¹⁵N Tracer Studies of Protein Metabolism in Low Birth Weight Preterm Infants: A Comparison of ¹⁵N Glycine and ¹⁵N Yeast Protein Hydrolysate and of Human Milk- and Formula-Fed Babies

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ABSTRACT. Nitrogen flux and protein synthesis and degradation were estimated using a single oral bolus of ¹⁵N glycine or ¹⁵N yeast protein hydrolysate and measuring the ¹⁵N enrichment of urinary ammonia in five low birth wt infants fed a low birth wt formula and in six who were receiving their own mother's breast milk. Results derived from using ¹⁵N-glycine and ¹⁵N-yeast hydrolysate tracers in a randomized crossover study in 10 studies on seven infants showed, with one exception, higher turnover rates and more interindividual variation with the ¹⁵N yeast. Both tracers showed good reproducibility in two infants who had repeated studies. Although wt gain was similar in both groups, nitrogen intake and retention were greater ($p < 0.01$) in the formula-fed group. Mean nitrogen turnover was similar in both groups, but there was a greater variance in the human milk-fed group which also had a greater nitrogen turnover/U absorbed nitrogen ($p < 0.025$) and a lower excretion of nitrogen/U flux. (*Pediatr Res* 25:167-172, 1989)

Abbreviations

FF, formula-fed
HMF, human milk-fed

Most measurements of whole-body protein synthesis and degradation in neonates have used a technique in which the subjects receive a prolonged intragastric infusion of a single ¹⁵N-amino acid (usually glycine), and the calculation of protein turnover is based on the "plateau" ¹⁵N enrichment of urinary urea (1). This approach is time consuming and is impractical for use in routine clinical pediatrics. The length of the infusion may result in erroneous values due to label recycling and the need to allow approximately 48 h after a study for the ¹⁵N abundance of urinary urea to regain background levels limits the use of this technique in studies of acute changes in protein metabolism.

The length of these studies can be shortened significantly if urinary ammonia is used as the end-product on which the calculations are based (2, 3), particularly if, rather than using a constant infusion of isotope, with measurements in the isotopic steady state, the technique is based on a single dose of isotope and the average isotope abundance in urinary ammonia excreted

over a short (~12 h) period. This approach has received relatively little attention in studies with infants (4).

The use of single amino acids as tracers in studies based on end-product labeling may give results that are biased by the peculiarities of the metabolism of the tracer amino acid (5), and it seems clear that different amino acids can give quite different estimates of whole-body amino-N flux in the same individuals (6, 7). This consideration may be particularly important when ¹⁵N-glycine is used in preterm babies, because in these infants glycine appears to behave as a limiting or semi-essential amino acid. As suggested by others (1, 8-10) one possible way, in theory at least, of avoiding this problem is to use mixtures of labeled amino acids as tracers.

In the present work, we have measured protein turnover in preterm neonates by examining the labeling of urinary ammonia after a single oral dose of tracer, as suggested by Waterlow and colleagues (2) and used in adult subjects (8, 11, 12). We compared two tracers—¹⁵N-glycine and ¹⁵N-yeast protein hydrolysate—and protein turnover in infants receiving a formula feed or their mother's milk.

MATERIALS AND METHODS

Subjects and experimental protocol. Eleven male infants were investigated (Table 1). Four FF infants (no. 1-4) and three HMF infants (no. 6-8) received, in separate studies and in a randomized sequence at least 48 h apart, either ¹⁵N-glycine or hydrolyzed ¹⁵N-yeast protein. The remaining one FF and three HMF infants received ¹⁵N-glycine alone (there being insufficient yeast for further studies).

The subjects were low birth wt infants and, with the exception of cases 1 and 11, had birth wt appropriate for their gestational ages (13). Gestational ages were assessed from the date of the last menstrual period, ultrasound cephalometry, and by clinical assessment (14). Informed parental consent was obtained, and the study was approved by the local ethical committee.

Infants were weighed daily. When studied, all had regained their birth wt and were established on enteral feeding. The FF infants received at least 130 ml/kg/24 h, and the HMF infants received at least 155 ml/kg/24 h for at least 48 h before and during the study periods. All infants gained wt and retained nitrogen throughout the balance periods (Tables 1 and 2). The formula used in these studies was a single batch of "Low Birth Weight" (LBW) SMA (Wyeth Laboratories, Taplow, UK) (334 kJ/100 ml, 2.0 g protein (N × 6.38)/100 ml). Each HMF infant received his own mother's milk either by breast feeding when intakes were calculated from the gain in body wt during a feed (Sartorius 3862 MP8 electronic balance, Scottish Scientific Instrument, Edinburgh, UK) or more commonly by naso- or

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Table 1. Clinical details of infants studied

Subject	Birth wt (g)	Gestational age (wk)	Wt at study (g)	Postnatal age at study (days)	Wt gain (g/kg/24 h)
FF infants					
1*	820	29	850	19	23.1
2 (i)	1080	27	1340	31	17.1
2 (ii)			1510	39	16.5
2 (iii)			1910	54	12.1
3 (i)	1440	31	1580	13	14.7
3 (ii)			1730	19	11.4
4	1500	32	1640	14	12.2
5	1550	32	1660	17	21.4
Mean (SD)	1278 (316)	30 (2)	1528 (320)	26 (14)	16.1 (4.4)
HMF infants					
6	1240	28	1330	30	5.0
7	1830	32	1830	9	16.4
8	1880	32	1880	18	20.8
9	1300	30	1710	40	5.8
10	1340	29	1470	25	22.1
11*	1480	32	1580	32	20.7
Mean (SD)	1512 (278)	30 (2)	1633 (194)	26 (11)	15.1 (7.8)

* Small for gestational age.

Table 2. Energy intakes and nitrogen balance data

Subject	Total energy intake (Kj/kg/day)	Intake (mg/kg/day)	Nitrogen		
			Absorption Retention		Excretion (mg/kg/day)
			(mg/kg/ day)	(mg/kg/ day)	
FF infants					
1	568	534	414	307	107
2 (i)	538	505	439	357	82
2 (ii)	544	510	453	366	87
2 (iii)	496	466	389	302	87
3 (i)	451	423	370	294	77
3 (ii)	473	443	401	318	83
4	486	456	345	250	95
5	579	544	444	378	66
Mean (SD) <i>n</i> = 5	524 (51)	492 (48)	403 (39)	317 (48)	87 (15)
HMF infants					
6	489	354	295	227	68
7	454	392	301	194	107
8	446	352	268	216	52
9	515	340	246	182	64
10	424	309	247	182	67
11	476	387	312	254	58
Mean (SD) <i>n</i> = 6	467 (33)	356 (31)	279 (28)	209 (29)	69 (19)

orogastric tube administration of expressed samples. The intakes of the other infants were weighed directly. The infants' intakes of energy (calculated) and nitrogen as well as their rates of nitrogen retention over the balance periods are shown in Table 2. All milk samples were stored at -20°C until analyzed.

Balances and tracer studies. Three-day nitrogen balance studies were carried out on all the infants; during this period they were fed at 2- to 4-h intervals as appropriate to their wt and gestational ages. The mattresses of the cots were covered in polythene sheeting on which Whatman 541 filter papers were placed to retain any vomits, regurgitated feeds, or spilled urine.

Stools were collected between carmine markers, given orally 72 h apart, onto similar filter papers. The collection of urine is described below. All material was collected immediately and stored at -20°C until analyzed.

Lipid and nucleic acid-free ^{15}N -yeast protein was hydrolyzed in sealed tubes in 5.4 M HCl at 105°C for 18 h. The hydrolysate was dried under vacuum and reconstituted in distilled water at least three times. In the final dry product, 98% of the N could be accounted for by amino acids and the remaining nitrogen as NH_4^+ . The excess abundance of ^{15}N was 92 atoms %, and the final solution was neutral. The amino acid composition (measured in four separate hydrolysates) of the final yeast sample is shown in Table 3. ^{15}N -glycine (96 atoms % excess) was obtained from the British Oxygen Company, Glasgow, UK.

Both tracers were dissolved in distilled water and were sterilized by filtration through a $0.2\ \mu\text{m}$ Millipore filter (Millipore, Harrow, Middlesex, UK). Each tracer was given as a single dose over a 3-min period via a polyvinyl nasogastric tube between 08:30 and 12:30, 45 min before a feed. The tube was then flushed with 2 ml of distilled water and sealed. The subsequent feed was given by nasogastric tube. The dose of ^{15}N -glycine supplied known amounts of 0.608–0.964 mg ^{15}N and that of ^{15}N -yeast provided 0.918–1.493 mg ^{15}N .

Urine collection condom devices were fashioned from soft polythene gloves and affixed to the penis with a drainage tube connected to a glove finger. After each tracer administration, urine was collected for a known period of approximately 12 h, and during this time it was removed immediately from the collection bag acidified with 10% H_2SO_4 to pH < 2 and stored at -20°C until analyzed. Thereafter, the urine for the residual balance period was collected as a single pool or as separate aliquots for a second tracer study. Urine passed immediately before the start of the balance period was used to measure background abundance of ^{15}N , and when a second tracer was used, urine passed between 33 and 36 h after completion of the first study was collected separately to measure background abundance for the second study; this had an NH_3 ^{15}N abundance which did not differ from natural abundance. In one subject (no. 8), separate 4-h urine samples were analyzed to investigate the pattern of ^{15}N -ammonia excretion over the first 12 h after administration of isotope: almost 75% of the excretion of ^{15}N -ammonia had occurred by 8.25 h of collection.

Sample analysis. The nitrogen content of the feeds urine and

Table 3. Amino acid analysis of yeast hydrolysate*

Amino acid	g amino acid/16 g nitrogen
Aspartate + asparagine	13.5 ± 0.7
Threonine	5.2 ± 0.5
Serine	7.0 ± 0.2
Proline	5.0 ± 0.5
Glutamate + glutamine	12.3 ± 0.8
Glycine	5.3 ± 0.4
Alanine	6.8 ± 0.4
Cysteine	0.4 ± 0.2
Methionine	2.4 ± 0.3
Valine	4.0 ± 0.2
Isoleucine	3.5 ± 0.4
Leucine	7.0 ± 0.5
Tyrosine	4.4 ± 0.7
Phenylalanine	4.9 ± 0.6
Histidine	3.5 ± 0.4
Lysine	9.9 ± 1.1
Arginine	5.1 ± 0.6

* Four samples of dry yeast (11–14 mg) were hydrolyzed for 18 h at 105°C in 5.4 M HCl containing L-norleucine as internal standard. The sample was dried under vacuum and reconstituted in 0.2 M Na citrate buffer, pH 2.2. The hydrolysate was then analyzed on a Rank Hilger amino acid analyzer.

stool were determined by the Kjeldahl method (15). The ammonia content of the urine was estimated by treatment of the urine with NaOH, steam distillation and titration (with potassium bi-iodate solution). ^{15}N in urinary ammonia was separated essentially by the method described by Read *et al.* (16). Resin-bound ammonia was subsequently oxidized to nitrogen gas by treatment with lithium hypobromite (17) in an evacuated tube connected to the inlet of a VG MM602 B double-collector isotope ratio mass spectrometer for the determination of the ^{15}N enrichments (18).

The fat content of human milk was measured by extraction with chloroform/methanol (2:1 vol/vol) under reflux with the subsequent measurement of the residue wt (19). Each human milk sample was also freeze-dried, and the carbohydrate content was calculated from the difference between the dry matter and the sum of the protein content ($\text{N} \times 6.38$) and the fat content. The energy content of the milk was then calculated as:

$$\text{energy (kJ)} = (\text{N} \times 147) + (\text{fat} \times 39.0) + (\text{carbohydrate} \times 17).$$

Calculations. The nitrogen flux (Q) was calculated by:

$$Q \text{ (mg N} \cdot \text{day}^{-1}\text{)} = \frac{\text{dose } ^{15}\text{N (mg)} \times 24}{^{15}\text{N abundance in NH}_3 \text{ (mg } ^{15}\text{N/mg total N)} \times \text{collection time (h)}}$$

Protein synthesis (S) and breakdown (B) were then determined from:

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

where I is the apparent absorbed N and E the excretion of N. Statistical analyses and comparisons were performed by paired and unpaired *t* tests, except where indicated otherwise in the text (20). Where replicate values were obtained from a child, the mean of these has been used for calculating group means for the tables and statistical evaluation.

RESULTS

Comparison of diets. The energy intakes of the FF infants was marginally greater than those of the HMF infants (Table 2) ($p < 0.05$), whereas the intakes, absorption, and retention of nitrogen of the FF group were much the larger of the two groups (Table 2) ($p < 0.001$). The nitrogen retention relative to that absorbed was similar in both groups, 79% (SD, 5%) in the FF infants and 75% (SD, 6.1%) in those receiving their own mother's milk, and there was no correlation between the amount of nitrogen absorbed or retained and the rate of nitrogen flux which was similar in both groups despite the greater variability in the breast-fed babies (Table 4). Thus the nitrogen flux measured by ^{15}N -glycine relative to absorbed nitrogen was higher in the HMF infants ($839 \pm 266\%$) than in those fed formula ($529 \pm 110\%$) ($p < 0.05$). Nitrogen excretion, both in total and in proportion to nitrogen flux, tended to be lower in the babies fed their own mother's milk than in the FF infants. Although the mean values for the derived rates of N flux and protein synthesis and degradation of the FF and HMF groups differed (Table 5), this difference was not statistically significant ($p > 0.05$).

Comparison of tracers. The calculated rates for protein synthesis and degradation and nitrogen flux (Table 5) were higher with ^{15}N yeast than with ^{15}N -glycine as tracer ($p < 0.05$), irrespective of the diet. There was no order effect. The values obtained with yeast protein were also more variable, although this was biased by one subject (no. 3). It was particularly interesting that the large difference in this subject was observed in two separate pairs of studies in which ^{15}N -yeast was administered first in one and second in the other.

When the rates derived from the different tracers were compared by Spearman's rank correlation (20), an agreement was found for the rates of protein breakdown ($p < 0.05$), but not for

Table 4. Comparison of calculated nitrogen flux (gN/kg/day), protein synthesis, and degradation (g protein/kg/day) measured with ^{15}N -glycine in FF and HMF infants

Subject	Nitrogen flux	Protein synthesis	Protein degradation
FF infants			
1	1.99	11.8	9.9
2 (i)	1.90	11.3	9.1
2 (ii)	1.96	11.7	9.4
2 (iii)	1.84	11.0	9.1
3 (i)	2.42	14.7	12.9
3 (ii)	2.46	14.9	12.9
4	2.32	13.9	12.3
5	1.98	12.0	9.6
Mean (SD) <i>n</i> = 5	2.13 (0.24)	12.8 (1.5)	10.8 (1.7)
HMF infants			
6	2.60	15.9	14.4
7	2.93	17.6	16.4
8	1.34	8.1	6.8
9	2.79	17.1	16.0
10	1.27	7.5	6.4
11	3.13	19.2	17.6
Mean (SD) <i>n</i> = 6	2.34 (0.82)	14.2 (5.1)	12.9 (5.0)

those of protein synthesis or nitrogen flux ($0.1 > p > 0.05$). These findings question any assumption that both tracers were measuring similar or related metabolic pools or processes.

Protein gain as a percentage of protein synthesis with ^{15}N glycine as tracer was 16% for the formula studies and 9% for the human milk studies (derived from data in Table 4). With ^{15}N yeast, the figure obtained was 9% for both groups (derived from data in Table 5).

DISCUSSION

The values for protein synthesis obtained in the present study lie within the range obtained in previous studies (Table 6). However, this is probably because of the wide range of values obtained in the earlier studies (5.9–18 g protein/kg/day); this observation is perhaps not surprising in view of the heterogeneous nature of the subjects, their different diets, and the variety of methods that have been used to investigate the metabolism of nitrogen.

Choice of tracer and end-product. With the exception of an early study by Nicholson (21), some recent reports from the German Democratic Republic (9, 10, 22, 23), and a study using ^{13}C -leucine (24), most studies of protein turnover in preterm infants have used variations of the "constant infusion" method developed by Picou and Taylor-Roberts (1); additionally most have depended on the use of ^{15}N -glycine as the tracer, and urinary ^{15}N -urea (or occasionally total N) as the end-product.

Apart from theoretical problems applicable in general to the study of protein metabolism (see below), the study of protein turnover in preterm infants presents particular practical and ethical problems. The infants require close care and may often be in a state in which their metabolic state is changing rapidly. For these reasons alone, methods that avoid prolonged administration of tracer and equally prolonged collection periods have a distinct advantage. To some extent, then, our choice of an approach involving a single dose of isotope and the use of urinary ammonia was influenced by these practical requirements since this method reduced the length of each study from times in excess of 30 h to less than 12 h. Another advantage of basing the calculation of protein turnover in HMF infants on the abundance

Table 5. Comparison of calculated nitrogen flux (g N/kg/day), protein synthesis, and degradation (g protein/kg/day), measured with ¹⁵N-yeast protein hydrolysate (Y) and ¹⁵N-glycine (G) in FF and HMF infants

Subject	Nitrogen flux		Protein synthesis		Protein degradation	
	Y	G	Y	G	Y	G
FF infants						
1	2.87	1.99	17.2	11.8	15.3	9.9
2 (i)	2.19	1.90	13.2	11.3	11.0	9.1
2 (ii)	2.37	1.96	14.2	11.7	11.9	9.4
2 (iii)	2.68	1.84	16.2	11.0	14.3	9.1
3 (i)	5.21	2.42	32.1	14.7	30.2	12.9
3 (ii)	5.4	2.46	33.2	14.9	31.3	12.9
4	2.72	2.32	16.4	13.9	14.9	12.3
Mean (SD)	3.33 (1.34)	2.16 (0.26)	20.2 (8.4)	13.0 (1.7)	18.4 (8.4)	11.1 (1.8)
HMF infants						
6	3.06	2.6	18.7	15.9	17.3	14.4
7	2.36	2.93	14.1	17.6	12.9	16.4
8	1.78	1.35	10.8	8.1	9.4	6.8
Mean (SD)	2.4 (0.64)	2.29 (0.83)	14.5 (4.0)	13.9 (5.1)	13.2 (4.0)	12.5 (5.1)

Table 6. Reported measurements (mean values) of protein synthesis in preterm neonates receiving either formula feeds or human

Source	No. of studies	Tracer	End-product	Protein synthesis (g/kg/day)
Formula diet				
Nicholson (1970) (21)	3	¹⁵ N-glycine	Urea	12.7
Pencharz <i>et al.</i> (1981) (26)	20	¹⁵ N-glycine	Urea	14.4
	20	¹⁵ N-glycine	Urea	18.3
Pencharz <i>et al.</i> (1983) (27)	20	¹⁵ N-glycine	Urea	12.7
Catzefflis <i>et al.</i> (1985) (28)	7	¹⁵ N-glycine	Urea	11.7*
				Mean 14.0 ± 2.6
Nicholson (1970) (21)	3	¹⁵ N-glycine	Ammonia	15.4
Jackson <i>et al.</i> (1981) (5)	1	¹⁵ N-glycine	Ammonia	11.1
Catzefflis <i>et al.</i> (1985) (28)	6	¹⁵ N-glycine	Ammonia	5.9†
Stack <i>et al.</i> (1989) (present study)	5	¹⁵ N-glycine	Ammonia	12.7
				Mean 13.1 ± 2.2‡
DeBenoist <i>et al.</i> (1984) (24)	3	¹³ C-leucine	Urinary ¹³ C-leucine	12.0
	4	¹³ C-leucine	Expired CO ₂	12.1
Stack <i>et al.</i> (1989) (present study)	4	¹⁵ N-yeast	Ammonia	20.1
			(omitting subject N.D.)	16.1
Human milk				
Pencharz <i>et al.</i> (1983) (27)	10	¹⁵ N-glycine	Urea	18.4
Catzefflis <i>et al.</i>	2	¹⁵ N-glycine	Urea	10.2*
				Mean 14.3 ± 5.8§
Jackson <i>et al.</i> (1981) (5)	3	¹⁵ N-glycine	Ammonia	12.3
Catzefflis <i>et al.</i> (1985) (28)	4	¹⁵ N-glycine	Ammonia	5.9†
Stack <i>et al.</i> (1989) (present study)	6	¹⁵ N-glycine	Ammonia	14.2
				Mean 13.2 ± 1.3‡
DeBenoist <i>et al.</i> (1984) (24)	3	¹³ C-leucine	Urinary ¹³ C-leucine	10.6
	3	¹³ C-leucine	Expired CO ₂	10.6
Plath <i>et al.</i> (1987) (10)	4	¹⁵ N-glycine	Urinary N	15.8 ± 2.6
	4	¹⁵ N-a.acid	Urinary N	11.3 ± 3.1
	10	¹⁵ N-yeast	Urinary N	10.2 ± 3.0
Stack <i>et al.</i> (1989) (present study)	3	¹⁵ N-yeast	Ammonia	14.5

* In two of nine studies with formula milk and in three out of five studies with human milk, urea was not labeled.

† The values calculated by Catzefflis *et al.* (28) were based on a second plateau. Values based on the "pseudo" plateau at 9 h are approximately 60% higher.

‡ Omitting the values of Catzefflis *et al.* (28).

§ The coefficient of variation within a group was approximately 17% for FF and >30% for HMF infants.

of urinary ammonia is that it minimizes artifacts that arise from the comparatively high urea content of human milk (24).

At the theoretical level, choices have to be made as to the tracer, the end-product, and the route of administration. Fern *et*

al. (25) have discussed the first two aspects in detail and noted the interaction between the route of administration of the tracer and the ¹⁵N-abundance of different end-products.

Glycine has been the tracer of choice in many previous studies

(26–28), principally because of its commercial availability, but there are general objections to the use of a single amino acid and to the use of glycine in particular, especially in premature infants. Jackson (29) has advanced the hypothesis that glycine may not only be a “semi-essential” amino acid but might also be in limited supply in the preterm infant (2, 5), especially when the infants are receiving human milk. Hence, glycine might not be an appropriate tracer for measuring protein synthesis in such individuals. The peculiarities of the catabolism of glycine may make it a poor substrate for urea synthesis and the N of ^{15}N -glycine may not be reliably transferred to urinary urea in human preterm infants (5, 28). By contrast, significant labeling of urinary ammonia has been a uniform finding in these and our subjects.

With ^{15}N glycine as tracer, protein gain (*i.e.* protein synthesis minus protein degraded) as a percentage of protein synthesis (data in Table 4) was 16% for the FF infants. This figure for FF infants is similar to those obtained by other workers using ^{15}N -glycine (5, 28) and ^{13}C -leucine (24). However, we found a lower value for the HMF infants (9%); similarly Pencharz and coworkers (27) obtained a higher figure for their FF infants (17%) than for their HMF (11%). The low protein gain (9%) as a percentage of protein synthesis in the FF infants studied with ^{15}N -yeast (data from Table 5) probably reflects the poorer transfer of ^{15}N to ammonia by some amino acids (see below).

Although the use of an amino acid mixture may provide a more generally representative tracer for amino N turnover, the choice of the mixture is important. Different ^{15}N -labeled amino acids label urea and ammonia to variable extents and can give quite different values for protein turnover (6, 7). Although this shows the danger of necessarily accepting results obtained with any single amino acid, a corollary is that, even with an amino acid mixture, the results are biased by the peculiarities of its composition (17). In this respect, yeast protein, which has high levels of the dicarboxylic amino acids and their amides (Table 3), is less than satisfactory. These amino acids and lysine will tend to label urea to a greater extent than ammonia, and this may underlie the fact that in the present study, in which ammonia was used as end-product, the estimates of rates of protein synthesis and degradation and of N-flux derived from yeast protein was higher than those derived from glycine in nine of the 10 studies (Table 5). Indeed in one infant (no. 3), the difference between the tracers was substantial, indicating that individual variations may occur in the metabolism of amino acids and that these differences should be remembered when interpreting studies of this nature, especially as the degree of between-study reproducibility seen with two subjects (nos. 2 and 3) and in other studies (7, 30) suggest the genuineness of such differences. In contrast to the present results, in previous comparisons of ^{15}N -glycine with labeled proteins or amino acid mixtures, glycine gave higher average values than amino acid mixtures, yeast, or egg protein (9, 10, 22). It seems that at this stage the “ideal” amino acid mixture cannot be defined, although a mixture close in composition to that of body protein may provide the generally most useful approach.

Comparisons of milks. Although there was no significant difference between the average rates of nitrogen turnover and protein synthesis in the FF and HMF infants, with ^{15}N -glycine as tracer, the latter group had wt-specific rates of protein synthesis that were 10% higher (Table 4). Even more strikingly, the results with the HMF subjects were considerably more variable (CV 35%) than those obtained in the infants that received the formula feeds (CV 11%) (Fisher test $p < 0.05$). The variations in the turnover of body nitrogen in the HMF infants did not correlate with either their estimated energy intake or with their individual nitrogen retention. This variability of protein turnover in HMF preterm infants, which is apparent in other studies (5, 27, 28), is unexplained but seems, nonetheless, to be a real difference between these two types of feeding.

To some extent, the results of the present and previous comparisons of protein turnover in breast-fed and FF infants are

compromised by the fact that HMF infants have generally received lower intakes of both nitrogen and total energy than those that received a formula feed. In this study, it was interesting that there was no significant difference in the rates of wt gain of the two groups of babies despite the fact that, allowing for body wt, the HMF group apparently received 11% less energy and respectively absorbed and retained 31% and 34% less nitrogen than the FF babies.

It would seem that the energy requirement of HMF infants is lower than that of infants receiving a formula feed, and, indeed, it has been calculated that the energy intakes of exclusively breast-fed full-term infants are approximately 25% lower than the currently accepted recommended requirements, yet they gain wt normally (31). Even when such infants received supplemental feeding, they achieved a normal rate of wt gain at energy intakes that remained below that recommended (32).

The present study demonstrates that a simple and minimally invasive approach to the measurement of protein turnover in preterm infants is practicable. The results obtained with the two different tracers highlighted some potentially important considerations with respect to individual variations in amino N metabolism. The simplicity of the approach suggests that it could be applied outside a specialist metabolic facility and could be used to investigate other metabolic problems in general pediatric practice, and to these ends the method merits further evaluation.

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