Postprandial intestinal and whole body nitrogen kinetics and distribution in piglets fed a single meal

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DURING THE POSTPRANDIAL PHASE, the amino acids absorbed by the gut are subjected to extensive metabolism during passage through the intestinal mucosa and liver before being available to peripheral tissues, particularly muscle. Tissue protein synthesis is enhanced in most protein pools in this absorptive state, and both dietary and endogenous amino acids are used to replete protein stores, which are depleted during the postabsorptive state (maintenance), and to allow the net accretion of tissue protein, especially in very young growing animals (11). However, uncertainties remain regarding the form in which dietary N is absorbed, metabolized within the enterocyte, released through the portal circulation, and made available for peripheral tissues after hepatic metabolism (45). The temporal fluxes of both dietary and proteolysis-derived amino acids through catabolic or synthetic pathways in different organs after a meal are also unclear and have received little attention despite evidence of a specific time frame during which stimulation of protein synthesis occurs (8, 36).

The lack of information about the kinetics and metabolic fate of dietary nitrogen is due to technical and ethical constraints for accessing different organs and tissues, particularly in nonsteady-state conditions, where studies are rare. Studies in humans are indirect, because it proves difficult to access compartments other than blood, giving insight into the circulating N compounds kinetics (free N, plasma protein, and urea) and urine, where the N end products can be quantified (14). Studies in rats provide useful and additional information regarding the tissue distribution of the amount of dietary N retained under different nutritional conditions, but the small size of this experimental animal model precludes performing kinetics studies with sequential sampling of blood and portal sampling in the same animal for several hours (38). The catheterized pig model, coupled with a portal ultrasonic flow probe and with tracer techniques, offers an ideal solution for the intensive study of postprandial dynamic N kinetics across the portal drained viscera (PDV: stomach, intestines, spleen, and pancreas). This model has proved useful for the study of specific dietary amino acid digestive and metabolic fate (50, 51) or total amino acid fate (47), and the measurements of amino N pool (44%), and the absence of urinary urea intravenous infusion and were studied for 8 h after a bolus mixed meal ingestion (46 mmol N/kg body wt) intrinsically labeled with 15N to trace dietary N fluxes. The real cecal digestibility of the formula was 94.3% (SD 1.8). PDV output of dietary N was found principally in the pool of circulating protein (51% of the measured dietary N PDV output), in the free α-amino N pool (44%), and to a lesser extent in ammonia (5%). Dietary N release in α-amino N and ammonia mainly occurred during the first 3 h. Total and exogenous postprandial urea productions were 5.8 and 2.0 mmol N/kg body wt, respectively. At the end of the postprandial period, losses of dietary N amounted to 10.3% of the dose: 5.7% through ileal losses and 4.6% by deamination and transfer to urea. Net postprandial retention of dietary N was 90.4% (SD 1.3), of which 20% was found in splanchnic zone (small intestine 10%, liver 5%, and plasma protein 3%) and 42% in peripheral zone (muscle 31%, skin 6%). In conclusion, our results show a high efficiency of dietary N utilization for muscular uptake and anabolic utilization. However, the results obtained point out the necessity to further explore the form of dietary N released into the portal blood.

dietary nitrogen; postprandial metabolism; portal drained viscera; nonsteady state; pigs

MATERIALS AND METHODS

Animals. The study used seven 4-wk-old female piglets (Large White × Hampshire × Duroc) purchased at the Texas Department of Criminal Justice (Huntsville, TX). The animals were received at the Children’s Nutrition Research Center at 2 wk of age. They were

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housed for a 1-wk adjustment period and fed a cow’s milk formula (Litterlife; Merrick, Union, WI) at a rate of 50 g dry mass (DM)-kg body wt⁻¹-day⁻¹, supplying lactose (26.4 g·kg body wt⁻¹-day⁻¹), protein (12.5 g·kg body wt⁻¹-day⁻¹), and fat (5 g·kg body wt⁻¹-day⁻¹). The protocol was approved by the Animal Care and Use Committee of the Baylor College of Medicine and conformed to current US Department of Agriculture guidelines.

**Study design.** At 3 wk of age, the pigs were prepared for surgery as previously described (52). Food was removed overnight before surgery. The piglets were placed under general anesthesia (5% isoflurane), and catheters were implanted in a carotid artery, the jugular vein, and the portal vein. An ultrasonic probe was implanted around the portal vein (Transonic Systems, Ithaca, NY). After surgery, the piglets received parenteral nutrition for 24–36 h and were then progressively returned to their preceding level of dietary intake for ≥5 days before the experimental protocol. At a postnatal age of 4 wk, the animals were studied for a 10-h experimental period. Their mean body weight was 7.80 kg (SD 1.12).

**Tracer protocol.** The pigs were fasted overnight before the experimental protocol. At 0700, a primed (100 μmol·kg⁻¹) continuous infusion of [¹⁸O]urea (99%, Cambridge Isotope Laboratories) was infused continuously at a mean rate of 11.7 (SD 0.4) μmol·kg⁻¹·h⁻¹ via the jugular vein. At 0900 (t = 0), the animals received a single mixed meal providing one-third of their previous daily intake. The meal fed to the piglets consisted of 46 ml/kg body wt of a mix of Litterlife powder and water (1:3) mixed with a trace of a uniformly and intrinsically [¹⁵N]-labeled soy protein isolate [150 mg/kg body wt, 98 atom % (AT%)]. The soy protein isolate was prepared as previously described (6, 19).

The total nitrogen content of the mixed meal was 46 mmol/kg body wt (SD 1) (3.1 AT%). The liquid meal was fed orally and ingested quickly, within 45 min. A baseline blood sample was drawn at time t = 0. Portal and arterial blood samples (2.4 ml) were obtained every 15 min for 2 h, every 30 min for the next hour, and then every hour for the last 5 h. Hematocrit was measured at regular intervals in blood samples. The portal blood flow was measured and recorded continuously throughout the experimental period by transit-time ultrasound. An aliquot (0.2 ml) of arterial and portal plasma was mixed with an equal volume of methionine sulfone (0.5 mmol/l) as an internal standard and stored at −70°C for later amino acid analysis. An aliquot of 0.8 ml of arterial and portal plasma was also taken for further isotopic analysis of the protein N, free N, ammonia, and urea enrichment. At the end of the tracer protocol, animals were killed with an arterial injection of pentobarbital sodium, and their intestinal contents (stomach, small intestine, and cecum) were collected, weighed, and dried (NPN fraction). The pellet was rinsed with 1 ml of saline water, centrifuged, weighed, and dried (PN fraction). The total N content and the isotopic enrichment of [¹⁵N] in tissue PN and NPN fractions in plasma protein and in intestinal contents were measured by elemental analysis coupled to IRMS (Optima; Fisons Instruments, Manchester, UK). Tissue samples were weighed, ground in four volumes of saline and divided into two aliquots. The first aliquot was used to separate the protein N (PN) and nonprotein N (NPN) fractions. The PN fraction was precipitated with the addition of trichloroacetic acid (TCA) solution so as to reach a final concentration of 10% TCA. Samples were centrifuged, and the supernatant was collected, weighed, and freeze-dried, and used for the determination of [¹⁵N] enrichment of plasma free N. Resin was washed three times with water. Ammonia was eluted from resin using 2.5 M KHSO₄, and [¹⁵N] enrichment of both urea and ammonia plasma was determined by IRMS (Optima; Fisons Instruments, Manchester, UK). Tissue samples were weighed, ground in four volumes of saline and divided into two aliquots. The first aliquot was used to separate the protein N (PN) and nonprotein N (NPN) fractions. The PN fraction was precipitated with the addition of trichloroacetic acid (TCA) solution so as to reach a final concentration of 10% TCA. Samples were centrifuged, and the supernatant was collected, weighed, and dried (PN fraction). The pellet was rinsed with 1 ml of saline water, centrifuged, weighed, and dried (PN fraction).

The isotopic enrichment of urea in plasma samples was determined by gas chromatography-mass spectrometry (GC-MS) of the 2-pyrimidinon-N-(tert-butylmethylsilyl) derivative. The analyses were performed with a 5890 series II gas chromatograph linked to a model 5989B (Hewlett-Packard, Palo Alto, CA) quadrupole mass spectrometer. We used electron ionization, and the isotopic enrichment of urea was determined by monitoring ions at a mass-to-charge ratio of 153 and 154 for the M + 0 and M + 1 isotopes of [¹⁵N]urea and 155 for the M + 2 isotope of [¹⁸O]urea. The relative amount of M + 1 isomers served to determine the isotopic enrichment of [¹⁵N]urea (tracing urea carrying an atom N of dietary origin), and the relative amount of M + 2 isomers served to determine the isotopic enrichments of [¹⁸O]urea (tracing endogenous total body urea). Plasma ammonia [¹⁵N] enrichment was calculated using measurements of both urea + ammonia fraction (IRMS) and urea alone (GC-MS).

**Calculations.** Data from all seven piglets are included in the calculations of tissue N incorporation, but PDV balances are restricted to the five animals with successful portal flow records.

**Quantification of dietary N in body N pools.** Dietary N incorporation (expressed as a percentage of the ingested amount) into the different body N pools monitored (tissues, plasma free N, plasma protein, plasma ammonia, body urea) was evaluated by the following equation:

\[
N_{\text{tot}}(t) = N_{\text{tot}}(0)N_{\text{ingested}} \times \left(1 - e^{-E(t)/E_{\text{meat}}}ight) \times 100
\]

where \(N_{\text{tot}}(0)\) is the N content of the pool (mmol N) at time point t, \(E(t)\) is the [¹⁵N] enrichment (expressed as AT%) in the N pool sampled at time t, \(E(0)\) is the [¹⁵N] enrichment at time 0 (AT%), \(E_{\text{meat}}\) is the [¹⁵N] enrichment of the meal (AT%), and \(N_{\text{ingested}}\) is the N content of the meal (mmol N). \(N_{\text{tot}}\) in the plasma free N or protein pool was determined as the plasma concentration of free amino N or protein N, respectively, multiplied by the plasma volume, estimated to be 5% of body weight (23). The body urea N pool size was calculated as the product of the urea concentration and total body water, estimated as 75.3% of the body weight (17). A factor of 0.92 was used to take account of the water content of plasma. Colon wall was estimated to weigh 17.5 g/kg body wt (26). Skin mass and subcutaneous fat were assumed to represent 15% of body weight (22, 54). Total skeletal muscle mass was difficult to evaluate due to the lack of specific data on piglets of this age. Considering that lean body mass was 90% of
body weight (17) from which were subtracted skin mass without subcutaneous fat [5\% (22)], visceral mass (15\%, measured), blood volume [9\% (30)], and bone mass [11\% (54)], muscle mass was estimated to reach 50\% of body weight. Possible errors introduced by the assumptions made on plasma volume, total body water volume, skin mass, and muscle mass impacted on the assessment of the distribution of total and dietary N in these pools (each percent error in determination of the pool size translates into 1\% error in tissue N content). However, these assumptions had no influence on PDV fluxes, urea kinetics, or the determination of the percentage of N in free or bound form in tissues.

**Meal N real cecal digestibility.** Amounts of dietary N recovered in the stomach, small intestine, and cecum lumen contents 8 h after the meal were summed and considered as the part of dietary N that was not absorbed during the postprandial period. Colon contents were assumed to contain only negligible amounts of dietary N 8 h after the meal and thus were not sampled. Digestibility of the meal N was then calculated as the part of ingested N that was absorbed. Recycling of dietary N through resorption of N compounds in the intestinal lumen within the postprandial period was not quantifiable and thus was neglected, slightly underestimating the absorbed quantity of dietary N.

**PDV balances.** PDV balances (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) of amino acid N, urea N, ammonia N, and plasma protein N were calculated by the product of portal plasma flow (PF, \(\text{1-kg}^{-1} \cdot \text{h}^{-1}\)) and the portoenteric difference in plasma concentrations (\(\mu\text{mol} \cdot \text{N/l}\)). PF was derived from the measured portal blood flow, corrected for the hematocrit value. The portal mass balances were expressed as percentages of the enteral flux. Portal tracer balances were derived in the same manner by using the tracer concentrations (\(\text{15N-labeled fractions}\)) in portal and arterial plasma samples. Plasma amino acid N (\(\mu\text{mol} \cdot \text{N/l}\)) was calculated by the sum of each amino acid molar concentration multiplied by the number of N atoms in each amino acid.

The absorption flux of dietary N was derived from published data of gastric emptying rate of cow’s milk formula obtained in piglets of the same age ingesting the same amount of food [13–16 g DM/kg body wt (39)], as previously described (6). The cumulative flux of absorbed N accounted for the digestibility of the dietary protein (average 94\%), which was measured directly by subtracting the amount of dietary N recovered in the intestinal lumen contents (stomach + small intestine + cecum) of each animal from the amount ingested. The stomach and small intestine retention times of the dietary nutrients were consistent with data obtained in 28- or 35-day-old piglets fed liquid milk diets, showing that the mean small intestine retention time was 

\[
\text{Average 94\%}
\]

Possible errors introduced by the use of an estimated dietary N absorption flux only concern the partition of dietary N absorbed between early and late postprandial periods.

**Systemic urea kinetics.** Urea concentrations and enrichments of [\(^{15}\text{O}\)]urea and \([^{15}\text{N}]\)urea in arterial plasma during the constant [\(^{15}\text{O}\)]urea intravenous infusion were used to calculate the urea systemic rate of appearance (Ra) in the peripheral circulation, using Steele’s equations (49) modified by Proietto et al. (43). The values (urea concentrations and enrichments) used in these equations were derived from fitted curves (as shown in Fig. 3), which avoid random experimental errors responsible for implausible values for the rate of change of concentration or enrichment. The use of two urea tracers allowed us to calculate the total Ra of urea (using [\(^{15}\text{O}\)]urea enrichments) and the exogenous (dietary) Ra of urea (using [\(^{15}\text{N}\)]urea enrichments). By the difference, the Ra of endogenous urea was derived. We used the following equations:

\[
\text{total } Ra \text{ (}\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = [IR - pV \times C(t) \times dE_{e}(t)/dt]/E_{e}(t) - IR
\]

where IR is the rate of [\(^{15}\text{O}\)]urea intravenous infusion corrected for isotopic purity, p is the pool fraction for instant mixing, V is the volume of distribution of urea in the body (l/kg), C(t) is the mean arterial urea plasma concentration at t and \(\Delta t\) (calculated as \([C(t) + C(t + \Delta t)/2]\)), and dE_{e}(t)/dt is the variation of the plasma [\(^{15}\text{O}\)]urea enrichment (mol % excess, MPE, calculated as \([[E(t + \Delta t) - E(t)]/\Delta t]]\), and dietary N enrichment is the \([^{15}\text{N}]\) enrichment of the meal (3.07 AT\%)

Endogenous systemic urea N Ra was calculated as the difference between the total urea N Ra and dietary urea N Ra:

\[
\text{endogenous urea N } Ra_{e} = \text{total urea N } Ra - \text{exogenous urea N } Ra_{e}
\]

**Statistics.** Results are expressed as means (SD). Changes of portal plasma nutrient fluxes over time were tested using contrast analysis under a mixed model, with time as a repeated factor (SAS 8.01; SAS Institute, Cary, NC). Single-planned comparisons between total and dietary N fractions in tissues were tested using Student’s t-test. Significance was set at \(P = 0.05\).

**RESULTS**

**Luminal intestinal N and dietary N absorption.** Total and dietary N in intestinal contents were quantified in the lumen of stomach, small intestine, and cecum at the end of the 8-h postprandial periods (Table 1). The dietary N represented 85\% of the total N present in these three intestinal luminal compartments. Nonabsorbed dietary N was principally found in cecum. Dietary N absorbed was assessed 8 h after the meal by the difference [43.4 (SD 0.5) mmol N/kg body wt] between dietary N ingested (46 mmol/kg body wt) and the total recovery of dietary N in the lumen of stomach, small intestine, and cecum. Thus the real cecal digestibility of the formula was 94.3\% (SD 1.8).

**PDV fluxes of N compounds after meal ingestion.** The fluxes of the different portal and arterial circulating forms of total and dietary N (i.e., circulating α-amino N, protein N, ammonia N, and urea N) have been calculated from their arterial and portal plasma concentrations, respectively, multiplied by the portal plasma flow after the meal (Fig. 1).

<table>
<thead>
<tr>
<th>Contents</th>
<th>Dry Matter, g/kg body wt</th>
<th>Total N, mmol N/kg body wt</th>
<th>Dietary N, mmol N/kg body wt</th>
<th>Dietary N, %Ingested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0.22 (0.16)</td>
<td>0.77 (0.65)</td>
<td>0.73 (0.67)</td>
<td>1.5 (1.1)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.06 (0.03)</td>
<td>0.21 (0.13)</td>
<td>0.14 (0.11)</td>
<td>0.3 (0.3)</td>
</tr>
<tr>
<td>Cecum</td>
<td>0.46 (0.26)</td>
<td>2.17 (0.96)</td>
<td>1.77 (0.62)</td>
<td>3.9 (1.3)</td>
</tr>
<tr>
<td>Total</td>
<td>0.74 (0.25)</td>
<td>3.16 (1.09)</td>
<td>2.64 (1.09)</td>
<td>5.7 (1.8)</td>
</tr>
</tbody>
</table>

Values are means (SD); \(n = 7\) piglets.

Table 1. Intestinal contents at end of 8-h postprandial period in piglets fed a single \(^{15}\text{N}-\text{labeled meal}

E438 DIETARY N METABOLISM IN FED PIGLETS

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As shown in Fig. 1A, total \( \alpha \)-amino N portal and arterial fluxes were fourfold significantly increased for 5 h after the meal and reached a maximum between 2 and 3 h; of these total N fluxes, dietary \( \alpha \)-amino N fluxes significantly increased for the 6 h following the meal and constituted up to 7.5% and up to 9% in arterial and in portal plasma at 5 h, respectively. Figure 1B shows that flux of total N in circulating protein rapidly increased and was transiently significantly higher than

Fig. 1. Portal drained viscera (PDV) arterial and portal plasma fluxes of total and dietary N in \( \alpha \)-amino N pool (A), circulating protein N pool (B), ammonia pool (C), and urea pool (D) in piglets fed a single mixed meal. Asterisks and horizontal lines indicate time points at which values differ from baseline (\( P < 0.05 \), by use of a mixed model).
the baseline flux and then remained relatively constant and similar in both arterial and portal circulations throughout the postprandial period; dietary N in circulating protein N flux increased for the first 6 h after the meal and then reached a plateau (41–42 mmol N·kg⁻¹·h⁻¹) for the last 2 h (significantly different from baseline from 1.5 to 8 h, \(P < 0.05\)). As seen in Fig. 1C, total ammonia N portal flux significantly increased from 0.25 mmol N·kg⁻¹·h⁻¹ in fasting conditions to 0.69 mmol N·kg⁻¹·h⁻¹ 3 h after the meal and was largely higher than ammonia arterial flux by a factor of three to four throughout the postprandial period; a major part of ammonia portal flux was of dietary origin for the first 3 h after the meal (significant increase in portal and arterial circulation for 90 and 30 min, respectively). Figure 1D illustrates that total urea N portal and arterial fluxes were not different and that both significantly increased twofold after the meal ingestion with a maximum at 3 h due to the significant rise of urea N fluxes of dietary origin from 1.5 h (arterial) or 2 h (portal) after the meal.

Cumulated PDV balances of total and dietary N over the 8-h postprandial period are depicted in Fig. 2. PDV total N balance after the meal was positive for both total \(\alpha\)-amino and ammonia N (significantly different from zero, \(P < 0.05\)), null for total urea N, and null or negative for total protein N (not significantly different from zero; Fig. 2A). For the dietary N forms, of the 42 mmol N·kg⁻¹·h⁻¹ absorbed, Fig. 2B shows that there was a null PDV balance of dietary N in the form of urea but a significant net PDV release of dietary N in the \(\alpha\)-amino N pool [5.2 (SD 1.4) mmol N/kg], circulating protein pool [6.4 (SD 2.5) mmol N/kg], and ammonia pool [0.6 (SD 0.4) mmol N/kg]. These cumulated dietary N PDV balances were differentially partitioned between the early and late postprandial periods (Table 2). \(\alpha\)-Amino N portal release in both total and dietary fractions occurred predominantly during the first 4 h following the meal. Portal N output in the circulating protein pool was higher during the late (4- to 8-h) period than the early period for both total and dietary fractions. Portal appearance of ammonia was equally partitioned between dietary and endogenous origins during the early postprandial period but was only endogenous for the late period. Finally, 27% (SD 8) of the absorbed dietary N appeared in the portal plasma circulation, predominantly in the circulating protein pool (15%), in the \(\alpha\)-amino N pool (12%), and in ammonia (1.3%).

Systemic kinetics of total and dietary N in deamination pools. Urea production was assessed at the systemic level by a tracer dilution method during continuous intravenous infusion of \([18O]\)urea. Appearance of the \(^{15}\)N label from the meal in the urea pool allowed for the discrimination between urea production rates from dietary or endogenous origins. As shown in Fig. 3A, arterial urea concentration increased between 2 and 6 h after the meal ingestion. Plasma enrichment of \([18O]\)urea decreased immediately after the meal until 4 h, whereas \([^{15}\text{N}]\)urea enrichment slowly but regularly increased during the postprandial period, reaching a plateau after 6 h (Fig. 3B). From these fitted experimental data and using Steele’s equations, we were able to calculate the rates of total, exogenous (dietary), and, by
difference, endogenous urea production in non-steady-state conditions. Total urea production was 313 μmol N·kg⁻¹·h⁻¹ in the fasting state and increased threefold at 3 h (Fig. 3C). This increase was mainly due to the raise of dietary N transfer to urea N, which was maximal (416 μmol N·kg⁻¹·h⁻¹) 3 h after the meal and then steadily declined. Endogenous urea N production increased rapidly after the meal and then slowly returned to the basal level for the 8-h postprandial period. At the end of the postprandial period, integrated total, exogenous, and endogenous urea productions were 5.8 (SD 0.9), 2.0 (SD 0.7), and 3.8 (SD 0.5) mmol N/kg, respectively, as calculated from the areas under the curves. Urea N production from dietary origin represented 4.4% (SD 1.5) of the ingested amount.

**Dietary N distribution in tissues and retention.** The amounts of total and dietary N present at the end of the postprandial period in both PN and NPN fractions of the major body N pools are depicted in Table 3. Dietary N was recovered mainly in skeletal muscle, small intestinal walls, skin, liver, and kidneys. The ratio of dietary N to total tissue N reflects the part of the N pool that was replaced by dietary N at the end of the 8 h. In the protein fraction, this ratio was maximal (6–10%) in small intestinal segments (higher in the distal portion than the proximal portion) and pancreas; intermediary (2.0–4.4%) in the other intestinal segments, liver, kidneys and spleen; and low (1.1%) in skeletal muscle and skin. This ratio was always higher in the NPN fraction and ranged from 3.4% in muscle to 11% in distal small intestine. On the sum of all the body N pools, the ratio of dietary N to total N was 1.6% for the PN fraction and 4.3% for the NPN fraction.

Except for pancreas and distal small intestine, dietary NPN-to-PN ratio (NPN/PN) was always significantly higher than total NPN/PN (Table 4). This ratio was up to threefold higher for dietary N in spleen, muscle mass, and skin. In skeletal muscle, for instance, 11.5% of total N was free N but 30% of dietary N retention was free N.

Table 5 summarizes the fate of dietary N 8 h after the ingestion of a single meal in piglets. Losses of dietary N amounted to 10.3% of the dose, 5.7% not absorbed at the intestinal level, and 4.6% by further transfer to urea. Thus the net postprandial retention, calculated by the difference of dietary N ingestion and losses, was 90.4% (SD 1.3). Dietary N retention was 19.8 and 41.8% in splanchnic and peripheral zones, respectively. Anabolic utilization (part of retention in the protein fraction) was 15.8 and 30.9% of the ingested N in splanchnic and peripheral tissues, respectively.

**DISCUSSION**

This work aimed to better characterize the postprandial N fluxes after administration of a single 15N-labeled mixed meal in a model of catheterized animals with a rapid growth rate. This is the first study giving a large overview of the postprandial non-steady-state events occurring in dietary N metabolism by reporting the concomitant measurement of dietary N fluxes in portal circulation, systemic urea production from both dietary and endogenous origin, and final regional distribution in the body of retained dietary N. In particular, by combination of arteriovenous balances across the PDV and labeling of dietary N, we were able to quantify the fluxes of N exiting the PDV via the portal circulation under physiologically relevant feeding conditions. The results indicated that ~27% of the dietary N was absorbed from the PDV, of which 51% was found in the protein N fraction, 44% was free α-amino N, and only 5% was ammonia N. No significant production of urea by the PDV was measured. Moreover, consistent with the fast-growing animal model used, the results also showed the high efficiency of dietary N utilization for peripheral tissue uptake and anabolic utilization, which represented 42 and 31% of the ingested N 8 h after the meal, respectively.

**N transactions across the PDV.** There was a positive α-amino N PDV balance for both dietary and total N, thus...
confirming that PDV play a major role in the postprandial distribution of free amino acids to peripheral tissues. As expected, an important part of the N released from the PDV after the meal is in the form of free amino acids. In contrast, an unexpected result was also that a large amount of dietary N released into the portal circulation was associated with the plasma protein fraction. The total amount of dietary N found in plasma protein was even higher than that measured in plasma free amino acid N and represented 15% of ingested N over the eight postprandial hours. Moreover, in contrast to α-amino N, a positive PDV balance was observed for dietary but not for total protein-associated N, the latter being near zero or even slightly negative. This large and circulating plasma protein pool may act as a carrier taking up dietary N compounds in the visceral tissues after the meal. The nature of dietary N associated with the plasma protein fraction after their passage through the PDV remains unclear and will require further research. Two main mechanisms can be responsible for this large amount of dietary N found in the circulating protein fraction, i.e., intestinal synthesis of circulating protein and fixation of free N compounds on these proteins. First, a fraction of dietary amino acids may be incorporated into newly synthesized proteins from PDV tissues and released in the plasma, but the nature of these proteins is unclear. Although enterocytes synthesize and secrete different proteins including apolipoproteins [i.e., apoA-I and apoB-48 (5, 9, 40)], this secretion mainly occurs in lymph and is probably not present in portal plasma. Another possible origin of the high level of plasma protein associated dietary N is the noncovalent binding on plasma proteins of free amino acids and small peptides carrying dietary N. The role of plasma proteins as interorgan carriers of amino acids has been shown in ruminants (34). Such a role remains to be clarified in monogastric species and humans.

At the whole body level, we recovered 72% of the dietary N in tissues and monitored N pools at the end of the 8-h protocol, which is much higher than the 48% of dietary N measured in the cumulated net dietary N fluxes exiting the PDV in either form (amino acids, ammonia, urea, and protein; Table 2), the cecal losses, and the amount of dietary N present in the PDV tissues. One explanation for this discrepancy could be that some components of the dietary N were transferred to peripheral tissues through lymph, but few data are available on this route. Second, this discrepancy may be due to an underestimation of portal output because of the use of plasma rather than blood metabolites concentrations (29, 53). A fraction of dietary N absorbed by the gut is probably incorporated into blood cells that are rich in free amino acids and protein. We can assume that dietary N incorporated in hemoglobin was approximately the same in arterial and portal blood; thus this fraction should not have disturbed the portal balance calculations. However, it is probable that changes in free amino acid concentrations occur in blood cells when traversing the PDV, and an important consumption of free amino acids from blood cells by the gastrointestinal tract was shown in calves (27). In our study, the phenomenon may have been amplified by the fact that we have measured fluxes across the PDV, and which include the spleen, an organ playing an important role in erythrocyte breakdown and disposal. Finally, underestimation of dietary N appearance in the portal circulation may be explained by the release of a substantial part of α-amino N in the form of peptides of different sizes, which are difficult to quantify. Indeed, it has been shown, mostly in ruminants, that the splanchnic output of peptide amino acids represents as much as 7 to 10 times the magnitude of the plasma free amino acid splanchnic output (21, 27). This magnitude may differ across species (48).
N transfer to catabolic pathways. In line with the early metabolism of dietary amino acid by the gut mucosa, as exemplified by lysine in a previous paper (6), the results indicated that, after the meal ingestion, 1) ammonia production by the PDV immediately shifted from endogenous to dietary origin and progressively returned to endogenous origin from 1 to 3 h after the meal, and 2) it was progressively followed by hepatic production of urea derived from dietary N, with a maximal production 3 h after the meal ingestion. In contrast to ammonia, no significant production of urea by the PDV, either from endogenous or from dietary origin, was observed. This is consistent with results obtained in older pigs (13). This zero rate of dietary urea N balance may, however, offset a parallel urea diffusion from enterocytes to the lumen and urea release in the portal vein after de novo synthesis in enterocytes, since both fluxes have been shown to occur (3, 47, 58).

We used a primed continuous intravenous infusion of [18O]urea to obtain an assessment of the systemic rate of urea N appearance in the non-steady-state conditions of the meal ingestion. This isotopic method offers the possibility to measure short-term variations in whole body urea kinetics (25, 32, 57). Furthermore, we were able to distinguish the endogenous and exogenous (dietary) components of this flux by measuring incorporation of 15N from the diet into the body urea N pool. We found a significant increase in the total rate of urea production after the meal, from 313 to 1,026 mmol urea N·kg⁻¹·h⁻¹ before and 2.5 h after the meal, respectively. Except for the early and probably artifactual increase in both total and endogenous urea production rates, the dynamics of this flux were consistent with the portal kinetics of dietary N in ammonia, which is incorporated into urea in hepatocytes. These urea production rates are consistent with that reported for growing pigs of different ages (10, 13). They are also in the

Table 3. Distribution of dietary N in piglet tissue protein pools

<table>
<thead>
<tr>
<th>Pool Weight, g/kg body wt</th>
<th>Total N, mmol N/kg body wt</th>
<th>Dietary N, mmol N/kg body wt</th>
<th>Ratio Dietary N/Total Tissue N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PN fraction</td>
<td>NPN fraction</td>
<td>PN fraction</td>
</tr>
<tr>
<td>Gut segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>3.4 (2.0)</td>
<td>33.2 (3.4)</td>
<td>3.2 (0.8)</td>
</tr>
<tr>
<td>Proximal small intestine</td>
<td>16.2 (2.6)</td>
<td>23.6 (3.2)</td>
<td>3.9 (0.6)</td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>18.8 (2.8)</td>
<td>20.2 (1.9)</td>
<td>5.2 (2.2)</td>
</tr>
<tr>
<td>Cecum</td>
<td>5.8 (2.1)</td>
<td>2.6 (0.3)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>Colon</td>
<td>17.5*</td>
<td>14.9 (2.6)</td>
<td>1.8 (0.4)</td>
</tr>
<tr>
<td>Other visceral organs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>33.2 (2.0)</td>
<td>51.3 (3.4)</td>
<td>6.8 (0.7)</td>
</tr>
<tr>
<td>Plasma proteins</td>
<td>2.9 (0.3)*</td>
<td>33.2 (2.9)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.4 (1.3)</td>
<td>8.4 (2.7)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.7 (0.4)</td>
<td>2.4 (0.5)</td>
<td>0.4 (0.4)</td>
</tr>
<tr>
<td>Total PDV</td>
<td>650 (5.9)</td>
<td>78.4 (7.4)</td>
<td>13.2 (2.5)</td>
</tr>
<tr>
<td>Muscle and skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>500*</td>
<td>932 (42)</td>
<td>120 (5)</td>
</tr>
<tr>
<td>Skin + subcutaneous fat</td>
<td>150*</td>
<td>201 (47)</td>
<td>18 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>764</td>
<td>1391 (66)</td>
<td>161 (19)</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 7. PN, protein N; NPN, nonprotein N. SD estimates for colon, plasma protein, muscle, and skin N pools only represent variations in total N content and dietary N percentages, because these pool sizes were estimated from published data. *Estimated values (see MATERIALS AND METHODS).

Table 4. Proportion of NPN in total or dietary N tissue pools 8 h after ingestion of a single meal in piglets

<table>
<thead>
<tr>
<th>%NPN in Tissue N Pools</th>
<th>Total N</th>
<th>Dietary N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut segment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>12.1 (1.4)</td>
<td>20.4 (2.3)*</td>
</tr>
<tr>
<td>Proximal small intestine</td>
<td>13.1 (0.5)</td>
<td>17.5 (1.7)*</td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>19.7 (4.7)</td>
<td>23.0 (2.9)</td>
</tr>
<tr>
<td>Cecum</td>
<td>11.4 (1.4)</td>
<td>21.5 (3.1)*</td>
</tr>
<tr>
<td>Colon</td>
<td>10.4 (1.4)</td>
<td>21.5 (5.4)*</td>
</tr>
<tr>
<td>Other visceral organs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>11.4 (2.0)</td>
<td>24.7 (3.2)*</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.9 (1.3)</td>
<td>15.7 (2.8)*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>14.1 (9.1)</td>
<td>12.6 (5.4)*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>9.9 (2.3)</td>
<td>24.5 (7.6)*</td>
</tr>
<tr>
<td>Muscle and skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>11.5 (0.4)</td>
<td>30.3 (3.5)*</td>
</tr>
<tr>
<td>Skin + subcutaneous fat</td>
<td>7.8 (4.3)</td>
<td>23.8 (10.3)*</td>
</tr>
<tr>
<td>All tissues†</td>
<td>10.4 (0.8)</td>
<td>24.9 (1.7)*</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 7. Proportion of tissue N in the form of PN or NPN was determined after separation of the 2 fractions by addition of trichloroacetic acid. Dietary N in each fraction was quantified by isotope analysis. *P < 0.001 between total (endogenous and dietary) and dietary pools. †Average percentages for all tissues sampled, weighted for tissue N content.

Table 5. Summary of dietary N utilization 8 h after ingestion of a single meal in piglets

<table>
<thead>
<tr>
<th>Dietary N</th>
<th>mmol N/kg</th>
<th>%Ingested N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingested N</td>
<td>45.0 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Cecal losses</td>
<td>2.6 (0.8)</td>
<td>5.7 (1.8)</td>
</tr>
<tr>
<td>Deamination</td>
<td>2.0 (0.7)</td>
<td>4.4 (1.5)</td>
</tr>
<tr>
<td>Retention in splanchic tissues</td>
<td>8.9 (1.2)</td>
<td>19.8 (2.6)</td>
</tr>
<tr>
<td>Including anabolic utilization</td>
<td>7.1 (1.0)</td>
<td>15.8 (2.1)</td>
</tr>
<tr>
<td>Retention in peripheral tissues</td>
<td>18.8 (3.7)</td>
<td>41.8 (8.0)</td>
</tr>
<tr>
<td>Including anabolic utilization</td>
<td>13.9 (3.3)</td>
<td>30.9 (7.2)</td>
</tr>
<tr>
<td>Total recovery</td>
<td>32.4 (4.3)</td>
<td>71.8 (9.5)</td>
</tr>
<tr>
<td>Including anabolic utilization</td>
<td>21.0 (3.7)</td>
<td>46.7 (8.30)</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 7.
same order of magnitude as that measured in fasting humans (25, 32).

From a methodological point of view, it seems that the priming dose was appropriate, because the ratio of the priming dose/infusion rate (8.5 h) was equal to the ratio of body pool size/rate of production (8.3 h), as previously discussed (32), and an adequate 2-h period between the priming dose and the sampling period (25). A slight overestimation of the fasting urea $N_R$, may have occurred, explaining the fast increase in endogenous urea production. Overall, the cumulated urea production was extremely low in piglets, in line with the fed values previously measured in 20-kg pigs (13). Finally, the postprandial dietary urea production represented only 4% of the ingested amount. Because such data on the specific dietary $N$ deamination are scarce in pigs, we are only able to compare the present results to that obtained in other species and conclude that the deamination is in the low range of that measured in young rats (5–25%) and is much lower than that measured in adult humans (~5%) (37, 38). Compared with splanchnic oxidation of specific dietary amino acids traced by $^{13}C$ measured in piglets of the same age, the deamination of dietary amino acids found is the present work is low as well (52, 55).

Such discrepancies may arise from differences between $N$ and $C$ metabolism/recycling or differences due to the mode of feeding (bolus vs. continuous enteral intake).

**Dietary $N$ digestibility, retention, and anabolic utilization in body tissues.** Using an intrinsically labeled $^{15}N$ meal, we found that the real cecal digestibility of the formula milk protein was 94%, a value close to those already found for milk protein in piglets or minipigs (18, 33) and consistently slightly inferior to the apparent protein digestibility (~98%) measured in piglets (35, 42). When summing these cecal losses and the transfer of dietary $N$ to urea production, we were able to estimate that the whole body postprandial retention of dietary $N$ is 90% of the ingested amount over 8 h, which represents a high efficiency of utilization. This result may be slightly overestimated because some pools were not sampled (for instance colon and fecal materials), as discussed below, but is consistent with the fast growth rate of piglets and the optimal ratio of energy to $N$ of their diet, as described previously (35). This result also compares well with the 85% daily $N$ retention-$N$ intake ratio in 22-day-old piglets (41) but is higher than the values of ~60% measured in older piglets (20 kg) (42), consistent with a decrease of the efficiency of $N$ utilization with growth.

We found a total recovery of the dietary $N$ amounting to 72% of the ingested dose, of which two-thirds was anabolic utilization in tissue protein fractions. The recovery of the dose of dietary $N$ was incomplete despite sampling of the body protein pools with the larger sizes or faster protein turnover rates. This incomplete recovery has already been observed in similar studies on the distribution in tissues of dietary $N$ after ingestion of mixed meals in rats (28, 31, 38) and, to a lesser extent, in newborn piglets infused with $^{[15]}N$alanine (44). The sum of organ masses of the piglets, either measured or estimated, amounted to 76% of their final body weight. The reasons for incomplete recovery of dietary $N$ thus certainly arise from underestimation of those protein pools, which were estimated but not weighed (i.e., total muscle mass, skin, colon content), from the sum of some small $N$ pools that were not sampled (bone mass, connective tissues, etc.) and from the underestimation of possible circulating peptides pools, as discussed earlier. Also an interesting finding was that incorporation of dietary $N$ into body protein pools 8 h after the meal ingestion represented, on average, 75% of the dietary $N$ retained, whereas when both endogenous $N$ in tissues are considered, the ratio between protein and nonprotein fractions was much higher, i.e., 90:10. This result indicates that a significant part of dietary $N$ is present in free form in tissues, presumably serving as a labile storage of amino acids during the postprandial phase and possibly in the form of peptides. The discrepancy between endogenous and dietary forms of $N$ in tissues was all the more pronounced, as tissues were far from the site of ingestion, with a particularly marked difference observed for muscle free $N$, which constituted 30% of the dietary $N$ retained, as opposed to endogenous $N$, which was composed of only 12% of free $N$. Such regional differences may be ascribed to the successive anatomic and metabolic steps of dietary $N$ process. A delayed appearance of dietary $N$ in the peripheral free amino acid pool compared with the splanchnic pool has been evidenced by modeling approaches (16).

The distribution of the dietary $N$ load into tissues at the end of the postprandial period was 10% in the small intestine segments, 5% in constitutive hepatic proteins, 3% in circulating protein, and 42% in the other tissues, of which ~37% was found in skeletal muscle and skin. Such a distribution was somewhat different from what we had measured in rats by use of similar methodology, with the main difference lying in the larger part of dietary $N$ recovered in intestine within splanchnic tissues: 54% in piglets compared with 28–40% in growing rats fed different levels of protein, respectively (28, 38). The present results can also be compared with the predicted tissue distribution of dietary $N$ obtained from a modeling approach using intestinal, plasma, and urine data to predict dietary $N$ postprandial kinetics in adult humans. Differences were related to the ratio between splanchnic and peripheral tissues and were characterized by a greater dietary $N$ incorporation into peripheral tissues in piglets (>45% of the dose ingested) than in humans [closer to 30–35% in conditions of a mixed-meal feeding (bolus vs. continuous enteral intake)].

**Fig. 4.** Correlation between percentage of tissue $N$ pool replaced by dietary $N$ in piglets 8 h after ingestion of a single meal (present study) and rates of protein synthesis (FSR) measured in 26-day-old fed piglets by the flooding-dose technique (11, 12).
ingestion (15, 16)]. Such a discrepancy obviously is ascribable to the net muscular anabolic utilization of protein in growing animals. However, the ratio of dietary N incorporation into plasma protein to total splanchnic constitutive protein compared well between the piglet results and human-modeled results (~19%), in line with the scarce other studies conducted in humans (1, 2, 24). In addition, the particular importance of intestinal tissue metabolism within splanchnic tissues in the piglet model is well recognized (4, 50–52) and seems even more pronounced for dietary N metabolism. As represented in Fig. 4, there was a good correlation between the ratios of tissue protein N pools replaced by dietary N at the end of the postprandial period and the fractional synthetic rates measured in piglets of the same age (11, 12). However, the ratio of dietary to total N was particularly high in the small intestine and rather low in the liver, which may be related to the fact that small intestinal metabolism of dietary amino acids is stimulated in conditions of a bolus (6). The low amount of dietary N anabolic utilization in liver protein pools found herein is also consistent with the quasi-null first-pass hepatic uptake of lysine measured in the same experimental conditions (6) or of valine in older pigs (13).

In conclusion, our study shows that some interorgan N fluxes deserve further research, notably the role of plasma protein in N transport, the importance of secreted intestinal protein, and the full quantification of dietary N distribution in tissues after a meal ingestion. These developments imply several experimental improvements or could be studied using an expanded modeling approach using such precious animal experimental data, including particularly the portal appearance of dietary N compounds derived from the meal absorption.

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REFERENCES


