is an indispensable



**FIGURE 6.** Mean ( $\pm$  SD) rates of urea production of dietary and endogenous origins after the ingestion of 298 mmol [ $^{15}\text{N}$ ]-labeled wheat protein in a single mixed meal in humans ( $n = 9$ ).

TABLE 2

Summary of the bioavailability and postprandial metabolic utilization of wheat protein nitrogen 8 h after its ingestion in a single mixed meal in humans<sup>1</sup>

	Percentage of ingested nitrogen
	%
Ileal losses	9.7 ± 4.3
Real ileal digestibility	90.3 ± 4.3
Deamination	
Body urea	11.9 ± 5.0
Urinary urea	11.0 ± 3.4
Urinary ammonia	0.8 ± 0.4
Total deamination losses	24.7 ± 6.8
Total losses	33.9 ± 5.8
Net postprandial protein utilization	66.1 ± 5.8
Postprandial biologic value	72.9 ± 6.9

<sup>1</sup> All values are  $\bar{x} \pm \text{SD}$ .

amino acid with large body stores (32, 33), it is possible that the metabolic utilization we measured reflects a maximum value. Nonetheless, not only serum lysine but also total serum amino acid amounts decreased throughout the postprandial period, which is not a typical postprandial response to a protein meal (32, 34). This observation suggests that there was an acute effect of lysine deficiency on peripheral amino acid availability, possibly limiting protein synthesis. Further investigations are required in subjects chronically exposed to sublimiting lysine amounts to assess the consequences of such deficiencies on wheat protein metabolic utilization.

As for the metabolic utilization of dietary AA for synthetic purposes, the incorporation of dietary nitrogen into the serum protein pool after ingestion of the wheat meal was 7%, which compares better with the amount found after milk protein ingestion than after soy protein ingestion (30, 34). The kinetics of nitrogen absorption is known to strongly influence the degree to

which dietary AAs are dispatched through the anabolic and catabolic pathways. The profile of dietary incorporation into the serum AA pool was as fast as that observed after soy protein but was associated to a lower incorporation of dietary nitrogen into plasma protein. This suggests that protein synthesis efficiency might be actually limited in the case of wheat protein ingestion by the lysine deficiency despite the fact that subjects had probably high intracellular lysine stores.

### Lysine requirements and wheat protein quality in human diets

It is difficult to assess the extent to which lysine deficiency of cereal products is limiting in cereal-based diets and has any effect on functional and immune status, growth, and body composition. A broad range of lysine requirements (12–45 mg · kg<sup>-1</sup> · d<sup>-1</sup>) was reported, but, according to the most recent data, the lower value of 12 mg · kg<sup>-1</sup> · d<sup>-1</sup>, which corresponds to the latest international recommendations published (8), is markedly underestimated. The true value for lysine requirements is thus thought to lie between 18 and 45 mg · kg<sup>-1</sup> · d<sup>-1</sup>, as assessed by using isotopic methods (3–7, 9, 14). Interestingly, the present study showed that an experimental wheat-containing meal providing 300 mmol N and 8.3 mg lysine/kg balanced postprandial nitrogen losses for the 5 h after ingestion (Figure 7). If lysine is considered to be the first limiting AA in wheat protein, this result may translate to a lysine requirement of 1.6 mg · kg<sup>-1</sup> · h<sup>-1</sup>—ie, 40 mg · kg<sup>-1</sup> · d<sup>-1</sup>. This value is derived from fed state measurements and as such probably overestimates the requirement. On the basis of the total nitrogen losses of 413 mmol at 8 h (Figure 7), the approximate lysine requirement would then be 1.4 mg · kg<sup>-1</sup> · h<sup>-1</sup>, or 33.6 mg · kg<sup>-1</sup> · d<sup>-1</sup>. This value would reach 31.6 mg · kg<sup>-1</sup> · d<sup>-1</sup> if corrected for a 25% recycling of ileal nitrogen (35). Although limited by the habitual high lysine intakes of the subjects, which probably led to the determination of an optimal wheat protein utilization and underestimated the derived lysine requirement, this value is consistent with published data.

On the basis of the postprandial utilization in humans, wheat protein (66% retention) was of lower nutritional quality than were milk (74%), soy (71%), pea (70%), and lupin (74%) proteins (20, 21, 30). To appreciate the incidence of the moderate nutritional value of wheat protein, it is crucial to consider the extent to which lysine deficiency is compensated for by other sources. All cereal-based diets include other types of protein, which are likely to compensate for the lysine deficiency of cereal proteins. It has long been acknowledged that adding lysine to gluten or wheat protein improves their biological value (10, 11, 36). It was also reported that subjects receiving for 50 d a predominantly (90–95%) wheat-based diet were able to maintain their nitrogen equilibrium but required a marked increase in their daily energy intake to achieve this (37). Such a diet was also shown to produce significant changes in the ratio of plasma indispensable AA to dispensable AA (38). The modalities of supplementation of cereal proteins with lysine-rich protein sources, if necessary, are not yet elucidated.

In conclusion, we showed that wheat protein has a RID within the current range of the values found for other plant protein sources but has a lower postprandial biological value. However, the relative deficit of net postprandial protein utilization of wheat protein nitrogen compared with milk protein was not as depressed as might be predicted from the ratios of lysine content between dietary proteins,

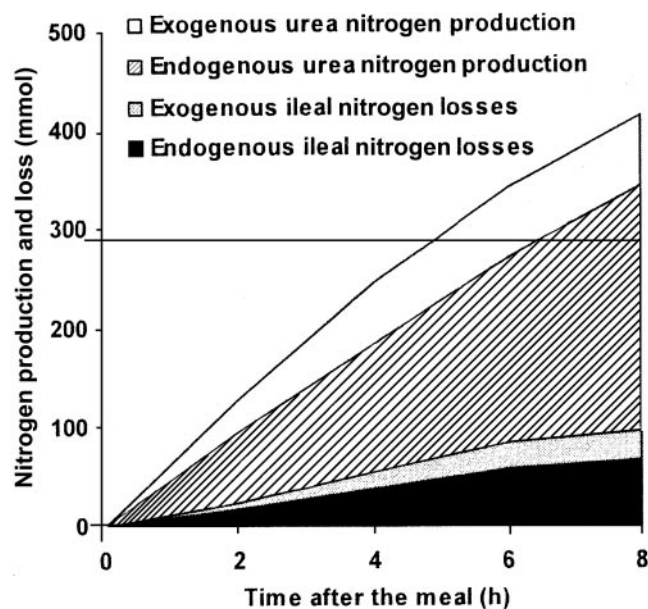



FIGURE 7. Mean ( $\pm$  SD) cumulative losses of nitrogen of dietary and endogenous origins during the postprandial period after the ingestion of 298 mmol [<sup>15</sup>N]-labeled wheat protein in a single mixed meal in humans ( $n = 9$ ).

which confirmed that AA score-based methods are problematic in assessing protein nutritional quality. 

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CB collected and analyzed the data and wrote the manuscript. BJ, LT, SD, and CL collected and analyzed the data. HF analyzed the data. RN and RB were responsible for the clinical management of the volunteers. NG provided the labeled wheat biscuits and contributed to manuscript preparation. DT and CG designed the study and participated in the writing of the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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