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Whole-Body Protein Parameters in Premature Infants: A Comparison of Different ^{15}N Tracer Substances and Different Methods

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ABSTRACT. [^{15}N]glycine, [^{15}N]leucine, and [^{15}N]yeast protein thermitase hydrolysate (YPTH) as tracers for investigating the protein turnover rates in premature infants were studied in nine human milk-fed neonates (born after 32 to 34 wk of gestation) by paired comparison of the tracers. The ^{15}N enrichment of total urinary nitrogen and ammonia after administration of a single oral dose of ^{15}N was measured by emission spectrometry. Flux rates were calculated using a three-compartment model and the ammonia end product method. The mean whole-body protein synthesis rates, as determined by the three-compartment model derived from the three ^{15}N tracers, differed significantly ($p < 0.01$) among [^{15}N]glycine (15.9 g/kg/d), [^{15}N]leucine (9.1 g/kg/d), and ^{15}N-YPTH (5.9 g/kg/d). When the corresponding rates were determined from the excretion of label in ammonia, the results showed the opposite tendency; the lowest apparent synthesis rates were found after [^{15}N]glycine (7.5 g/kg/d), followed by [^{15}N]leucine (14.4 g/kg/d), and the highest figure resulted after [^{15}N]YPTH (16.7 g/kg/d). The results of this comparison substantiate the assumption that there are methodologic errors in connection with the use of different tracers and models for the calculation of whole-body protein parameters in preterm infants, with respect to the main requirement for tracer kinetic studies; the tracer nitrogen must be representative of total amino acid nitrogen. Seen in this light, mixtures of completely labeled amino acids such as YPTH may represent the most reliable tracer substance. On the basis of the data obtained with ^{15}N-labeled YPTH and with total ^{15}N as the end product, there is no major difference in the order of magnitude of the protein flux between preterm and full-term infants. (*Pediatr Res* 31: 95–101, 1992)

Abbreviations

HMF, human milk feeding
YPTH, ^{15}N-yeast protein thermitase hydrolysate
TCM, three-compartment model
AEPM, ammonia end product method

There is currently general confusion with regard to protein turnover analysis in preterm infants. This confusion may be attributed to the fact that there is considerable methodologic variation in the analytical methods used. Different tracers may be used, *e.g.* [^{15}N]H_4Cl (1), [^{15}N]glycine (2–7), [1-^{13}C]leucine (8, 9), ^{15}N-amino acid mixtures (10), ^{15}N-yeast protein pepsin-trypsin hydrolysate (11), and ^{15}N-yeast protein acid hydrolysate (12). There may also be different routes of administration, *e.g.* i.v. or intragastric infusion of L-[1-^{13}C]leucine with the tracer dilution measured and tracer lost quantified as urinary [^{13}C]leucine and expired $^{13}CO_2$, respectively (8, 13). Alternatively, a continuous intragastic infusion of [^{15}N]glycine to attain an enrichment plateau for the urinary [$^{15}N_2$]urea or [^{15}N]ammonia as end products of the protein metabolism (2–4, 6) may be used or labeling of urinary urea and ammonia may be measured after a single injection of a single oral dose of [^{15}N]glycine (5, 7, 11, 12). Finally, there are different ways of calculating the protein turnover rates in preterm infants, including the approach described by Picou and Taylor-Roberts (14) [modified by Steffee *et al.* (15)], the model of Waterlow *et al.* (16), the plasma method of Matthews *et al.* (17), and the compartment model developed by Winkler and Faust (18).

Most of the papers published do not take into consideration that the tracer must be representative of the protein metabolism (19); this is one of the basic requirements to be met by substances used as tracers in the investigation of parameters of the whole-body protein metabolism in preterm infants. Despite many theoretical and practical objections raised in relation to this requirement, [^{15}N]glycine probably still continues to be the most commonly used tracer (2, 4–6, 20). Although [^{15}N]glycine appears to satisfy the requirement when used in adults, it manifestly fails to do so in the case of those infants born prematurely; reported synthesis rates range between 6.1 and 23.3 g/kg/d (1–4).

Extremely high protein turnover rates found using [^{15}N]glycine in preterm infants led Jackson *et al.* (4) to the conclusion that glycine is semiessential in character in this age group. Mixtures of fully labeled amino acids (10) might be an alternative to [^{15}N]glycine, but the prohibitive cost precludes widespread use. ^{15}N-labeled yeast protein and its pepsin-trypsin hydrolysate, which might be an alternative to these expensive amino acid mixtures, are poorly absorbed (11). The almost complete enzymatic breakdown of ^{15}N-yeast protein all the way down to the tripeptide, dipeptide, and amino acid level with thermitase, as recommended by our group (11), seems to hold promise for further improvement.

Several authors (5, 12) claim that the end product method with [^{15}N]ammonia as the final metabolite is suitable for assessing the whole-body protein parameters in this age group; they emphasize the ensuing benefits, such as reproducibility, fast urine collection, rapid monitoring, and minimal invasion. The main drawback of this approach is that it has not yet been validated by comparing it to conventional compartment methods as applied to the same subject.

The objective of the present study is, therefore, to investigate the protein turnover rates by paired comparison between preterm neonates after labeling with three different ^{15}N-tracers (YPTH,

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leucine, and glycine) and two different methods, the TCM and the AEPM.

MATERIALS AND METHODS

Subjects. Nine healthy, male, appropriate-for-gestational-age preterm infants were studied. Their mean weight at birth was 2028 ± 158 g. Their post-conceptual and postnatal age was 33 ± 1 wk and 18 ± 3 d, respectively. Their body weight was 2146 ± 244 g (glycine study), 2137 ± 186 g (leucine study), and 2143 ± 203g (YPTH study) (Table 1). The infants were weighed daily. Four of the subjects had not yet fully regained their birth weight at the beginning of the study and during the initial tests (this was true of subject 1 during the glycine and leucine tests, of subject 2 during the glycine and YPTH tests, of subject 6 during the YPTH test, and of subject 8 during the glycine test; these were the respective initial tests performed according to the randomization principle). All infants were established for enteral HMF. With one exception (subject 2, glycine group), all infants were gaining weight during the study. The testing protocol was approved by the Committee on Ethics of the University of Rostock. All parents had given their consent for their children to be included in our study.

Feeding. All subjects were on HMF (pooled raw human milk collected from voluntary healthy donors in the 2nd to 12th wk of lactation). The mandatory quality tests were performed on every batch from each donor before the milk was added to the pool. After pooling, the milk was deep-frozen immediately. The food volume per subject and its nitrogen and energy content are shown in Table 2. The HMF infants received the pooled human milk either by bottle feeding or by nasogastric tube. At the time of the study, all infants were being nursed in an incubator and had been free of any major clinical problems.

Analysis showed that the pooled human milk had a mean nitrogen content of 149.6 mmol/L (2.096 g/L), corresponding to a protein equivalent of 13.1 g/L. The calorimetric energy content, measured by anisothermic bomb calorimetry, was 2920.0 kJ/L (698 kcal/L).

Tracer substances. The tracer substances used in the experiments were [^{15}N]glycine (95.0 atom %), [^{15}N]leucine (95.0 atom %), both manufactured by Berlin-Chemie, Germany, and ^{15}N-YPTH. The latter was produced by hydrolyzing ^{15}N-yeast protein with the protease thermitase for 4 h at pH 7.5 and 55°C. The proportion of substrate to enzyme was approximately 200 to 1. After inactivation by heating to 100°C, the insoluble sediment was removed by centrifugation and the supernatant was lyophilized. The degree of hydrolysis was 43%, the mean chain length being 2.44. The percentage of pure amino acids was 18%. ^{15}N abundance was 91.1 atom %, and the nitrogen content was 13.54 mg/100 mg (21). The amino acid composion of the YPTH has been published previously (11).

Study protocol. In all cases, the three different tracers were dissolved in 2 mL of human milk. Each tracer was given as a single dose, by nasogastric tube, at 1400 h, immediately preceding a feed. The three stages of testing began on the infants' 14th, 18th, and 22nd d of life, respectively. On each of these days, one of the tracers was given, but the order in which the nine subjects received the ^{15}N-tracer substances was determined by a permutation table (Table 1). The dosage of the tracer corresponded to a ^{15}N excess of 2 mg ^{15}N per kg body weight. None of the subjects regurgitated after receiving the tracer. Condom urinals were used to collect the urine 2, 4, 6, 8, 12, 16, 20, 24, 30, and 36 h after administration of the tracer. The urine was removed from the collection bag immediately, acidified with 6 N H_2SO_4 to attain a pH of <2, and stored at −20°C until analysis. Urine passed immediately before the start of the next tracer administration was analyzed to determine the background abundance of ^{15}N (baseline) remaining from the preceding test.

Feces were collected over 36 h, scraped from polyvinyl film, homogenized, and kept at −20°C for later analysis. Fecal ^{14}N and ^{15}N losses were subtracted from the total nitrogen intake and the ^{15}N excess dose, respectively.

Analytical techniques. The nitrogen content of the urine, feces, and milk was determined by the Kjeldahl digestion method. The protein content of the milk was calculated by multiplying the total nitrogen by a factor of 6.25.

Ammonia was separated in microdiffusion vessels through 12-h diffusion, following alkalization of the urine with 6 N NaOH, and trapped in 0.1 M boric acid. This was followed by titration with 0.1 N HCl in the presence of Tashiro indicator.

The ^{15}N enrichment of the total nitrogen in the urine and feces and the urinary ammonia was subsequently determined by emission spectrometry (NOI 6E; Central Institute of Isotope and Radiation Research, Leipzig, Germany). This procedure includes oxidation of the residual ammonium chloride from the Kjeldahl digestion to nitrogen gas using sodium hypobromide (22).

TCM. Winkler and Faust's (18) open three-pool model was used to calculate the parameters of the protein metabolism, *e.g.* protein synthesis, protein breakdown, and protein turnover, using a factor of 6.25 for conversion.

Our numerical treatment of the experimental data differed from that used by Sprinson and Rittenberg (23) in that the cumulative ^{15}N excess in the urine 48 h after administration was used as the plateau value for nonlinear regression, rather than the excess excreted by time infinity. Figure 1 is an example of the computer fitting of the excretion curve (q_3) and also shows

Table 1. *Clinical details of infants studied*

			Tracer group								
			[^{15}N]glycine			[^{15}N]leucine			^{15}N-YPTH		
Subject	Birth weight (g)	Gestational age (wk)	Weight at study (g)	Postnatal age at study (d)	Weight gain during study (g/kg/d)	Weight at study (g)	Postnatal age at study (d)	Weight gain during study (g/kg/d)	Weight at study (g)	Postnatal age at study (d)	Weight gain during study (g/kg/d)
1	2310	33	2220	14	8.8	2300	18	19.8	2490	22	9.8
2	1900	32	1870	14	−2.7	1980	22	6.2	1850	18	17.0
3	1950	32	2220	22	7.8	1970	14	8.7	2040	18	18.7
4	1910	33	2040	18	15.4	1950	14	11.3	2170	22	12.4
5	2260	34	2600	22	5.7	2510	18	8.8	2390	14	12.2
6	2030	34	2090	18	10.5	2180	22	6.8	1990	14	12.2
7	2070	33	2400	22	11.2	2220	18	19.5	2190	14	3.4
8	1920	31	1830	14	20.9	2100	22	10.5	1990	18	13.4
9	1900	34	2060	18	14.2	2020	14	4.9	2180	22	2.3
Mean	2028	33	2146	18	10.2	2137	18	10.7	2143	18	11.3
SD	158	1	244	3	6.6	186	3	5.4	203	3	5.5

Table 2. *Food volume, nitrogen supply, and energy supply during three studies*

	Volume (mg/kg/d)			Nitrogen supply (mg/kg/d)			Energy supply (kJ/kg/d)		
Subject	[^{15}N]glycine	[^{15}N]leucine	^{15}N-YPTH	[^{15}N]glycine	[^{15}N]leucine	^{15}N-YPTH	[^{15}N]glycine	[^{15}N]leucine	^{15}N-YPTH
1	180	191	177	377	400	371	526	558	517
2	193	182	195	405	381	409	564	531	569
3	182	183	196	381	384	411	531	534	572
4	216	185	221	453	388	463	631	540	645
5	173	179	184	363	375	386	505	523	537
6	191	183	201	400	384	421	558	534	587
7	175	192	183	367	402	384	511	561	534
8	197	190	180	413	398	377	575	555	526
9	175	178	183	367	373	384	511	520	534
Mean	187	185	191	392	387	401	546	540	558
SD	14	5	14	29	11	27	41	15	40

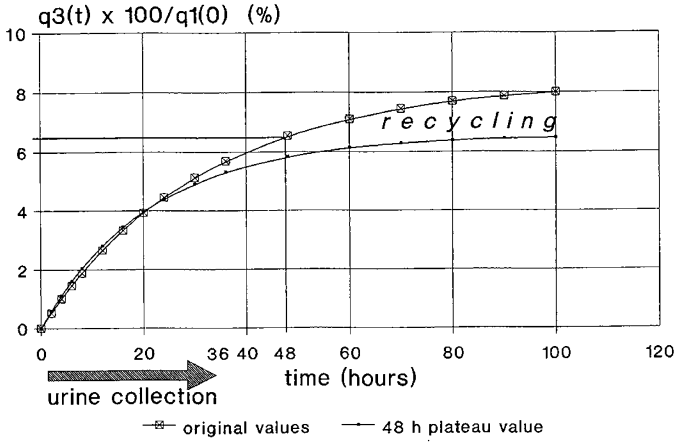

Fig. 1. Fitted cumulative urinary ^{15}N excretion curves.

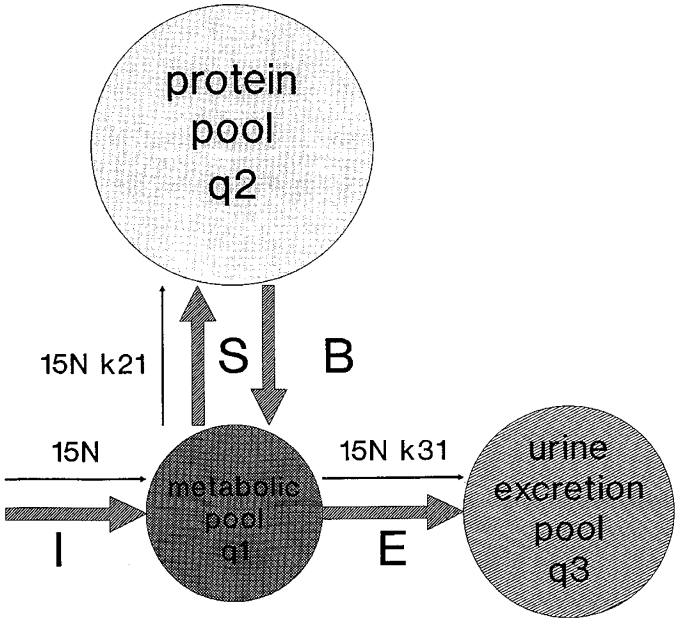

Fig. 2. TCM. See text for description.

the corrected curve obtained if q_3 (t = infinity) is equated to the observed value for q_3 at 48 h. The difference between the observed and corrected curves represents the recycling of tracer. This approach, which has been suggested by Waterlow *et al.* (16) is a reasonable compromise.

The three pool model is shown in Figure 2. The inflow of nutrient into the nonprotein nitrogen pool (metabolic pool) is denoted by *I*. Some of this is used for protein synthesis (*S*), and some is excreted (*E*). The flow of nitrogen out of the protein nitrogen pool consists of nitrogen resulting from protein breakdown (*B*). The quantity of tracer in one compartment is denoted by q. The rate constants k_{21} and k_{31} denote the proportion of compartment 1 transferred per unit time to compartments 2 and 3, respectively. Neglecting the return of the tracer from the protein pool to the metabolic pool, the following differential equations will result:

$$dq_1/dt = -k_{21}q_1 - k_{31}q_1$$

$$dq_2/dt = k_{21}q_1$$

$$dq_3/dt = k_{31}q_1$$

$$q_1(t = 0) > 0;\ q_2(t = 0) = q_3(t = 0) = 0$$

The kinetics of nitrogen after a single oral dose is therefore described by the following equations:

$$q_1 = q_1(0)e^{-(k_{21}+k_{31})t}$$

$$q_2 = q_1(0)\frac{k_{21}}{k_{21} + k_{31}}[1 - e^{-(k_{21}+k_{31})t}]$$

$$q_3 = q_1(0)\frac{k_{31}}{k_{21} + k_{31}}[1 - e^{-(k_{21}+k_{31})t}]$$

In these equations, $q_1(0)$ is the amount of tracer in the nonprotein nitrogen pool q_1 at t = 0. Therefore, $q_1(0)$ is the excess dose of ^{15}N and is 100% at t = 0. The quantities q_2 and q_3 are, respectively, the amount of tracer dose in the protein pool and the cumulative renal excretion of excess ^{15}N up to time t. The following parameters of protein turnover are, therefore:

$$\text{Nitrogen input (absorbed)} = I$$

$$\text{Urinary nitrogen excretion} = E$$

$$\text{Protein synthesis rate (S)} = E \cdot \frac{k_{21}}{k_{31}}$$

$$\text{Protein breakdown rate (B)} = S + E - I$$

$$\text{Protein nitrogen turnover rate (Q)} = S + E = B + I$$

$$\text{Net protein gain (N)} = S - B$$

$$\text{Half-life of } q_1 \ (t_{1/2}) = \frac{1n\ 2}{k_{21} + k31}$$

$$\text{Reutilization rate (R)} = 100\ \frac{(1 - E)}{Q}$$

AEPM. In addition to using the TCM, we measured protein turnover in preterm infants by examining the labeling of urinary ammonia after a single oral dose, as described by Waterlow *et al.* (16) and other authors (12, 24).

The nitrogen flux (Q) was calculated as

$$Q = \frac{d}{a \cdot t}$$

where d is the dose ^{15}N (mg)·24, a is the ^{15}N abundance in NH_3 (mg ^{15}N/mg total N), and t is the collection time (h).

Protein synthesis (S) and breakdown (B) were determined in the same way as in the TCM:

$$Q = S + E = B + I$$

RESULTS

Clinical details, nitrogen, and energy supply (Tables 1 and 2). The infants were closely matched for birth weight and gestational age. There were no statistical differences in weight at the time of testing, weight gain during testing, intake of human milk, or nitrogen and energy supply among all the tracer groups (27 separate test events).

Urinary and fecal excretion (Table 3). Given these practically identical conditions, the renal excretion percentages of the three different tracer substances were glycine, 2.8%; leucine, 3.4%; and YPTH, 5.4%. Renal ^{15}N excess excretion increased from glycine to leucine to YPTH, with statistically significant differences between glycine and YPTH ($p < 0.05$). Fecal excretion for the different ^{15}N tracers was glycine, 0.6%; leucine, 0.9%; and YPTH, 2.2%. We found significant differences between glycine and YPTH ($p < 0.001$) and between leucine and YPTH ($p < 0.01$). Figures 3 and 4 show the ^{15}N enrichment, measured in atom percent excess in ammonia nitrogen and total nitrogen.

Rate constants (Table 4). The rate constants for renal nitrogen excretion and protein synthesis calculated by the TCM also show differences among the tracer groups. The highest values for the protein synthesis rate constant were obtained after glycine (10.9 %/h) and the lowest, after administration of YPTH (5.1%/h) (glycine *vs* leucine and glycine *vs* YPTH, $p < 0.01$).

Reutilization rates and half-life periods (Table 5). We observed the highest reutilization rates after administration of glycine (97.3%), and the lowest were seen after YPTH (93.5%) (glycine *vs* leucine, $p < 0.05$; glycine *vs* YPTH, $p < 0.01$).

The half-life periods of ^{15}N in the nonprotein nitrogen pool were significantly ($p < 0.01$) shorter after administration of glycine (7.2 h) than after leucine (12.5 h) or YPTH (13.6 h).

Protein synthesis and breakdown rates (Table 6). The calcu-

Table 3. *^{15}N excretion after administration of three different ^{15}N-tracer substances in % of dose**

	[^{15}N]glycine		[^{15}N]leucine		^{15}N-YPTH	
Subject	Renal	Fecal	Renal	Fecal	Renal	Fecal
1	2.76	0.31			4.14	
2	1.70	0.19	2.80	0.95	5.53	1.80
3	1.75	0.39	3.18	1.28	2.74	1.86
4	3.84	0.99			4.67	1.19
5	2.96	0.74	4.65	1.25	13.58	3.45
6	3.66	0.74	4.00	0.73	3.30	3.13
7	1.90	0.50	2.72	1.18	4.67	1.98
8	3.78	0.67	3.67	0.20	4.67	1.68
9	2.21	0.84	2.77	0.56	5.38	2.70
Mean	2.79†	0.60‡	3.40§	0.88‖	5.41¶	2.22**
SD	0.92	0.26	0.74	0.40	3.19	0.78

* Symbols indicate significance as follows: † *vs* §, NS; † *vs* ¶, $p < 0.05$; ‡ *vs* ‖, NS; ‡ *vs* **, $p < 0.001$; § *vs* ¶, NS; and ‖ *vs* **, $p < 0.01$.

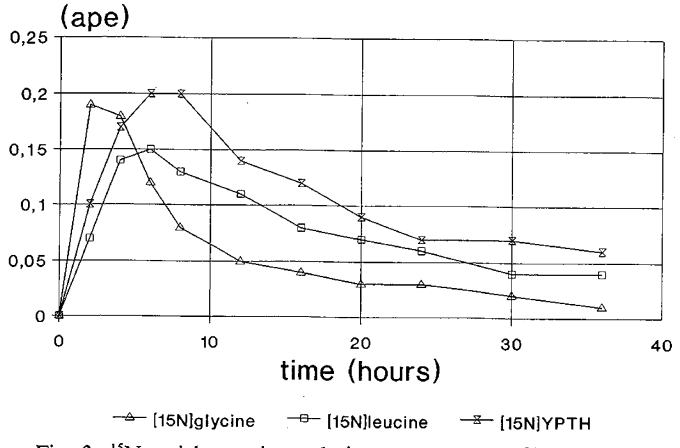

Fig. 3. ^{15}N enrichment in total nitrogen. ape, atom % excess.

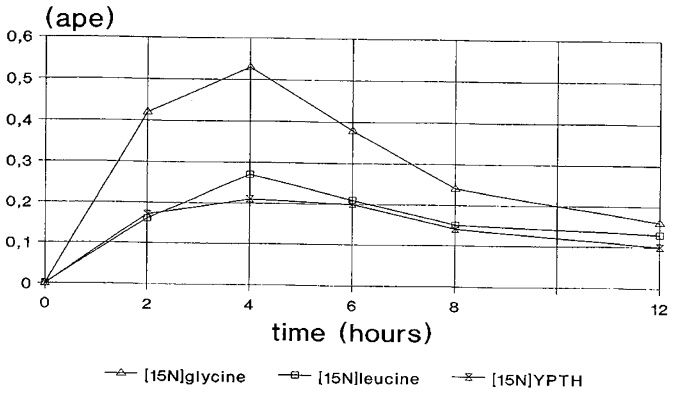

Fig. 4. ^{15}N enrichment in ammonia nitrogen. ape, atom % excess.

Table 4. *Rate constants of protein synthesis k_{21} and urinary nitrogen excretion k_{31} in %/h, calculated by TCM**

	[^{15}N]glycine		[^{15}N]leucine		^{15}N-YPTH	
Subject	k_{21}	k_{31}	k_{21}	k_{31}	k_{21}	k_{31}
1	10.10	0.29			2.97	0.24
2	4.59	0.09	3.85	0.13	4.36	0.31
3	8.39	0.16	4.75	0.19	3.95	0.18
4	14.28	0.55			6.18	0.34
5	18.87	0.52	6.05	0.32	4.47	0.83
6	9.63	0.37	5.60	0.26	5.18	0.21
7	13.88	0.25	5.83	0.17	5.47	0.30
8	7.57	0.31	6.15	0.25	5.34	0.30
9	10.64	0.25	6.06	0.19	7.98	0.46
Mean	10.88†	0.31	5.47‡	0.22	5.10§	0.35
SD	4.23	0.15	0.86	0.06	1.43	0.20

* Symbols indicate significance as follows: † *vs* ‡, $p < 0.01$; † *vs* §, $p < 0.001$; and ‡ *vs* §, NS.

Table 5. *Reutilization rate (R) and $t_{1/2}$ of ^{15}N in nonprotein nitrogen pool calculated by TCM**

	[^{15}N]glycine		[^{15}N]leucine		^{15}N-YPTH	
Subject	R (%)	$t_{1/2}$ (h)	R (%)	$t_{1/2}$ (h)	R (%)	$t_{1/2}$ (h)
1	97.3	6.7			92.6	21.6
2	98.1	14.8	96.7	17.4	93.5	14.9
3	98.1	8.1	96.2	14.1	95.7	16.8
4	96.3	4.7			94.8	10.6
5	97.3	3.6	95.0	10.9	84.4	13.1
6	96.3	6.9	95.6	11.8	96.1	12.9
7	98.2	4.9	97.1	11.6	94.8	12.0
8	96.1	8.8	96.2	10.8	94.7	12.3
9	97.7	6.4	97.0	11.1	94.5	8.2
Mean	97.3†	7.2‡	96.3§	12.5‖	93.5¶	13.6**
SD	0.3	3.3	0.8	2.4	3.6	3.9

Symbols indicate significance as follows: † *vs* §, $p < 0.05$; † *vs* ¶, $p < 0.01$; ‡ *vs* ‖, $p < 0.01$; ‡ *vs* **, $p < 0.01$; § *vs* ¶, NS; and ‖ *vs* **, NS.

Table 6. *Comparison of protein synthesis (S) and protein breakdown (B) rates in g protein/kg/d, calculated by TCM and AEPM**

	[^{15}N]glycine				[^{15}N]leucine				^{15}N-YPTH			
	S		B		S		B		S		B	
Subject	TCM	AEPM	TCM	AEPM	TCM	AEPM	TCM	AEPM	TCM	AEPM	TCM	AEPM
1	19.7	7.9	18.1	6.2					6.3	17.6	4.6	15.8
2	23.3		21.4		11.3	18.3	9.6	16.5	5.7	19.3	3.7	17.3
3	21.6	8.0	19.8	6.2	10.7	21.3	8.9	19.5	9.3	17.7	7.6	16.0
4	11.6	7.8	9.4	5.7					5.7	12.5	3.6	10.0
5	12.5	7.8	10.7	6.0	7.2	11.2	5.4	9.4	2.4	9.3	1.7	7.5
6	9.4	6.1	7.4	4.1	8.6	8.0	6.7	6.1	7.7	26.3	5.5	24.1
7	12.0	9.2	10.1	3.3	7.7	11.4	5.5	9.3	5.4	16.0	3.6	14.2
8	14.0	8.2	12.2	6.4	8.5	13.1	6.5	11.1	5.4	17.7	3.7	15.9
9	10.9	8.9	9.1	7.1	9.6	17.8	7.6	15.9	5.5	14.1	3.5	12.1
Mean	15.0†	7.5‡	13.1	5.6	9.1§	14.4‖	7.2	12.5	5.9¶	16.7**	4.2	14.8
SD	5.1	1.2	5.2	1.3	1.5	4.7	1.6	4.8	1.9	4.8	1.6	4.7

* Symbols indicate significance as follows: † *vs* §, $p < 0.02$; † *vs* ¶, $p < 0.001$; ‡ *vs* ‖, $p < 0.01$; ‡ *vs* **, $p < 0.001$; § *vs* ¶, $p < 0.01$; and ‖ *vs* **, NS.

lated protein synthesis values according to the TCM differed significantly between glycine (15 g/kg/d) and leucine (9.1 g/kg/d) ($p < 0.02$), glycine and YPTH (5.9 g/kg/d) ($p < 0.001$), and leucine and YPTH ($p < 0.01$).

When the protein synthesis rates were derived from the AEPM, the differences did not agree with the values obtained using the TCM; on the contrary, their rank order was reversed. We found the lowest values for glycine (7.5 g/kg/d), followed by leucine (14.4 g/kg/d), and the highest protein synthesis values were found for YPTH (16.7 g/kg/d). The differences between glycine and leucine ($p < 0.01$) and glycine and YPTH ($p < 0.001$) were statistically significant.

The calculated protein breakdown rates from both approaches were for TCM: glycine, 13.1 g/kg/d; leucine, 7.2 g/kg/d; and YPTH, 4.8 g/kg/d and for AEPM: glycine, 5.6 g/kg/d; leucine, 12.5 g/kg/d; and YPTH, 14.8 g/kg/d. The statistical significance was of the same order as for the protein synthesis rates.

Flux rates calculated from the AEPM with ^{15}N enrichment of total nitrogen in the denominator of the equation $Q = d/(a \cdot t)$ showed the expected overestimation derived from the lower ^{15}N enrichment of total nitrogen in all ^{15}N tracers (Table 7).

Any interference of ^{15}N recycling from protein breakdown between the first and the third study was negligible, as shown by the natural urinary ^{15}N abundance at the onset of the next study.

Flux rates and net protein gain (Table 8). The flux rates derived from the different tracers and both approaches had an effect of the same order of magnitude, whereas the net protein gain was practically the same for all tracer groups (1.89 to 1.99 g/kg/d).

Table 7. *Comparison of flux rates in g protein/kg/d calculated by AEPM with ^{15}N enrichment of ammonia (NH_3) and total nitrogen (N), respectively, in denominator of equation $Q = d/(a \cdot t)$*

	[^{15}N]glycine		[^{15}N]leucine		^{15}N-YPTH	
Subject	NH_3	N	NH_3	N	NH_3	N
1	8.4	34.2			18.1	21.6
2		50.0	18.7	32.9	19.7	19.4
3	8.4	27.8	21.7	27.5	18.1	26.3
4	8.3	15.0			12.8	15.0
5	8.2	16.1	11.6	16.6	9.7	7.3
6	6.5	16.0	8.4	16.6	26.6	25.5
7	5.4	20.5	11.6	18.3	16.3	14.8
8	8.8	22.3	13.4	18.0	19.0	16.2
9	9.2	23.8	18.1	34.7	14.4	12.2
Mean	7.9	25.1	14.8	23.5	17.1	17.7
SD	1.3	11.2	4.8	8.0	4.8	6.2

Table 8. *Comparison of flux rates (Q) and net protein gain (N) in g protein/kg/d calculated by TCM and AEPM*

	[^{15}N]glycine			[^{15}N]leucine			^{15}N-YPTH		
	Q			Q			Q		
Subject	TCM	AEPM	N	TCM	AEPM	N	TCM	AEPM	N
1	20.3	8.4	1.68			1.93	6.8	18.1	1.76
2	23.7		1.91	11.7	18.7	1.75	6.1	19.7	2.01
3	22.0	8.4	1.82	11.2	21.7	1.86	9.7	18.1	1.70
4	12.0	8.3	2.19			2.59	6.1	12.8	2.16
5	12.8	8.2	1.81	7.6	11.6	1.77	2.8	9.7	1.74
6	9.8	6.5	2.05	9.0	8.4	1.91	8.0	26.6	2.18
7	12.2	5.4	1.90	7.9	11.6	2.13	5.7	16.3	1.87
8	14.7	8.8	1.83	8.8	13.4	2.01	5.7	18.0	1.75
9	11.2	9.2	1.86	9.9	18.1	1.96	5.8	14.4	1.96
Mean	15.4	7.9	1.89	9.4	14.8	1.99	6.3	17.1	1.90
SD	5.2	1.3	0.15	1.6	4.8	0.25	1.9	4.8	0.11

DISCUSSION

The use of the ^{15}N-tracer technique for obtaining information relating to the protein metabolism is based on the assumption that the tracer is representative of the system as a whole (25). The validity of the tracer used has been repeatedly questioned since the 1970s, when investigation of the protein metabolism in prematurely born infants was begun on the basis of different models of calculation (1–4, 6–9, 11, 12). At present, [^{15}N]glycine is the most commonly used tracer, primarily because it is easily available commercially.

Especially for premature infants, there are principal objections to the use of [^{15}N]glycine. These children are in particular need of glycine for skeletal protein synthesis during the phases of relatively rapid growth between the 30th and 34th postconceptual weeks. Because the glycine supply is limited under HMF, a substantial part of the tracer is retained for protein synthesis, especially for incorporation within cartilage (with a glycine content of 24%). Under these conditions, protein turnover may be overrated because of a low level of urea labeling, urea being the final product of the protein metabolism.

In addition to glutamine, glycine participates in ammonia formation to a greater extent than other amino acids (26). Furthermore, according to Matthews *et al.* (27), the transamination of the glycine-$^{15}NH_2$ group to other amino acids is very poor, with the exception of serine and glutamine/glutamate. We have tried to overcome these problems by using YPTH.

When considering the results obtained from the TCM, the differences among the three tracer groups become obvious: Administration of [^{15}N]glycine resulted in exaggerated estimates of

up to 2.5 times the flux rate as compared to YPTH (15.4 g/kg/d *vs* 6.3 g/kg/d; $p < 0.001$), as had been expected. This typical glycine response (overestimate of protein nitrogen turnover rate, protein synthesis rate, and protein breakdown rate, as seen in Tables 6 and 8, significantly higher reutilization rates, and shorter half-life periods of ^{15}N in the nonprotein pool) reflects the fact that glycine is additionally involved in the synthesis of cartilage, hemine, choline, purine, and hippuric acid. This assertion agrees well with the results of Golden *et al.* (28).

If, however, the rates are calculated from the excretion of label in ammonia, the results are the exact opposite: The apparent rates of protein nitrogen turnover rate, protein synthesis rate, and protein breakdown rate derived from ammonia were about twice as high with YPTH as with glycine: protein nitrogen turnover rate with glycine, 7.9 g/kg/d; and protein nitrogen turnover rate with YPTH, 17.1 g/kg/d ($p < 0.001$). Several investigators (4, 5, 16, 29) have observed lower flux, synthesis, and breakdown rates when analyzing ammonia rather than urea in the majority of children and infants when [^{15}N]glycine was administered continuously or in a single dose.

These results do not agree with data recently published by Stack *et al.* (12). After administration of [^{15}N]glycine and acid hydrolyzed ^{15}N-yeast protein to three premature infants fed on human milk, the authors did not find any differences in protein turnover rates. The results of our study are not directly comparable to those of Stack *et al.* Our study groups differ in gestational age, postnatal age, body weight, and the number of subjects observed. The resultant ratios of both tracers on formula feeding of four premature infants were of approximately the same order of magnitude as the results from the present study: protein nitrogen turnover rate with [^{15}N]glycine, 13.8 g/kg/d; and protein nitrogen turnover rate with acid hydrolyzed ^{15}N-yeast protein, 21.2 g/kg/d.

The main drawback of a yeast protein hydrolysate as used by Stack *et al.* (12) could be the destruction of some amino acids, *e.g.* glutamine or tryptophan, through treatment with concentrated acids. The conversion of the glutamine to glutamate induced by acid hydrolysis is suspected to reduce the urinary ammonia excretion rate. To avoid any decomposition of amino acids, we prefer safe enzymatic hydrolysis with thermitase, a protease from *Thermoactinomyces vulgaris*. The degree of absorption of ^{15}N from this short-chain peptide/amino acid mixture was only slightly lower (97.8%) than that from leucine (99.1%) or from glycine (99.4%). The fecal loss was so low that the analysis of the feces becomes dispensable in determining the turnover rates in prematurely born infants.

Regardless of which of the different methods was used, the calculated protein nitrogen turnover, synthesis, and breakdown rates after administration of [^{15}N]leucine were always between the values derived for [^{15}N]glycine and ^{15}N-YPTH (Tables 6 and 8).

Labeled leucine has commonly been used as a "representative" amino acid (19, 25). One of the indispensable amino acids, leucine is preferably transaminated via α-ketoglutarate and glutamic acid in the muscles, kidneys, and brain. Compared to glycine, ammonia formation from leucine is less intense. This might suggest that [^{15}N]leucine is relatively model-independent.

Considerable differences in the flux rates obtained using a variety of tracers have also been reported by Fern *et al.* (30), with either urea or ammonia as end product. To avoid discrepancies between calculated flux rates derived from urea or ammonia, the authors emphasize the use of arithmetics and harmonic end product averages. Comparison of the flux rates as well as the protein synthesis and breakdown rates calculated by AEMP and [^{15}N]glycine as tracer and by TCM and ^{15}N-YPTH as tracer shows that there are practically no differences (Tables 6 and 8). However, this observation does not justify replacing the combination TCM/YPTH with AEPM/glycine. The disadvantages of the AEPM with [^{15}N]glycine are based on the assumption of the existence of a single metabolic pool. Meanwhile, however, it has been generally accepted that the precursor pool of ammonia is not representative of the precursor pool for protein synthesis (30, 31).

Our results obtained with the ammonia-based calculation indicate preferential utilization of glycine nitrogen by the premature infant's kidneys for ammonia genesis (5). The relatively low protein synthesis rates (5.9 g/kg/d) derived from labeling the total nitrogen after administration of YPTH and using the TCM agree well with data (7.9 g/kg/d) recently published by Beaufrére *et al.* (9) calculated by the plasma method with L-[1-^{13}C]-leucine. Pencharz *et al.* (13) concluded that the premature infant needs an optimum ratio between the net protein gain and the lowest possible rate of synthesis, inasmuch as protein synthesis requires energy input. The use of ^{15}N-YPTH is a way of avoiding overestimates of the protein turnover rate and major individual variations due to disproportionally high glycine amino nitrogen retention.

Considering the uniformity of our three tracer groups with respect to gestational age, postnatal age, and body weight, it can be assumed that the "real" protein turnover rates were similar in all infants. It is therefore to be suspected that the wide range of protein synthesis and breakdown rates as determined by the different tracers and models are to be considered methodologic errors. The major difficulty in assessing protein flux rates in preterm infants is the absence of an objective "gold standard" combination of tracers, techniques, models, and assumptions.

The results of this comparison substantiate the assumption that there are methodologic errors in connection with the use of different tracers and models for the calculation of whole-body protein parameters in preterm infants, with respect to the main requirement for tracer kinetic studies; the tracer nitrogen must be representative of total amino acid nitrogen (19, 25). Seen in this light, mixtures of completely labeled amino acids such as YPTH may represent the most reliable tracer substance inasmuch as, on the basis of the data obtained with ^{15}N-labeled YPTH and with total ^{15}N as the end product, there was no major difference in the order of magnitude of the protein flux between preterm and full-term infants (32).

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