CONTRIBUTION OF INTESTINAL MICROBIAL LYSINE TO LYSINE HOMEOSTASIS IS REDUCED IN MINIPIGS FED A WHEAT GLUTEN–BASED DIET

Gunda Backes, Ulf Hennig, Klaus J Petzke, Angelika Elsner, Peter Junghans, Gerd Nürnberg, and Cornelia C Metges

ABSTRACT

Background: We previously reported microbial lysine contribution to plasma lysine homeostasis in humans with an adequate lysine intake.

Objective: We sought to explore whether the low lysine intake from a wheat gluten–based diet is balanced by enhanced microbial lysine contribution in a pig model.

Design: Twenty miniature pigs (minipigs) fitted with ileo-ileal cannulas were fed 2 wheat gluten–based diets. One diet provided 2.7 g lysine/kg diet (WG diet) and one diet was supplemented with crystalline lysine to provide 6.6 g lysine/kg diet (WG+Lys diet). Both diets were fed for 10 or 100 d (n = 5 per group): 10WG, 10WG+Lys, 100WG, 100WG+Lys, and 100WG diets. Ileal microbial lysine, which we considered to be the precursor pool for absorption, was labeled by oral administration of 15NH4Cl for the final 10 d. On days 10 and 100, a 10-h fast-fed tracer protocol with [1,13C]lysine was performed.

Results: Lysine rates of appearance decreased by 25% with the WG diet in the fed state but increased by 50% with the WG+Lys diet in the fasted state (P < 0.05). Daily gross microbial lysine contribution was lower (P < 0.05) with the WG diet (205.3 μmol · kg⁻¹ · d⁻¹) than with the WG+Lys diet (370.7 μmol · kg⁻¹ · d⁻¹), irrespective of the adaptation period and was similar to the ileal lysine loss with the WG diet. In the WG groups, incorporation of microbial lysine increased in the duodenum and liver (P < 0.05) but not in whole-body and muscle proteins.

Conclusion: Minipigs fed the WG diet did not adapt by showing an enhanced absorption of microbial lysine to the extraplanchonic tissues, presumably because microbial lysine continues to be used for splanchnic protein synthesis. Am J Clin Nutr 2002;76:1317–25.

KEY WORDS: Miniature pig, microbial amino acids, ileal cannula, plasma lysine kinetics, small intestine, microflora, low-lysine diet, wheat gluten, [15N]lysine, gas chromatography–combustion isotope ratio mass spectrometry, GC-CIRMS

INTRODUCTION

The gastrointestinal tract of monogastric animals and humans is colonized by > 400 bacterial species (1). Among their various biosynthetic properties is the ability to synthesize indispensable amino acids (2). It has been suggested that the lysine requirement is not only met through the diet but possibly by lysine derived from de novo microbial synthesis (3). We and others showed previously that microbially derived lysine is absorbed and incorporated into the body protein of the mammalian host (4–8).

Diet in developing countries are low in lysine (31 mg/g protein) because the intake of proteins is mainly from grains, particularly wheat (9). The habitual lysine intake in parts of the developing world is below the FAO/WHO/UNU recommendation, which is based on the protein digestibility–corrected amino acid score (10, 11). Various metabolic adaptation and accommodation processes ensure survival if the protein or indispensable amino acid intake is not severely compromised (12). Therefore, we hypothesized that at low dietary lysine intakes, as is the case with the consumption of a wheat gluten–based diet, the body may be supplied by an additional amount of lysine made available by intestinal microbial lysine synthesis due to an adaptational response. Generally, it is believed that physiologic adjustments to alterations in the intake of an indispensable amino acid are complete within ≈1 wk. Because lysine is an amino acid that is particularly well conserved in the body, we anticipated that an increased contribution of microbial lysine might only occur after long-term adaptation (13–15).

Hence, the objectives of our study were to (1) compare the microbial lysine contribution (MLC) to plasma lysine turnover and whole-body and intestinal proteins with a wheat gluten–based semisynthetic diet (WG diet) and the WG diet supplemented with lysine (WG+Lys), and (2) determine whether changes in whole-body and microbial lysine and nitrogen metabolism occurring within 10 d of adjustment to the WG diet are maintained over a 100-d dietary adaptation time (10 or 100 d). We used minipigs as a human model.

1 From the Unit Protein Metabolism, German Institute of Human Nutrition, Bergholz-Rehbrücke, Germany (GB, KJP, AE, and CCM), and the Research Unit Nutritional Physiology “Oskar Kellner” (UH, PJ, and CCM) and the Research Unit Genetics and Biometrics (GN), Research Institute for the Biology of Farm Animals, Dummerstorf, Germany.

2 Supported by a grant from the Deutsche Forschungsgemeinschaft (Bonn, Germany) and a travel grant from NATO (Brussels).

3 Reprints not available. Address correspondence to CC Metges, Research Unit Nutritional Physiology, Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany. E-mail: metges@fbn-dummerstorf.de.

Accepted for publication January 31, 2002.
MATERIALS AND METHODS

Experimental design and 15N labeling

Twenty male, castrated minipigs (Minilewe strain) were obtained from the animal facilities of the Humboldt University of Berlin and the Technical University of Dresden, Germany, at a mean age of 8 wk and a mean body weight of 8 kg. The experimental protocol was approved by the Ethics Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, Schwerin, State Mecklenburg-Vorpommern, Germany (permission no. VI 522a-7221.31-1-031/97). The minipigs were cared for according to the European Union guidelines on the use of animals for experimental purposes (86/609/EU). Before the start of the experiment, the minipigs were fed a commercial cereal-based diet (barley, wheat, rye, triticale, and soy; Masta Universal, Nordkom Agrarhandel GmbH, Karstädt, Germany). The diet provided 13 MJ/kg metabolizable energy and was composed of 15.5% crude protein, 6.1% crude fat, 5.7% crude fiber, and 0.88% lysine. When the minipigs reached a body weight of ≈23 kg (10-d adaptation) or 17 kg (100-d adaptation), they were randomly assigned to 4 feeding groups (see below) and then housed individually. At termination of the experiment, all animals were 6 mo old.

The maintenance requirement for lysine is similar in humans and pigs, and pigs are suitable model animals for nutritional studies in humans (16, 17). Furthermore, the absorption of microbial lysine due to coprophagy only, as shown in rats, could be excluded (8). We used minipigs as experimental subjects because tracer material can be used more economically because of the lower adult body weight of minipigs than of conventional pigs.

To simulate a diet with a low lysine intake, such as is consumed in developing countries, a diet with wheat gluten (2.7 g lysine/kg diet, protein digestibility–corrected amino acid score 0.5, and 25 mg lysine/g protein; 11) as the only protein source was chosen (Table 1). Because there is no specific data on the lysine requirement of adult minipigs at a body weight of 15–25 kg (19), we assumed that the developmental stage of the minipigs is comparable with that of young adult conventional pigs, and pigs are suitable model animals for nutritional studies and blood sampling, respectively. Catheters were kept open by a constant infusion (0.7 mL/h) of physiologic saline containing heparin (1000 IU/L), heparin-sodium; B Braun Melsungen AG, Melsungen, Germany) and sulfadimidine (2 mL/L; Sulfadimidin as sodium salt; Serumwerk Bernburg AG, Bernburg, Germany).

Tracer-infusion studies

On day 10 (100WG+Lys and 10WG groups) and day 100 (1000WG+Lys and 1000WG groups) of the experimental period, a 10-h tracer protocol was conducted after an overnight fast. Tracer solutions were prepared under sterile conditions in physiologic saline. At 0800, a constant intravenous infusion of L-[1-13C]lysine·HCl (99 AP, 5 μmol·kg⁻¹·h⁻¹; MassTrace, Woburn, MA) was started after intravenous priming doses of NaH¹³CO₃ (99 AP, 1.0 μmol/kg; CIL, Andover, MA) and [¹³C]lysine (7.5 μmol/kg) with the use of a screw-driven syringe pump (model 22; Harvard Apparatus, South Natick, MA). During the first 4 h of the infusion, no food was given, and then the minipigs were fed meals every half hour from 1200 to 1800. One-twelfth of the daily intake was consumed per meal; the first and last meals contained ¹⁵NH₄Cl.

Sample collection and analysis

Postabsorptive blood samples were drawn from the carotid catheter into lithium heparin–containing tubes (Monovette; Sarstedt AG & Co, Nürnbergch, Germany) before (day −1) and on days 3, 5, 7, 9, and 10 of the ¹⁵NH₄Cl administration period. These time points corresponded to days 89, 93, 95, 97, 99, and 100 of the experimental period in the groups adapted to the diets for 100 d (100WG+Lys and 100WG diets). On the day of the [¹³C]lysine infusion, baseline blood samples were obtained, and then blood was withdrawn every 30 min during the last 2 h of the fasted (120–240 min) and fed (480–600 min) periods, respectively. Plasma was separated by centrifugation (10 min, 4°C, 900 × g) and stored frozen at −80°C until analyzed.

By means of ileo-ileal cannulas, 24-h samples of ileal chyme were collected into chilled containers on days 8, 9, and 10 of the ¹⁵NH₄Cl administration period; a baseline sample was collected on day −1. Immediately after being collected, the ileal chyme was divided into aliquots of 15% and 85% (by wt). The 15% sample was stored at −20°C and then freeze-dried, whereas the 85% sample was homogenized and conserved in an equivalent volume of 10% sodium lauryl sulfate:1% formaldehyde:physiologic saline (0.1:5:5, by vol) at 2°C until analyzed further. The minipigs were killed by intravenous injection of hexobarbital (2 g per animal, Hexobarbital-Natrium AWD; VEB Arzneimittelwerk, Dresden, Germany) on days 11 and 101. Then, the complete gastrointestinal
tract was removed to avoid contamination of tissue samples with 15N-labeled digesta. Tissue samples of the duodenum, ileum, liver, and skeletal muscle (longissimus dorsi) were frozen in liquid nitrogen immediately after being rinsed with cold physiologic saline. Mucosa of the ileum and duodenum was sampled by scraping with a horn spatula and frozen immediately in liquid nitrogen. All organ and mucosa samples were kept frozen at −20 °C for analysis of whole-body protein.

The microbial fraction of the ileal chyme was isolated by differential centrifugation for 40 min at 400 × g and 4 °C to remove food particles and then for 40 min at 22 000 × g at 4 °C (22, 23). The pellet was isolated by isopropanol, precipitated with 10% trichloroacetic acid, and subsequently washed with acetone and ether. Microbial and tissue proteins (trichloroacetic acid precipitates) were hydrolyzed (6 mol HCl/L, 24 h, 110 °C), and amino acids were isolated and measured for [15N]lysine enrichment as previously described (5).

Plasma was acidified (0.1 mol HCl/L, pH 2) and poured onto a cation exchange column (Dowex 50WX8, Na+ form, 200–400 mesh; Fluka, Steinheim, Germany). Free amino acids were eluted with 4 mol NH4OH/L. The eluate was dried at 60 °C under nitrogen (24).

To measure [15N]lysine enrichments, plasma and protein amino acids were derivatized to form N-pivaloyl-isopropyl esters as described in detail (5, 24). Briefly, amino acids were esterified with the use of acetylchloride and isopropanol. The product was dried under nitrogen and dissolved in ethylacetate and kept at −20 °C until analyzed. The plasma free [15N]lysine enrichment was analyzed with gas chromatography–mass spectrometry (GC-MS) and chemical ionization by methane (SSQ710; ThermoFinnigan, Bremen, Germany). Lysine N-trifluoro-acetyl-propyl esters were separated on a DB-5MS column (30 m × 0.25 mm internal diameter, 0.25-μm film thickness; J&W Scientific, Folsom, CA). Both unlabeled and labeled lysine were measured by single ion monitoring in the range of m/z 339 to 341 [diagnostic ion lysine (M+H+)-42]. By analyzing at m/z 304/339 and 341/339 in baseline samples before the [15N]lysine infusion, it was established that there was no interference of [15N]lysine with [13C]lysine enrichment. Samples (1 μL) were injected splitless (injector temperature: 280 °C; temperature program: 100–280 °C, ramp 10 °C per minute; held for 8 min at 300 °C). With the use of splitless mode, 0.5 μL of sample was injected with an autosampler (CTC A200S; CTC Analytics, Zwillenberg, Germany). Under these conditions, the lysine derivative eluted after 60 min. Measured ratios of 15N to 14N were derived from mass-to-charge ratio (m/z) 29 to m/z 28 ion current signals of the mass spectrometer, and the standard gas used was calibrated against the international air standard. Enrichments were converted to 15N AP (5). For the calculation of atom percent excess (APE), the respective 15N baseline enrichment was subtracted.

Plasma [13C]lysine enrichments were determined after conversion to N-trifluoro-acetyl-propyl esters (25). Free amino acids were isolated from 250-μL plasma samples by cation exchange. The dried eluate was combined with acetylchloride:isopropanol (1:4, by vol), and the mixture was heated at 110 °C for 30 min. Trifluoroacetic anhydride and dichloromethane were added, and, after heating at 60 °C for 30 min, the mixture was dried and redissolved in ethylacetate and kept at −20 °C until analyzed. The plasma free [13C]lysine enrichment was analyzed with gas chromatography–mass spectrometry (GC-MS) and chemical ionization by methane (SSQ710; ThermoFinnigan, Bremen, Germany). Lysine N-trifluoro-acetyl-propyl esters were separated on a DB-5MS column (30 m × 0.25 mm internal diameter, 0.25-μm film thickness; J&W Scientific, Folsom, CA). Both unlabeled and labeled lysine were measured by single ion monitoring in the range of m/z 339 to 341 [diagnostic ion lysine (M+H+)-42]. By analyzing at m/z 304/339 and 341/339 in baseline samples before the [15N]lysine infusion, it was established that there was no interference of [15N]lysine with [13C]lysine enrichment. Samples (1 μL) were injected splitless (injector temperature: 280 °C; temperature program: 100 °C for 0.5 min and 100–280 °C, ramp 30 °C per minute). The enrichment calculations were based on tracer-trace ratios and calibration curves, derived from standard mixtures (0–10 moles percent excess).
The nitrogen contents in lyophilized samples of ileal chyme, the microbial fraction of ileal chyme, and tissue samples were determined with a combustion analyzer (Vario EL; Elementar Analysensysteme GmbH, Hanau, Germany).

Plasma d-lysine concentrations were determined as \(N\)-(O)-pentafluoropropionyl 2-propyl esters by GC-MS (impact ionization; SSQ710). Briefly, to 200 \(\mu\)L plasma, 8 nmol \(\text{trans-4-(aminomethyl)cyclohexanecarboxylic acid}\) was added as internal standard, and the sample was precipitated with sulfosalicylic acid (5\% wt:vol). For derivatization, 1 mL 2-propanol in acetyl chloride (8:2, by vol) was combined with the supernatant fluid and the reaction mixture was esterified at 100 \(^\circ\)C for 1 h. The sample was subsequently dried under nitrogen at 60 \(^\circ\)C. Dichloromethane (200 \(\mu\)L) and pentafluoropropionic acid anhydride (50 \(\mu\)L) were added, and the mixture was heated (20 min at 100 \(^\circ\)C). The sample was evaporated and redissolved in 250 \(\mu\)L dichloromethane. d- and l-Lysine were separated on a Chirasil-D-Val column (25 m \(\times\) 0.25 mm internal diameter, 0.08-\(\mu\)m film thickness; Chrompack, Middelburg, Netherlands). A 1-\(\mu\)L sample was injected splitless (injector temperature: 270 \(^\circ\)C; temperature program: 60–70\(^\circ\)C, ramp 2.5 \(^\circ\)C per minute; 70–90\(^\circ\)C, ramp 8 \(^\circ\)C per minute; 190 \(^\circ\)C for 5 min; carrier gas, helium) and the diagnostic ions \(m/z\) 230 and 258 for lysine and \(\text{trans-4-(aminomethyl)cyclohexancarboxylic acid}\) derivatives, respectively, were monitored. The d-lysine concentration was calculated by internal standard calibration. We measured both plasma and microbial \([^{13}\text{C}]\)lysine enrichments without consideration of the enantiomeric form, because we had no indication that d-lysine contributes largely to the plasma lysine pool. That this approach was justified was confirmed by measurements in postabsorptive and postprandial plasma samples taken during the \([^{13}\text{C}]\)lysine infusion, which resulted in a concentration of <1\% d-lysine.

The concentrations of lysine and other amino acids in plasma and proteins were determined by ion-exchange chromatography with HPLC (System Gold; Beckman Instruments GmbH, Munich, Germany) as reported previously (26). The plasma urea concentration was determined with a coupled colorimetric ultraviolet test that used glutamate dehydrogenase (EC 1.4.1.4) (Olympus System Potsdam, Germany) with a coupled colorimetric ultraviolet test that used glutamate dehydrogenase (EC 1.4.1.4) (Olympus System Potsdam, Germany) as reported previously (26). The plasma urea concentration was determined with a coupled colorimetric ultraviolet test that used glutamate dehydrogenase (EC 1.4.1.4) (Olympus System Potsdam, Germany)

The plasma D-lysine concentrations were determined as \(N\)-(O)-pentafluoropropionyl 2-propyl esters by GC-MS (impact ionization; SSQ710). Briefly, to 200 \(\mu\)L plasma, 8 nmol \(\text{trans-4-(aminomethyl)cyclohexanecarboxylic acid}\) was added as internal standard, and the sample was precipitated with sulfosalicylic acid (5\% wt:vol). For derivatization, 1 mL 2-propanol in acetyl chloride (8:2, by vol) was combined with the supernatant fluid and the reaction mixture was esterified at 100 \(^\circ\)C for 1 h. The sample was subsequently dried under nitrogen at 60 \(^\circ\)C. Dichloromethane (200 \(\mu\)L) and pentafluoropropionic acid anhydride (50 \(\mu\)L) were added, and the mixture was heated (20 min at 100 \(^\circ\)C). The sample was evaporated and redissolved in 250 \(\mu\)L dichloromethane. d- and l-Lysine were separated on a Chirasil-D-Val column (25 m \(\times\) 0.25 mm internal diameter, 0.08-\(\mu\)m film thickness; Chrompack, Middelburg, Netherlands). A 1-\(\mu\)L sample was injected splitless (injector temperature: 270 \(^\circ\)C; temperature program: 60–70\(^\circ\)C, ramp 2.5 \(^\circ\)C per minute; 70–90\(^\circ\)C, ramp 8 \(^\circ\)C per minute; 190 \(^\circ\)C for 5 min; carrier gas, helium) and the diagnostic ions \(m/z\) 230 and 258 for lysine and \(\text{trans-4-(aminomethyl)cyclohexanecarboxylic acid}\) derivatives, respectively, were monitored. The d-lysine concentration was calculated by internal standard calibration. We measured both plasma and microbial \([^{13}\text{C}]\)lysine enrichments without consideration of the enantiomeric form, because we had no indication that d-lysine contributes largely to the plasma lysine pool. That this approach was justified was confirmed by measurements in postabsorptive and postprandial plasma samples taken during the \([^{13}\text{C}]\)lysine infusion, which resulted in a concentration of <1\% d-lysine.

The concentrations of lysine and other amino acids in plasma and proteins were determined by ion-exchange chromatography with HPLC (System Gold; Beckman Instruments GmbH, Munich, Germany) as reported previously (26). The plasma urea concentration was determined at a commercial laboratory (Hygiene-Institut, Potsdam, Germany) with a coupled colorimetric ultraviolet test that used glutamate dehydrogenase (EC 1.4.1.4) (Olympus System Reagent 500; Olympic Diagnostica GmbH, Hamburg, Germany).

All chemicals used were of analytic grade and obtained from several suppliers (Fluka Chemie AG, Buchs, Switzerland; Sigma-Aldrich Chemie GmbH, Steinheim, Germany; Merck, Darmstadt, Germany).

### Evaluation of primary data

With the use of a steady state model, the rate of appearance of plasma \([^{13}\text{C}]\)lysine (Lys \(R_a\)), ie, lysine turnover, was calculated at half-hourly intervals as follows:

\[
Lys \ R_a \ (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{30 min}^{-1}) = \frac{i \times (E_i/E_p)}{i} \tag{1}
\]

where \(i\) is the tracer infusion rate (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{30 min}^{-1}\)), \(E_i\) is the \(^{13}\text{C}\) enrichment of the administered tracer, and \(E_p\) is the \(^{13}\text{C}\) enrichment (MPE) in plasma under fasted (120–240 min) and fed (540–600 min) conditions. Steady state conditions were defined by the absence of a significant slope.

The MLC to Lys \(R_a\) was calculated from the \([^{15}\text{N}]\)lysine enrichment in the WG+Lys group in the fed state than in the fasted state. \(\frac{\text{MLC}}{\text{LysILCH}}\) was measured on days 10 and 100 only, assuming that there was no significant difference in lysine concentrations between days 8 and 10 or days 98 and 100.

### Statistical methods

Data were analyzed with a mixed model, with 2 fixed effects (lysine concentration and adaptation period), a repeated factor (time \((-30\) min, fasting, and fed)), a random factor animal, and corresponding interactions by using the PROC MIXED procedure of SAS (SAS Systems, release 8.2; SAS Institute Inc, Cary, NC) (27). A two-factor fixed-effects (lysine concentration and adaptation period) and one-interaction ANOVA were performed with an SAS general linear models procedure when measurements occurred only once (eg, 24-h MLC). When significant interactions were present, post hoc tests of subclasses with a Tukey-Cramer correction (to ensure a multiple test risk of first kind \(\leq 0.05\)) were applied. Significance was set at \(P = 0.05\).

### RESULTS

#### Body weight

At the end of the experiment, mean body weights were not significantly different between groups (100WG: 25.9 ± 1.5 kg; 100WG+Lys: 26.2 ± 1.9 kg; 100WG: 22.8 ± 2.1 kg; and 100WG+Lys: 22.5 ± 1.4 kg). The original mean values in the 4 experimental groups (10WG, 10WG+Lys, 100WG, and 100WG+Lys) are shown in Table 2 and Figures 1 and 2, whereas statistical evaluation and least-squares means are shown in Table 2 and Tables 3–5.

### Lysine concentrations and lysine kinetics

Plasma lysine concentrations measured during the fed state were significantly affected by the dietary lysine content; values were significantly lower with the WG diet (Tables 2 and 3). A significant time effect resulted in a higher plasma lysine concentration in the WG+Lys group in the fed state than in the fasted state or at \(-30\) min (Tables 2 and 3). We found a higher Lys \(R_a\) in the WG+Lys groups than in the WG groups in the fed state (Table 3). In the WG+Lys groups, Lys \(R_a\) was significantly higher in the fed than in the fasting state, whereas it was significantly lower in the fed than in the fasting state in the WG groups (Table 3). A significant lysine \(\times\) adaptation period \(\times\) time interaction was observed for Lys \(R_a\) (Tables 2 and 3). However, the subgroup analysis did not confirm significant differences between 10 and 100 d (Table 3). Lysine concentrations in whole-body (carcass) protein did not differ significantly between groups (overall mean: 87 mg/g protein; data...
not shown). The same was observed for tissue samples (skeletal muscle, liver, ileum, ileal mucosa, duodenum, and duodenal mucosa) (data not shown). Lysine concentrations in total ileal chyme were not significantly different among groups on day 10 of the $^{15}$NH$_4$Cl administration (Figure 1). Lysine concentrations in the ileal microbial protein fraction amounted to 50% of that in the total ileal chyme (Figure 1). An adaptation effect was also significant for the ratio of glycine to lysine in liver tissue, with higher values in the WG group (Table 4). Ileal microbial lysine was $\approx$10 times more enriched than was plasma free lysine in the same group (Table 4; Figure 2). Tissue $[^{15}]$Nlysine enrichments are shown in Tables 4 and 5. MLC was affected only by the lysine intake, resulting in a reduced MLC with the WG diet (Tables 3 and 4).

**DISCUSSION**

This investigation was the first to explore whether the MLC to plasma lysine homeostasis and whole-body protein is affected by the dietary lysine intake and the adaptation period to the diet. On the basis of the fact that lysine is not transaminated in mammalian

TABLE 2

Mean plasma lysine concentrations and kinetics, whole-body microbial lysine contribution (MLC), plasma urea concentrations, total ileal lysine loss, and glycine-lysine and alanine-lysine ratios in body proteins in 4 groups of minipigs fed wheat gluten–based diets with (WG+Lys) or without (WG) lysine supplementation for a 10- or 100-d adaptation period (Adapt)$^1$

<table>
<thead>
<tr>
<th></th>
<th>10 d</th>
<th>100 d</th>
<th>$P^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WG+Lys</td>
<td>WG</td>
<td></td>
</tr>
<tr>
<td>Plasma lysine concentration (µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−30 min</td>
<td>71.3 ± 21.1</td>
<td>66.6 ± 33.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasted</td>
<td>97.4 ± 21.5</td>
<td>105.1 ± 22.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fed</td>
<td>352.9 ± 113.4</td>
<td>54.8 ± 17.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma lysine rate of appearance (µmol·kg$^{-1}$·30 min$^{-1}$)$^3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>44.2 ± 4.9</td>
<td>45.0 ± 3.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fed</td>
<td>88.7 ± 8.9</td>
<td>30.9 ± 3.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ileal microbial $[^{15}]$Nlysine enrichment (APE)$^4$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−30 min</td>
<td>0.21 ± 0.16</td>
<td>0.26 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Fasted</td>
<td>0.19 ± 0.14</td>
<td>0.12 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fed</td>
<td>0.12 ± 0.08</td>
<td>0.14 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MLC (µmol·kg$^{-1}$·30 min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>7.4 ± 5.5</td>
<td>5.1 ± 2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Fed</td>
<td>10.4 ± 6.5</td>
<td>4.3 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Daily MLC (µmol·kg$^{-1}$·24 h$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>427 ± 269</td>
<td>225 ± 99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma urea concentration (mmol/L)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>−30 min</td>
<td>2.16 ± 0.98</td>
<td>2.98 ± 0.54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fasted</td>
<td>1.58 ± 0.68</td>
<td>2.14 ± 0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fed</td>
<td>2.30 ± 1.04</td>
<td>3.22 ± 0.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Daily ileal lysine loss (µmol·kg$^{-1}$·24 h$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>124 ± 34</td>
<td>144 ± 41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycine-lysine ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-body protein</td>
<td>3.74 ± 0.23</td>
<td>3.89 ± 0.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver protein</td>
<td>1.58 ± 0.07</td>
<td>1.58 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alanine-lysine ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-body protein</td>
<td>1.74 ± 0.10</td>
<td>1.80 ± 0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver protein</td>
<td>1.39 ± 0.06</td>
<td>1.38 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^1$$^1$SEM: $^2$SEM: $^3$SEM:

$^4$Measured on day 8 of the $^{15}$NH$_4$Cl administration (equivalent to days 8 and 98 of the experimental feeding, respectively).

[^15]Nlysine enrichments and microbial lysine contribution

During the 10-d period of $^{15}$NH$_4$Cl administration, plasma free $[^{15}]$Nlysine enrichments increased in all groups but were not significantly different on day 10 (Figure 2). We detected no significant differences in $[^{15}]$Nlysine enrichments in the ileal microbial fraction between groups on day 10 (data not shown). However, on day 8 of $^{15}$NH$_4$Cl administration, the effect of the dietary lysine intake was significant, with higher values in the WG group (Table 4). Ileal microbial lysine was $\approx$10 times more enriched than was plasma free lysine in the same group (Table 4; Figure 2). Tissue $[^{15}]$Nlysine enrichments are shown in Tables 4 and 5. MLC was affected only by the lysine intake, resulting in a reduced MLC with the WG diet (Tables 3 and 4).
tissues (28), we labeled microbial lysine via oral administration of inorganic $^{15}$N. The appearance of $[^{15}$N$] $lysine in plasma and body proteins indicates its microbial origin and can be used to calculate the MLC (6, 28). However, it remains unclear whether the estimates of microbially derived lysine correspond to a net contribution (2, 6), although this has been claimed (7). Nevertheless, this approach can be used to make a comparison of the MLC when diets with different lysine contents are consumed. We were interested to know whether microbial lysine derived from the small intestine might contribute to the lysine conservation process (29, 30) when a wheat gluten diet is ingested.

Plasma lysine concentrations during the fed state were affected by the dietary lysine intake. Lower plasma lysine concentrations as a response to a low-lysine diet were also found in other studies (31–34). Plasma urea concentrations markedly responded to dietary lysine intakes, thus reflecting increased amino acid catabolism and urea production with the WG diet, which is a consequence of the low protein quality (35). Previous studies showed that the lysine intake is inversely associated with plasma urea concentrations (36, 37). Lys $R_b$ decreased with meal feeding at the lower lysine intake, in contrast with the increase that occurred during the fed state when the wheat gluten diet was supplemented with lysine (Tables 2 and 3). These observations are consistent with those from human studies and suggest that flux is stimulated when meals containing a more generous amount of amino acids are consumed (31, 38, 39). The time effect observed on some indexes was caused by the additional lysine input due to either intravenous lysine infusion or meal intake (Tables 2 and 3).

It is important to know the degree of lysine limitation with the WG diet because it might have implications for the MLC and thus for the interpretation of studies aimed at determining the lysine requirement. Body weight gain in minipigs in the WG groups was not significantly different from that in the WG+Lys groups. Additional information can be derived from preliminary lysine oxidation data (not shown), which suggests that although lysine oxidation was strongly suppressed in the WG groups, lysine balance was still positive. Thus, the WG diet, as compared with the WG+Lys diet, did not seem to be severely lysine limiting in these animals.

Because it is thought that microbially derived lysine is absorbed mainly at the terminal ileum (2, 40), we cannulated minipigs at the ileal site to gain access to this precursor pool. Consequently, the ratio of plasma $[^{15}$N$] $lysine to ileal microbial $[^{15}$N$] $lysine measures directly the contribution to the Lys $R_b$. However, because circulating $[^{15}$N$] $lysine enrichment had not achieved equilibrium, our calculations are only estimates. Nevertheless, between 9% and 16% of the microbial $[^{15}$N$] $lysine enrichment appears in the circulation, which is consistent with earlier results (2). Thus, the
we measured the incorporation of microbial [15N]lysine into the turnover than to decreased absorption of microbial lysine. However, it appears that this was due more to changes in plasma lysine intake, suggesting a lower absorption rate, lower synthesis, or a pool (Table 3). By contrast, the ratios were not affected by lysine quantitative data. Assuming, for the sake of argument, that if the increased microbial lysine synthesis, although it does not provide increased microbial lysine synthesis may benefit more from this source.

to further explore the fate of microbially synthesized lysine, we measured the incorporation of microbial [15N]lysine into the protein of splanchnic tissues, skeletal muscle, and whole body. [15N]Lysine enrichment in tissue protein of the duodenum and the liver was higher at the lower lysine intake (Table 4), whereas it did not differ significantly in whole body and muscle. This indicates that at the lower lysine intake, tissues close to the site of microbial lysine synthesis may benefit more from this source. Thus, the intestinal lysine requirement for pigs could be met in part by microbial lysine. This agrees with observations in protein-restricted piglets that lysine oxidation in portal-drained viscera is virtually absent, whereas the total lysine use of the portal-drained viscera was largely unaffected by low-protein feeding (41). Hence, this explains why the MLC did not increase with the WG diet. Any additional lysine input derived from the microflora was used for gut protein synthesis, and, although intestinal lysine oxidation was suppressed, even enhanced amounts of microbial lysine could not reach the peripheral circulation.

Another important question was whether lysine losses at the terminal ileum were affected by the dietary lysine supply, because a lower dietary lysine input might be counterbalanced by decreased ileal lysine losses. The total mean lysine loss did not differ among groups and ranged between 18 and 22 mg·kg⁻¹·d⁻¹. This is 3 times the daily lysine loss as measured in human subjects with an ileostomy (6). Interestingly, the ileal lysine loss measured in the present study was similar to the daily MLC in the WG group (Table 2). This indicates that the MLC might be important in counteracting ileal lysine losses but that it is not a net source of lysine in addition to the diet, as discussed previously (2, 6). It appears that there are 2 main factors that determine whether microbial lysine synthesis is nutritionally relevant. One factor is the composition of intestinal microflora and the amount of microbial lysine produced that is affected by the type of the diet, and possibly the subsequent endogenous nitrogen secretions. The second factor is the first-pass lysine metabolism, because it determines the amount of dietary and microbial lysine eventually reaching the periphery.

Lysine concentrations in ileal chyme, the ratio of glycine to lysine in liver tissue, and [15N]enrichments in whole body and muscle protein responded to long-term adaptation (Table 5, Figure 1). It appears that when animals had been previously adapted to the diets long term, lysine concentrations in ileal chyme were lower than in animals fed the preexperimental diet with its higher lysine content immediately before the start of the experimental feeding (Figure 1). This may indicate that the lysine supply from both

### Table 4
Effects of dietary lysine intake [wheat gluten–based diet with (WG+Lys) or without (WG) lysine supplementation] on daily microbial lysine contribution (MLC) to whole-body lysine homeostasis and [15N]lysine enrichments in ileal microbial protein and tissues in minipigs.

<table>
<thead>
<tr>
<th></th>
<th>WG+Lys (n = 10)</th>
<th>WG (n = 10)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily MLC (μmol·kg⁻¹·d⁻¹)</td>
<td>370.7 ± 50.9</td>
<td>205.3 ± 47.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>[15N]Lysine enrichments (APE)³</td>
<td>0.086 ± 0.0014</td>
<td>0.0170 ± 0.0013</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1. Least-squares means ± SE. Subclass comparisons were not performed when no significant interaction was present.
2. p values of F tests derived from a two-factor fixed-effect ANOVA of measurements made only once.
3. Measured after 10 d of [15NH₄]Cl administration unless noted otherwise.

### Table 5
Effects of adaptation period (Adapt; 10 or 100 d) on [15N]lysine enrichments in the whole body and muscle, the glycine-lysine ratio in liver, and the lysine concentration in ileal chyme in minipigs.

<table>
<thead>
<tr>
<th></th>
<th>Adaptation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 d (n = 10)</td>
</tr>
<tr>
<td>[15N]Lysine enrichments (APE)³</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0024 ± 0.0003</td>
</tr>
<tr>
<td>Whole body</td>
<td>0.0049 ± 0.0004</td>
</tr>
<tr>
<td>Liver glycine-lysine ratio</td>
<td>1.575 ± 0.015</td>
</tr>
<tr>
<td>Ileal chyme lysine concentration, day −1 (g lysine/g protein)</td>
<td>0.127 ± 0.011</td>
</tr>
</tbody>
</table>

¹ Least-squares means ± SE. APE, atom percent excess.
² P values of F tests derived from a two-factor fixed-effect ANOVA of measurements made only once.
³ Measured after 10 d of [15NH₄]Cl administration.
diets was not optimal, a conclusion that is supported by the increased glycine-lysine ratio after the 100-d adaptation (Table 5). That body amino acid concentrations are not constant and that they respond to differences in dietary intakes was shown previously (29, 42). These observations might be related to a depletion of proteins particularly rich in the deficient indispensable amino acid (3, 42). [15N]Lysine enrichments in muscle and whole body were affected by the adaptation period but in the opposite direction (Table 5). This finding appeared to be due to experimental and biological factors that we could not identify. Overall, the dietary lysine intake had more of an effect on the observed changes than did the adaptation period (Tables 2–5).

To summarize, we observed intestinal de novo synthesis and absorption of microbial lysine when adult mini pigs were fed wheat gluten–based diets for 10 or 100 d. The daily gross MLC to plasma lysine homeostasis was lower in the WG groups than in the WG+Lys groups; the adaptation period had no effect. Splanchnic proteins benefited more from the incorporation of microbial lysine when the WG diet was fed. By contrast, microbial lysine incorporation in muscle and whole body was not affected by the dietary lysine intake. This might be explained by previous findings in piglets (41), i.e., when dietary lysine intakes are low, intestinal lysine extraction is maintained at a level observed with an adequate protein (lysine) intake and, thus, increased amounts of microbial lysine cannot reach the peripheral circulation. Lysine losses at the terminal ileum were not affected by lysine intake. It is suggested that the MLC helps to counteract ileal lysine losses, which contribute to intestinal lysine requirements being met. In conclusion, it appears that adult mini pigs do not adapt to a wheat gluten–based diet via enhanced absorption of microbial lysine because of concurrent changes in lysine metabolism in the portal-drained viscera.

We thank the staff of the Unit Protein Metabolism of the German Institute of Human Nutrition, Bergholz-Rehbrücke, and the staff of the Research Unit Nutritional Physiology “Oskar Kellner” at the Research Institute for the Biology of Farm Animals (Dummerstorf, Germany) for excellent technical assistance.

REFERENCES

32. El-Khoury AE, Pereira PCM, Borgonha S, et al. Twenty-four–hour oral tracer studies with L-[1-13C]lysine at a low (15 mg·kg⁻¹·d⁻¹) and intermediate (29 mg·kg⁻¹·d⁻¹) lysine intake in healthy adults. Am J Clin Nutr 2000;72:122–30.