Z Ernährungswiss 37: 368–375 (1998)

ORIGINAL CONTRIBUTION

K.J. Petzke
J.G. Grigorov
O.V. Korkushko
N.K. Kovalenko
T.G. Semesko
C.C. Metges

Incorporation of urea nitrogen into fecal protein and plasma protein amino acids in elderly human volunteers after ingestion of lactic acid bacteria

Inkorporation von Harnstickstoff in Proteine von Plasma- und Stuhlproben bei älteren Menschen nach Gabe von Lactobazillen

Summary Health effects of fermented milks have been associated with the metabolic activity of lactic acid bacteria in the gastrointestinal tract. It has been proposed that an increased excretion of urea nitrogen via microbial protein may decrease the workload on kidneys and liver. Therefore, a study was carried out in healthy elderly human subjects to investigate the incorporation of [$^{15}N_2$]urea nitrogen into plasma and fecal proteins and amino acids. Over a period of 10 d 13 healthy elderly subjects ingested daily a freeze-dried microbial preparation which contained different genera of lactic acid bacteria and is used to produce fermented milk products. One of the strains was originally isolated from stool samples of elderly people from the Caucasus region (*Lactobacillus plantarum*). No stimulation of fecal protein-nitrogen excretion and no increase in ^{15}N-abundances in fecal protein was measured following the administration of the viable microbial preparation and a [$^{15}N_2$]urea bolus. Tentatively, it was concluded that this may have been caused by the inability of the microbial culture to survive the gastro-intestinal passage and (or) by the absence of additional fermentable carbohydrates in the diet as energy source for bacterial protein synthesis in the large intestine. However, using a highly sensitive GC-C-IRMS method we observed a significant incorporation of ^{15}N into plasma protein amino acids. ^{15}N-Enrichments in single amino acids were found according to their participation in transamination reactions. The slight enrichment of lysine which is not transaminated in mammalian tissues may indicate a microbial synthesis and absorption of bacterial lysine.

Received: 29 May 1998
Accepted: 17 September 1998

Supported in part by grant 07ERG16/8 from Federal Secretary of State for Education and Research, Germany. Authors are responsible for the content.

Dr. K.J. Petzke (✉) · C.C. Metges
Deutsches Institut für Ernährungsforschung
Potsdam-Rehbrücke
Arthur-Scheunert-Allee 114–116
D-14558 Bergholz-Rehbrücke
e-mail. petzke@www.dife.de

J.G. Grigorov · O.V. Korkushko
T.G. Semesko
Institut für Gerontologie
Vyshgorodskaja 67
254114 Kiev
Ukraine

N.K. Kovalenko
Institut für Mikrobiologie und Virologie
Zabolotnovo 154
252143 Kiev
Ukraine

Zusammenfassung Gesundheitlich vorteilhafte Auswirkungen einer Ernährung mit fermentierten Milchprodukten werden mit der gleichzeitigen Aufnahme von Lactobazillen, die den Gastrointestinaltrakt passieren und besiedeln, und deren metabolischen Aktivitäten in Zusammenhang gebracht. So sollte durch eine vermehrte Ausscheidung von Harnstoff-Stickstoff über mikrobielles Protein eine Entlastung von Leber und Niere erreicht werden können. Wir haben Untersuchungen an 13 älteren, gesunden Menschen durchgeführt und die Inkorporation von ^{15}N aus [$^{15}N_2$]-Harnstoff in Proteine von Plasma- und Stuhlproben nach Gabe eines probiotischen lyophilisierten Keimpräparates (Starterkultur mit isolierten Keimen aus dem Darm Langlebiger im Kaukasus zur Herstellung von fermentierten Milchprodukten) durchgeführt. Es wurde jedoch keine Stimulierung der Inkorporation von ^{15}N aus [$^{15}N_2$]-Harnstoff in Fäzesproteine durch die Gabe der getrockneten Kultur beobachtet. Immer wurde aber eine signifikante Ausscheidung von Harnstoff-Stickstoff in Form von fäkalem Protein gemessen. Wahrscheinlich überleben die Keime des verwendeten Präparates die gastro-intestinale Passage nicht und (oder) der nicht veränderte Anteil

fermentierbarer Kohlenhydrate in der Diät erlaubt kein zusätzliches intestinales bakterielles Wachstum. Die Messung einzelner Plasmaprotein-Aminosäuren mittels GC-C-IRMS ergab ^{15}N-Anreicherungen entsprechend ihrer Beteiligung an Prozessen der Transaminierung. Das nicht an Transaminierungsprozessen des Organismus beteiligte Lysin zeigte ebenfalls eine sehr geringe ^{15}N-Anreicherung, was auf mikrobielle Synthese und Absorption bakteriellen Lysins hinweisen kann.

Key words Urea – nitrogen metabolism – plasma protein amino acids – intestinal microflora – fermented milk – stable isotope ^{15}N – gas chromatography-combustion-isotope-ratio-mass spectrometry (GC-C-IRMS)

Schlüsselwörter Harnstoff – Stickstoff-Stoffwechsel – Aminosäuren – Darmflora – fermentierte Milch – stabiles Isotop ^{15}N – GC-C-IRMS ("gas chromatography-combustion-isotope-ratio-mass spectrometry")

Introduction

There is increasing evidence of the importance of the physiological microbial population in the gastrointestinal tract for the host. Beneficial effects on the health status of human subjects have been shown after ingestion of fermented dairy products containing live bacterial organisms. These health effects have been associated with the metabolic activity of lactic bacteria passing through the gastrointestinal tract and adhering to the mucosal surface in order to colonize and to exert interference with other microorganisms, as well as with the host (2, 7, 25, 34). Improved lactose digestion in lactase-deficient individuals and the alleviation of certain types of diarrhea following ingestion of lactic acid bacteria was reported. Other studies suggested that diet-induced changes in the intestinal microflora are involved in hypocholesterolemic effects, the prevention of human colon cancer, and effects on the human immune system (2, 7, 25, 34).

Therefore, several attempts have been made to enhance the selective proliferation and colonization of specific bacteria in the gut. The supplementation of diets with fiber and fermentable carbohydrates stimulated the growth of intestinal bacterial mass and the metabolic activity of the intestinal microflora (10, 18, 20, 33). Furthermore, fermented milk containing live bacterial cultures or capsules with pure cultures have been used (3, 11–12, 15, 29, 34). The nutritional advantages of fermented milk (e.g. source of calcium and vitamins, better toleration than milk, growth restriction of potential pathogenic and putrefactive bacteria) make them a valuable nutritional supplement particularly for the elderly population with its specific age related complex of health problems (19–20, 29, 31).

A consequence of stimulated growth of the intestinal microflora should be an increased utilization of urea and ammonia nitrogen, given that there is sufficient ATP to drive microbial amino acid and protein synthesis. Ureolytic anaerobes provide urea nitrogen which is used for bacterial protein synthesis. As an overall result an increased nitrogen excretion in the feces may occur while the urinary nitrogen excretion remains the same or is slightly decreased. This mechanism has been proposed to relieve both the liver and the kidneys from their excretion functions, and therefore could be useful as part of the treatment of uremic and cirrhotic patients (1, 26–27, 32, 41). Furthermore, it could be beneficial for the health status of elderly persons since anatomical change and progressive detoriation of renal function is inevitable with aging (26, 28).

Ammonia from urea-N degradation in the gastrointestinal tract can also be channeled away from the luminal ammonia pool and its nitrogen can enter the amino acid and urea pool of the body (21, 32). This process is well known and urea hydrolysis was estimated to be about 20 % of its production in man (5) where 70 % of the urea nitrogen is returned to the urea pool (21). Following administration of ^{15}N-labeled urea ^{15}N was incorporated into dispensable and indispensable amino acids in plasma or tissue proteins of rats, minipigs or man (1, 22, 24, 39). This suggests that either amino acids synthesized by the intestinal microflora from non-specific nitrogen sources may be absorbed by the gastrointestinal tract of the host or ammonia from breakdown of ^{15}N substrates in the gut is absorbed and used for transamination of dispensable amino acids (16, 24, 42). Using ^{15}N-labeled bifidobacteria as a tracer instilled in the large intestine it was observed that 70 % of ^{15}N was estimated to be retained in the infant's protein nitrogen pool. However, this does not necessarily mean that nitrogen is absorbed in form of intact amino acids from the colon (14). We have shown a low but significant ^{15}N-enrichment in lysine of serum albumin in minipigs and plasma free lysine of human subjects after ingestion of non-specific nitrogen which is indicative for an intestinal microbial synthesis and consecutive absorption of this indispensable amino acid (22, 24).

The objective of the present study was to examine whether the ingestion of a lactic acid bacteria preparation alone may affect the fecal and urinary excretion of nitrogen from urea in elderly human subjects. Although it has been shown that ingested lactic acid bacteria can colonize the intestinal tract (11, 15), a potential modification of microbial nitrogen and amino acid metabolism was not yet investigated. Furthermore, it was shown by means of a new, highly sensitive gas chromatograph-combustion-isotope-ratio-mass spectrometry method (GC-C-IRMS) that urea-nitrogen is incorporated into plasma protein amino acids.

Materials and methods

Subjects

13 healthy elderly subjects (Table 1) had been recruited as inpatients at the Ukrainian Institute of Gerontology (UIG), Clinical Department, Kiev, and were in generally good health as determined by medical history, physical examination, routine blood biochemistry profile and urinalysis. Informed consent was obtained and the protocol for the study was approved by the local ethics committee. The subjects received a controlled mixed diet based on local products, as usually provided to the clinic patients. This diet contained per kg body weight and per d: energy 32 kcal, protein of vegetable origin 0.78 g, protein of animal origin 0.24 g, vegetable fat 0.32 g, animal fat 0.44 g and carbohydrates 6 g.

Administration of bacteria and ^{15}N-tracer

The cultures of the preparation Gerosan belong to several genera of optional anaerobes of homofermentative lactic acid bacteria (Lactobacilli, Streptococci). Gerosan was kindly provided by the Institute of Technology and Food Industry, Kiev, and is usually used as a starter for the production of fermented milk. The freeze-dried bacterial preparation contained cultures of a strain of *Lactobacillus plantarum* (50–70 %) and in addition cultures of a strain of *Lactobacillus bulgaricus* and two strains of *Streptococcus thermophilus* (37–38). *Lactobacillus plantarum* was originally isolated from stool samples of long living people in the Caucasus region and was found also in traditionally home-made fermented milk of this region (19). The subjects ingested 1.5 g Gerosan per day, resuspended in drinking water, over a total period of 10 d under the supervision of the dietary staff.

Single doses of [$^{15}N_2$]urea (25 mg x kg body wt^{-1}, 95 AP ^{15}N, Berlin Chemie, Berlin, Germany) were administered orally on 3 occasions: 1) prior to Gerosan supplementation (A-period), 2) immediately after the Gerosan supplementation period (10 d, G-period), and 3) 10 d after completing the Gerosan supplementation period (B-period).

Sample preparation, nitrogen analysis, derivatization of amino acids

24 h collections of urine and feces were made after consumption of each [$^{15}N_2$]urea bolus. Fecal samples were pooled, weighed and mixed to obtain a representative aliquot of the 24 h collection. Fecal aliquots were dried at 110 °C until weight constancy and stored at -24 °C. The dried samples were homogenized and the proteins were precipitated by trichloracetic acid (0.6 mol/L, final concentration) and centrifuged (3 000 g for 10 min). The precipitate was washed with trichloracetic acid (0.6 mol/L), ethanol and ether, and was dissolved in 2 mol/L sodium hydroxide and agitatated at 50 °C for 2 h. Nitrogen was determined by a routine micro-Kjeldal method using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany) and a titration system (T110-TR160-TA10-TM120, Schott-Geräte GmbH, Hofheim, Germany).

Fasted blood samples were obtained 1 wk prior to the administration of [$^{15}N_2$]urea (baseline) and 7 h after each [$^{15}N_2$urea ingestion in EDTA containing tubes (Monovette, Sarstedt, Nürnbrecht, Germany) by venipuncture from an arm vein. Plasma was separated by centrifugation at 1 000 x g for 10 min and stored frozen (-24 °C) until analysis. Proteins of 50 µL plasma were precipitated by trichloracetic acid (0.6 mol/L, final concentration) and centrifuged (3 000 x g for 10 min). The

Table 1 Characteristics of subjects

Subject	Weight (kg)	Height (cm)	BMI (kg/m²)	Age (y)	Sex	glucose (mmol/L)	total cholesterol (mmol/L)
1	73.0	174	24.1	66	male	3.65	–
2	62.0	162	23.6	65	female	3.53	–
3	87.0	172	29.4	71	male	3.86	–
4	75.5	170	26.1	65	male	3.56	3.9
5	62.5	152	27.1	70	female	3.41	5.5
6	62.3	164	23.2	77	female	3.36	5.5
7	57.7	157	23.4	71	female	3.82	4.7
8	76.5	174	25.3	69	male	3.59	4.3
9	71.5	174	23.6	72	male	3.26	3.8
10	80.0	180	24.7	74	male	4.09	4.3
11	72.0	–	–	63	male	4.01	4.9
12	56.5	150	25.1	82	female	3.42	5.0
13	52.0	162	19.8	68	female	3.34	4.0
Mean ± SD	68.3±8.9	166±9	24.6±2.0	70±4		3.61±0.27	4.6±0.7

precipitate was washed with trichloracetic acid (0.6 mol/L), ethanol and ether. The dried protein pellet underwent a 24 h acid hydrolysis in 2 mL of distilled 6 mol/L hydrochloric acid at 110 °C in capped Pyrex vials. α-Amino adipic acid (100 µL, 1 µmol/mL in 0.1 mol/L hydrochloric acid) was added prior hydrolysis as internal standard.

Amino acid derivatization was performed as recently described (23–24). In brief, amino acids were treated with thionylchloride solution in i-propanol and heated for 60 min at 100 °C. The product was dried and dissolved in pyridine. After adding pivaloylchloride the solution was acylated 30 min at 60 °C and dichloromethane was added after cooling. The mixture was then passed over a silica gel column and the eluate was dryed in a nitrogen stream and the N-pivaloyl-i-propyl esters were redisolved in ethylacetate for injection.

Chemicals and amino acid standards were purchased from several suppliers (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany; Fluka Chemie AG, Buchs, Switzerland; Merck, Darmstadt, Germany) and were all of analytical grade.

Isotopic analysis

The measurement of the ^{15}N-abundance in fecal samples and urinary nitrogen was performed by emission-spectrometry using an automatic analyzer (NOI-6-PC, Fischer Analysen Instrumente GmbH, Leipzig, Germany) (6). An integrated system for sample preparation transforms the ammonium salts obtained by the Kjeldal digestion method, by means of hypobromite reduction into molecular N_2. ^{15}N-abundances in urinary ammonia and urea were obtained by emission-spectrometry after a prior separation of ammonia and urea using a microdiffusion method (6, 17).

The measurement of ^{15}N abundances in plasma protein amino acids was carried out on a Finnigan delta S isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) coupled on-line with a gas chromatograph (GC, HP 5890, Hewlett Packard, Waldbronn, Germany) via a combustion interface, generating and purifying N_2 gas from GC separated compounds to be introduced into the isotope ratio mass spectrometer (23–24). The introduction of a standard N_2 gas with known isotopic composition at particular timepoints during the gas chromatographic run is used for calibration of the sample amino acid nitrogen. An Ultra 2 capillary column (50 m; Hewlett Packard, Waldbronn, Germany) with He as carrier gas (1 mL He min^{-1}) was used for separation of the amino acids. A volume of 0.5 µL was injected splitless by an autosampler (CTC A200S; CTC Analytics, Zwingen, Switzerland). The injector temperature was 280 °C and the following oven temperature gradient program was used: 70 °C, held 1 min; 70–220 °C, ramp 3 °C min^{-1}; 220–300 °C, ramp 10 °C min^{-1}, held 8 min.

Nitrogen isotopic composition is given as $\delta^{15}N$ in ‰ or as atom-% ^{15}N (AP ^{15}N) or as atom-%-excess ^{15}N (APE ^{15}N). In the range of natural abundance (0.3626 to 0.3736 AP ^{15}N) the $[^{15}N]/[^{14}N]$ ratio is usually expressed as $\delta^{15}N$ as follows: $\delta^{15}N$ (‰) = $\{R_{sample}/R_{standard} - 1\} \times 10^3$, where R is the $[^{15}N]/[^{14}N]$ ratio. $[^{15}N]/[^{14}N]$ ratios are derived from respective ratios of m/z 29 to m/z 28 ion current signals of the mass spectrometer. The international standard for nitrogen is AIR (0.3663 AP ^{15}N) which has been assigned a $\delta^{15}N$ value of 0.0 ‰. Data processing was performed by the vendor-provided software "ISODAT" (Finnigan MAT, Bremen, Germany). Slope sensitivity for peak start and stop definition was set to be 0.2 and 0.4 mV/s, respectively. Integration time was 0.25 s (23–24).

Statistical analysis

Values are given as means ± SD. Means were compared using the unpaired or paired t-test. $P < 0.05$ was considered as significance limit (36). Each sample was prepared and analyzed in duplicate.

Results and discussion

The analysis of fecal samples revealed a distinct ^{15}N-enrichment in the fecal protein fraction following the administration of a single oral $[^{15}N_2]$urea bolus (Table 2). However, a higher excretion of proteins and a stimulation of the incorporation of ^{15}N from $[^{15}N_2]$urea into fecal proteins was not observed after a 10 d administration of the starter culture (G-period). Surprisingly, the total fecal nitrogen and protein-nitrogen-excretion was significantly lower after the G-period than prior to the administration (A-period) which can not be explained satisfactorily. Also, the values for urinary nitrogen excretion and urinary ^{15}N abundances were not significantly different for the A- and G-period, respectively, while the ^{15}N abundances were found to be lower in the ammonia-nitrogen fraction of urine in the G-period. These results indicate that the administration of the starter culture did not affect the microbial urea degradation in those elderly subjects.

Thus, under our experimental conditions the administration of the starter culture containing lactic acid bacteria only, did not affect the nitrogen excretion pattern in man. Although microbial assays were not performed, it is possible that the lactic acid bacteria did not survive the gastrointestinal passage and that the residential microflora was rather stable. Data on the survival of bacterial cultures during their passage through the gastrointestinal tract are controversial. On the one hand, survival over at least a few hours or days has been shown (11, 29). On the other hand, great stability of the human colonic flora to dietary interventions was reported when yogurt enriched with bifidobacteria was consumed (3). Another study

shows that lactic acid bacteria ingested in capsules are found in the colon of healthy subjects and in the ileal effluent of ileostomy patients. However, there was neither a change in the fermentation pattern or in the main fermentation products in ileostomy homogenates or in fecal homogenates, nor relieve of symptoms of lactose malabsorption (15). Even the survival of ingested bacterial cultures does not necessarily modify intestinal fermentation because of their inability to grow and to colonize the gastro-intestinal tract. How effectively a bacterial culture colonizes the gastro-intestinal tract may be determined by its ability to adhere to the mucosal surface (2, 25, 29). Although *L. bulgaricus* does not adhere to the human gut wall, the main components of the bacterial culture used are strains of *L. plantarum*, which are known to have strong adhesive characteristics (2). Whether there is adhesion of strains of *L. plantarum* to the mucosal surface remains to be examined in future studies.

It is well known from animal (1, 18, 40–41) and human (4, 29, 33) studies that the growth of the intestinal bacterial mass is dependent on the availability of energy sources and consequently on the consumption of fiber and fermentable carbohydrates with the diet. It was shown that pectin added to the diet decreased urea production and recycling in malnourished children (4) which was explained by an increased bacterial growth and ammonia consumption. The addition of fermentable carbohydrates to a diet provides energy fuels for increased bacterial growth and fermentation processes when entering the colon, increasing the fecal nitrogen excretion. Phillips et al. (33) reported an increased total fecal output and a higher excretion of fecal butyrate and acetate after two weeks of consumption of about 40 g of resistant starch compared to a control diet. Hence, in the present study the lack of an effect on fecal nitrogen excretion by administration of the starter culture only, may also indicate that energy sources for microbial protein synthesis were not sufficient to convert urea nitrogen into additional microbial protein.

A significant incorporation of ^{15}N in fecal proteins after [$^{15}N_2$]urea administrations occured in all experimental periods (Table 2) whether or not the bacterial culture was administered. This suggests, that urea nitrogen was liberated by microbial hydrolysis and incorporated into microbial protein amino acids in the gut either directly or indirectly via a recycling of ^{15}N labeled urea or amino acids (8). The latter is supported also by our finding that plasma protein amino acids became ^{15}N enriched (Table 3), which requires that the ^{15}N label is present in hepatic and extra-hepatic precursor pools for α-ketoglutarate amination.

Table 3 compares the $\delta^{15}N$ patterns of plasma protein amino acids at natural abundance level to their respective values obtained after three single doses of [$^{15}N_2$]urea administered prior (A-period) and after the 10 d Gerosan supplementation (G-, B-period). We have reported recently data on natural ^{15}N abundances in plasma free and protein bound amino acids (23, 30), which compares favorably with the $\delta^{15}N$ values at natural abundance in this study. There is a relatively low natural abundance in threonine, phenylalanine, lysine and histidine (Table 3). In contrast, proline and glutamic acid show the highest ^{15}N abundance. There is a relatively good agreement between the $\delta^{15}N$ pattern in plasma protein amino acids and collagen amino acids in pigs reared either on a corn based or grain-soybean diet (13). The comparison with amino acids of rat liver after 50 weeks on a soybean diet shows

Table 2 Nitrogen- and ^{15}N-excretion in stools and urine of elderly human volunteers prior to Gerosan supplementation (A-period), immediately after a 10 d Gerosan supplementation (1.5 g d^{-1}, G-period), and 10 d after completing the Gerosan supplementation period (B-period) during 24 h after single oral boluses of [$^{15}N_2$]urea (25 mg x kg body wt^{-1}, 95 AP ^{15}N)[1]

Parameter	A-period (prior to Gerosan supplementation)	G-period (immediately after the 10 d Gerosan supplementation)	B-period (10 d after completing the Gerosan supplementation)
Total nitrogen-excretion and ^{15}N-abundance in stool samples			
g/d	2.48 ± 1.38^{B}	1.63 ± 0.69^{A}	2.28 ± 1.39^{AB}
AP ^{15}N	0.442 ± 0.084	0.441 ± 0.076	0.429 ± 0.067
mg/d ^{15}N	10.6 ± 5.1^{B}	7.4 ± 3.7^{A}	11.3 ± 5.8^{AB}
Protein-nitrogen-excretion and ^{15}N-abundance in total proteins of stool samples			
g nitrogen/d	1.44 ± 0.86^{B}	0.97 ± 0.40^{A}	1.40 ± 0.87^{AB}
AP ^{15}N	0.450 ± 0.082	0.447 ± 0.072	0.430 ± 0.064
mg/d ^{15}N	6.29 ± 3.41	4.45 ± 2.27	6.16 ± 3.88
Total nitrogen-excretion and ^{15}N-abundance in urine			
g/d	8.55 ± 4.28^{B}	8.31 ± 4.22^{B}	5.89 ± 1.82^{A}
AP ^{15}N	5.140 ± 1.334	5.069 ± 1.479	4.888 ± 1.172
mg/d ^{15}N	414 ± 164^{B}	426 ± 150^{B}	292 ± 116^{A}
APE $^{15}NH_3$	0.255 ± 0.102^{AB}	0.191 ± 0.096^{A}	0.265 ± 0.101^{B}
APE [$^{15}N_2$]urea	5.74 ± 1.77	5.14 ± 1.78	5.30 ± 1.41

[1] Each value is the mean± SD, n = 13. Means within a row not sharing a superscript letter are significantly different, $P < 0.05$

Table 3 ^{15}N enrichments of amino acids in plasma proteins of elderly human volunteers prior to (baseline values) and 7 h after a single oral [$^{15}N_2$]urea administration (95 AP ^{15}N; 25 mg kg^{-1} body weight) prior to Gerosan supplementation (A-period), immediately after the 10 d Gerosan-supplementation (G-period), and 10 d after completing the Gerosan-supplementation (B-period) respectively measured by GC-C-IRMS[11)]

Amino acid	Baseline n = 13	A-period (prior to Gerosan supplementation) n = 6	G-period (immediately after the 10 d Gerosan supplementation) n = 6	B-period (10 d after completing the Gerosan supplementation) n = 6
	(δ ^{15}N [‰ vs. AIR])			
Alanine	11.81 ± 1.74^{A}	86.93 ± 32.08^{B}	125.05 ± 31.85^{C}	163.01 ± 40.26^{D}
Glycine	11.24 ± 1.30^{A}	50.48 ± 19.59^{B}	73.31 ± 21.50^{C}	97.36 ± 29.38^{D}
Valine	7.35 ± 3.38^{A}	18.76 ± 2.21^{B}	33.58 ± 8.00^{C}	49.24 ± 12.83^{D}
Leucine	10.14 ± 1.22^{A}	17.92 ± 4.86^{B}	36.47 ± 7.99^{C}	53.50 ± 12.96^{D}
Proline	16.88 ± 0.94^{A}	20.98 ± 1.44^{B}	28.61 ± 2.96^{C}	35.81 ± 5.18^{D}
Aspartic acid	11.41 ± 1.12^{A}	62.93 ± 22.53^{B}	87.63 ± 21.65^{C}	110.11 ± 27.01^{D}
Threonine	-3.75 ± 2.04	nd[2)]	nd	nd
Serine	8.94 ± 1.20^{A}	37.36 ± 14.06^{B}	57.79 ± 15.52^{C}	77.14 ± 19.35^{D}
Methionine	9.34 ± 2.24^{A}	22.06 ± 8.57^{B}	32.64 ± 8.47^{C}	43.65 ± 16.94^{D}
Glutamic acid	15.04 ± 0.79^{A}	78.93 ± 26.95^{B}	115.75 ± 29.61^{C}	149.32 ± 37.57^{D}
Phenylalanine	-0.01 ± 4.06^{A}	8.67 ± 3.53^{B}	13.84 ± 3.27^{BC}	18.06 ± 9.28^{C}
Lysine	4.57 ± 1.32^{A}	5.29 ± 1.84^{B}	5.46 ± 2.03^{ABC}	7.91 ± 2.04^{C}
Histidine	4.22 ± 0.85^{A}	6.82 ± 1.84^{B}	10.24 ± 3.03^{B}	12.96 ± 3.51^{C}
Tyrosine	6.26 ± 1.19^{A}	16.64 ± 4.64^{B}	26.40 ± 6.01^{C}	33.84 ± 4.74^{D}

1) Each value is the mean± SD. Means within a row not sharing a superscript letter are significantly different, $P < 0.05$
2) nd: not determined

a similar pattern (9). The same is true for data in pig's serum albumin fed with a casein and corn starch diet (24, unpublished data by Metges et al.), and for $\delta^{15}N$ values in plasma free amino acids of subjects on a crystalline L-amino acid-based diet (23).

The plasma protein amino acid ^{15}N enrichment patterns above natural abundance in the present study were similar to those obtained in serum albumin of a minipig following daily oral [$^{15}N_2$]urea doses over a period of 10 days (24). Transamination accounts for the labeling of most amino acids, but the most highly labeled are those amino acids that are transamination products of ketoacids. Thus the highest enrichments were found in glutamic and aspartic acids (peaks containing also about 30 % glutamine and asparagine, respectively, due to the acid hydrolysis performed). The branched-chain amino acids, phenylalanine and tyrosine represent a second group of amino acids which show only 25–50 % of the ^{15}N enrichment indicating that a significant fraction of these amino acids is derived from unlabeled leucine entering via the diet and from tissue protein turnover (Table 3). Lowest enrichments in proline, histidine, phenylalanine and lysine suggest a limited or no participation in transamination reactions. Lysine does not undergo transamination in mammalian tissues. Hence, its slight enrichment may indicate a microbial synthesis and absorption of microbial lysine.

It is noteworthy that the ^{15}N abundances in plasma protein amino acids are increasing during the experimental period. Due to tracer recycling within the nitrogen pools of the body, the ^{15}N abundances in protein amino acids do not return to their baseline values within 10 d, even after these comparably small single doses of [$^{15}N_2$]urea. In an earlier study we observed that even 42 d after the ingestion of a single dose of [$^{15}N_2$]urea some amino acids were still significantly enriched compared to baseline values (30). Hence, immediately before the subjects ingested the second [$^{15}N_2$]urea dose the level of ^{15}N enrichment in plasma protein amino acids may still have been 50 % of what was observed in the A-period (compare Table 1 in Petzke et al. (30)). Therefore, the ^{15}N enrichments of plasma protein amino acids in the G- and B-period reflect an accumulation of ^{15}N labeled nitrogen in the body presumably due to nitrogen exchange or reversible transamination.

In summary, the ingestion of a freeze-dried preparation only containing different strains of lactic acid bacteria does not stimulate fecal protein-nitrogen excretion and the incorporation of urea nitrogen into fecal protein in elderly subjects. Two reasons for this observation are envisaged which are (i) that the culture used here did not survive the gastro-intestinal passage and (ii) that the energy supply, presumably in form of additional fermentable carbohydrates, was not sufficient to provide for an increased microbial protein synthesis and growth in the large intestine.

Acknowledgments The excellent technical assistance of P. Albrecht, A. Macher, and D. Ulbricht is highly appreciated.

References

1. Assimon SA, Stein TP (1994) Digestible fiber (Gum Arabic), nitrogen excretion and urea recycling in rats. Nutrition 10:544–550
2. Bengmark S (1998) Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 42:2–7
3. Bertram H-P, Scheppach W, Gerlach S, Ruckdeschel G, Kelber E, Kasper H (1994) Does yoghurt enriched with Bifidobacterium longum affect colonic microbiology and fecal metabolites in healthy subjects? Am J Clin Nutr 59:428–432
4. Doherty J, Jackson AA (1992) The effect of dietary pectin on rapid catchup weight gain and urea kinetics in children recovering from severe undernutrition. Acta Paediatr 81:514–517
5. El-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, Gleason RE, Chapman TE, Young VR (1994) Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1–13C]leucine and [^{15}N-^{15}N]urea. Am J Clin Nutr 59: 1000–1011
6. Faust H, Bornhak H, Hirschberg K, Jung K, Junghans P, Krumbiegel P, Reinhardt R (1981) Klinisch-chemische und isotopenanalytische Methoden zur Untersuchung des Stickstoff-Stoffwechsels mit N-15 beim Menschen. Methodenkatalog Zfl-Mitt, Leipzig, Nr. 36
7. Fuller R, Gibson GR (1997) Modification of the intestinal microflora using probiotics and prebiotics. Scand J Gastroenterol 32 (Suppl 222):28–31
8. Fuller MF, Reeds PJ (1998) Nitrogen cycling in the gut. Annu Rev Nutr 18:385–411
9. Gaebler OH, Vitti TG, Vukmirovich R (1966) Isotope effects in metabolism of ^{14}N and ^{15}N from unlabeled dietary proteins. Can J Biochem 44:1249–1257
10. Gibson GR, Beatty ER, Wang X, Cummings JH (1995) Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin. Gastroenterol 108:975–982
11. Goldin BR, Gorbach SL, Saretin M, Barakat S, Gualteri L, Salminen S (1992) Survival of lactobacillus species (strain gg) in human gastrointestinal tract. Dig Dis Sci 37:121–128
12. Guerin-Danan C, Chabanet C, Pedone C, Popot F, Vaissade P, Bouley C, Szylit O, Andrieux C (1998) Milk fermented with yogurt cultures and Lactobacillus casei compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants. Am J Clin Nutr 67:111–117
13. Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC (1991) The Isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Arch Sci 18:277–292
14. Heine WE, Mohr C, Wutzke KD (1992) Host-microflora correlations in infant nutrition. Progr Food and Nutr Sci 16:181–197
15. Hove H, Nordgaard-Andersen I, Mortensen PB (1994) Effect of lactic acid bacteria on the intestinal production of lactate and short-chain fatty acids, and the absorption of lactose. Am J Clin Nutr 59:74–79
16. Jackson AA (1993) Chronic malnutrition: protein metabolism. Proc Nutr Soc 52:1–10
17. Jung K, Faust H, Matkowitz R (1989) [^{15}N]Ammonium test for liver function diagnosis. Z Med Lab-diagn 30: 169–174
18. Kleesen B, Stoof G, Proll J, Schmiedl D, Noack J, Blaut M (1997) Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. J Anim Sci 75:2453–2462
19. Kvasnikov EI, Grigorov JG, Kovalenko NK, Medovar BJ, Shishlevskaja TN, Palecha SI, Kozlovskaja SM (1984) Lactic acid bacteria in the digestive tract and food of long-living people in Abkhazia. Mikrobiol Zhurnal (Moskau) 46:11–17
20. Kumemura M, Hashimoto F, Fujii FC, Matsuo K, Kimura H, Miyazoe R, Okamatsu H, Inokuchi T, Ito H, Oizumi K, Oku T (1992) Effects of administration of 4-β-D-galactosylsucrose on fecal microflora, putrefective products, short-chain fatty acids, weight, moisture and pH, and subjective sensation of defecation in the elderly with constipation. J Clin Biochem Nutr 13: 199–210
21. Long CL, Jeevanandam M, Kinney JM (1978) Metabolism and recycling of urea in man. Am J Clin Nutr 31: 1367–1382
22. Metges CC, El-Khoury AE, Petzke KJ, Bedri S, Fuller MF, Young VR (1997) The quantitative contribution of microbial lysine to lysine flux in healthy male subjects. FASEB J 11:A149
23. Metges CC, Petzke KJ (1997) Measurement of ^{15}N/^{14}N isotopic composition in individual plasma free amino acids of human adults at natural abundance by gas chromatography-combustion isotope ratio mass spectrometry. Anal Biochem 247:158–164
24. Metges CC, Petzke KJ, Hennig U (1996) Gas chromatography/combustion/isotope ratio mass spectrometric comparison of N-acetyl- and N-pivaloyl amino acid esters to measure ^{15}N isotopic abundances in physiological samples: A pilot study on amino acid synthesis in the upper gastrointestinal tract of minipigs. J Mass Spectrom 31:367–376
25. Mital BK, Garg SK (1995) Anticarcinogenic, hypocholesterolemic, and antagonistic activities of Lactobacillus acidophilus. Crit Rev Microbiol 21: 175–214
26. Mitch WE (1978) Effects of intestinal flora on nitrogen metabolism in patients with chronic renal failure. Am J Clin Nutr 31:1594–1600
27. Mortensen PB (1992) The effect of oral-administered lactulose on colonic nitrogen metabolism and excretion. Hepatol 16:1350–1356
28. Niederstadt C, Steinhoff J (1997) The kidneys in aging. Z Gerontol Geriatr 30:200–207
29. Pedrosa MC, Golner BB, Goldin BR, Brakat S, Dallal GE, Russel RM (1995) Survival of yoghurt-containing organisms and Lactobacillus gasseri (ADH) and their effect on bacterial enzyme activity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. Am J Clin Nutr 61:353–359
30. Petzke KJ, Korkushko OV, Semesko TG, Metges CC (1997) N-isotopic composition in human plasma protein amino acids at natural abundance level and after a single [$^{15}N_2$]urea administration measured by GC-C-IRMS. Isotopes Environ Health Stud 33:267–275
31. Petzke KJ, Mooz R, Grigorov JG, Albrecht V (1991) Zum Problem der Bedeutung der Darmflora für die Lebenserwartung. Ernährungsforschung 36:78–80
32. Petzke KJ, Semesko TG, Proll J, Grigorov JG (1995) Changes in nitrogen excretion of uraemic rats fed with a fermented milk product. Ernährungsforschung 40:137–143
33. Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'dea K, Young GP (1995) Effects of resistant starch on fecal bulk and fermentation-dependent events in humans. Am J Clin Nutr 62:121–130
34. Saloff-Coste CJ (1994) Health benefits of lactic acid bacteria. DANONE World Newsletter 6:2–6
35. Saxelin M, Pessi T, Salminen S (1995) Fecal recovery following oral administration of Lactobacillus strain GG (ATCC 53103) in gelatine capsules to healthy volunteers. Int J Food Microbiol 25:199–203
36. Snedecor GW, Cochran WG (1989) Statistical methods, 8th edition. Iowa State University Press, Ames, Iowa, USA
37. SU-Patent No 1795571 (1990) Verfahren zur Herstellung des Sauermilchproduktes „Laktogerovit"
38. SU-Patent No 1795570 (1990) Verfahren zur Herstellung der Starterkultur „Gerosan" für die Herstellung von fermentierten Milchprodukten
39. Tanaka N, Kubo K, Shiraki K, Koishi H, Yoshimura H (1980) A pilot study

on protein metabolism in the Papua New Guinea highlanders. J Nutr Sci Vitaminol 26:247–259

40. Younes H, Demigné C, Behr SR, Garleb KA, Rémésy C (1996) A blend of dietary fibers increases urea disposal in the large intestine and lowers urinary nitrogen excretion in rats fed a low protein diet. J Nutr Biochem 7:474–480
41. Younes H, Rémésy C, Behr S, Demigné C (1997) Fermentable carbohydrate exerts a urea-lowering effect in normal and nephrectomized rats. Am J Physiol 272:G515–G521
42. Young VR, El-Khoury AE (1995) The notion of the nutritional essentiality of amino acids, revisited, with a note on the indispensable amino acid requirements in adults. In: Cynober LA (ed) Amino acid metabolism and therapy in health and nutritional disease. CRC Press Inc, Boca Raton, pp 191–232