Changes in Protein Turnover after the Introduction of Parenteral Nutrition in Premature Infants: Comparison of Breast Milk and Egg Protein-Based Amino Acid Solutions

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ABSTRACT. Rates of protein turnover were measured in 20 infants receiving either Vamin Infant (group A) or Vamin 9 glucose (group B) as the amino acid source in total parenteral nutrition. A constant infusion of L-[1-¹³C] leucine was used to measure whole body leucine flux, and leucine oxidation rates were derived from measurements of total urinary nitrogen excretion. Infants were first studied when receiving only i.v. glucose and again on each of the next 4 d as total parenteral nutrition was gradually increased to a maximum of 430 mg nitrogen/kg/d and 90 nonprotein kcal/kg/d. Net protein gain and protein synthesis and breakdown rates increased progressively for all infants taken together over the study period as i.v. nutrition was increasing ($p < 0.001$). There were no differences between groups in the changes in net protein gain and rates of protein synthesis and breakdown throughout the study period. Nitrogen retention on d 5 for the two groups was similar ($60 \pm 16\%$ and $67 \pm 11\%$ in groups A and B, respectively). In a subgroup of infants, measurements were repeated on d 8, when the intake had been constant for 3 d. Protein retention was the same as on d 5, but both synthesis and breakdown were increased. It is concluded that rates of protein turnover increase significantly in response to increasing i.v. nutrition and that this elevation was not influenced by the composition of the amino acid mixture given. (*Pediatr Res* 32: 447-454, 1992)

Abbreviations

TPN, total parenteral nutrition
LBWN, low birth weight neonate
N, nitrogen

The controversy continues over which amino acid solution should be used for parenteral nutrition in the newborn, particularly for LBWN. There have been reports of potentially neurotoxic levels of phenylalanine (1-3) and high levels of other amino acids (4) in LBWN receiving Vamin 9 glucose (Kabi Pharmacia, Milton Keynes, UK), the amino acid profile of which is based on that of egg protein and was originally designed for adults. After these reports, amino acid solutions based on the profiles of

breast milk protein or on the plasma profiles of postprandial breast-fed neonates have been made, resulting in lower plasma amino acid levels (5, 6).

The majority of studies comparing different amino acid solutions have examined the effects on N balance and/or amino acid profiles (6-8). An alternative, however, is to examine the effect on whole body protein turnover, which can be measured using stable isotopes. [¹⁵N]glycine has been used in LBWN receiving either a casein hydrolysate or a crystalline amino acid solution, Vamin (9), showing that the percentage N retention was greater, and the fraction of N flux coming from endogenous protein breakdown was significantly less in LBWN who received Vamin. It was therefore inferred that Vamin was more efficacious. However, reservations regarding the use of [¹⁵N]glycine as a tracer in neonates have been expressed (10, 11). The alternative procedure is the constant infusion of carbon-labeled leucine (12). We have recently used a modification of this technique in sick, premature infants (13) and have now used this approach both to measure the progression of whole body protein turnover rates with increasing nutritional intake and to compare these rates in LBWN receiving two different amino acid solutions in TPN. The profiles of the solutions given were based on either breast milk protein (Vamin Infant, Kabi Pharmacia) or egg protein (Vamin 9 glucose), and their constituent amino acids, together with those of breast milk, are listed in Table 1.

MATERIALS AND METHODS

Patients. Twenty LBWN were randomly allocated to receive Vamin Infant (group A, $n = 11$) or Vamin 9 glucose (group B, $n = 9$). The gestational ages (mean $\pm$ SD) were 29.4 ± 2.0 and 29.5 ± 1.9 wk and the birth weights were 1.28 ± 0.34 and 1.38 ± 0.4 kg in group A and B, respectively. Infants could only be studied if there was an i.v. cannula *in situ* and if they were not receiving enteral feeds at the time of study. Because accurate urine collections were necessary, only male infants were included. Exclusion criteria were the presence of any metabolic disorders, renal failure, and insulin administration. Informed parental consent was obtained in all cases, and the study had the approval of Camberwell, Riverside East and West ethics committees. The clinical characteristics of all infants are listed in Table 2. All required varying degrees of ventilatory support for respiratory distress.

Infants were first studied just before starting TPN, when they were receiving only i.v. glucose (study d 1). The d 1 data from eight of the infants (nos. 8-13, 17, and 18) have been reported previously (13). The same experimental protocol was then repeated daily in each infant over the next 4 consecutive d (d 2-5) and again in some infants on d 8. Thus, a total of six studies were performed on each, when this was feasible. Details of the

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Table 1. Amino acid composition of Vamin 9 glucose, Vamin Infant, and human milk protein (expressed as g/16 g N)

	Vamin 9 glucose	Vamin Infant	Breast milk*
L-Alanine	5.1	10.8	5.5
L-Arginine	5.6	7.0	5.5
L-Aspartic	7.0	7.0	11.7
L-Cysteine	2.4	1.7	1.8
L-Glutamic acid	15.3	12.2	20.3
Glycine	3.9	3.6	3.6
L-Histidine	4.1	3.6	3.6
L-Isoleucine	6.6	5.3	5.4
L-Leucine	9.0	12.0	11.9
L-Lysine	6.6	9.6	9.6
L-Methionine	3.2	2.2	2.2
L-Phenylalanine	9.4	4.6	4.7
L-Proline	13.8	9.6	9.6
L-Serine	12.8	6.5	6.5
L-Threonine	5.1	6.2	6.2
L-Tryptophan	1.7	2.4	2.3
L-Tyrosine	0.9	0.8	3.7
L-Valine	7.3	6.2	6.2
Taurine			1.9

* Mature mothers' milk protein.

TPN regimens used are given in Table 3. The input of TPN was increased in an incremental fashion, to reach a maximum on study d 5, the 4th d of TPN. The decision to start TPN was made independently by the clinician concerned: this included an assessment of both the duration of time that intensive care would be needed and whether a particular neonate was likely, because

of the severity of the illness, to tolerate sufficient enteral feeds to provide adequate nutrition. Infants had been maintained on i.v. glucose alone since birth, the glucose providing the amount of calories listed in the TPN regimen (Table 3) for study d 1.

Stable isotope administration and N balance. Each study day, infants were given a 6-h i.v. infusion of [$1-^{13}\text{C}$]leucine at a dose of $6.1 \mu\text{mol/kg/h}$, preceded by a priming dose of $15.3 \mu\text{mol/kg}$. The infusion was started at the same time each morning, which was at least 12 h after the TPN regimen had been changed. Heparinized blood samples (0.5 mL) were collected from indwelling arterial lines at 0, 2, 4 and 6 h, immediately centrifuged and the plasma stored at -20°C until analysis. An accurately timed urine collection of about 12 h duration was started at the beginning of the infusion, using a closed circuit driven by a constant air pump, a modification of a method described previously (14). With this method, air is continually blown into the urine bag, preventing development of a vacuum, while the urine is sucked into a dependent syringe outside the incubator. An additional 0.2 mL of blood were taken at 0 and 12 h for measurement of plasma urea concentration to correct N excretion values for any change in total body urea N during the urine collection (15).

Laboratory analyses. The ^{13}C enrichment of plasma leucine was measured with a VG 12-250 gas chromatograph-mass spectrometer (VG Masslab, Manchester, UK), after conversion to the tertiarybutyldimethylsilyl derivative. The ion peaks measured were m/e 302 and 303. The total N content of urine was determined by the Kjeldahl method (16). Plasma urea measurements were made using a urease method on a Boehringer Mannheim Hitachi 717 (Boehringer Mannheim, Lewes, East Sussex, UK).

Calculation of whole body turnover rates. The basic principles

Table 2. Clinical characteristics of infants*

Patient no.	Gestational age (wk)	Birth wt (kg)	Age 1st studied (h)	Ventilation (d)	
				Duration	Air
Group A: infants given Vamin Infant					
9	30-31	1.39	33	1-3	7
10	30	1.34	43	0-16	89
11	30	1.54	46	0-47	49
12	31	1.70	24	0-16	
13	31	1.75	47	0-16	16
22	30	1.21	62	1-7	8
25	32	1.47†	24	0-5	8
27	30	1.02†	9	0-4	6
28	29	1.10	44	0-27	40
30	25	0.61	34	0-38	
34	27	0.98	47		
Mean ± SD	29.6 ± 2	1.28 ± 0.34	37 ± 15		
Group B: infants given Vamin 9 glucose					
8	32	1.74	44	0-3	4
17	28	0.83†	52	0-7	50
18	32	1.63	48	0-25	
20	31	1.80	44	0-12	
21	29	1.63	48	0-25	32
23	29	1.36	56	0-10	
29	29	1.14	34	0-9	12
33	26	0.94	97	0-8	5
35	28	0.92	59	0-4	8
Mean ± SD	29.3 ± 2	1.33 ± 0.4	54 ± 18		
Significance A vs B	NS	NS	<i>p</i> = 0.014		

* Further clinical details: No. 9 developed respiratory distress at age 4 d. Right middle lobe collapse on chest x-ray and C-reactive protein raised. Started antibiotics on study d 3. No. 12 was transferred d 29 in 23% O_2 . No. 18 later developed bronchopulmonary dysplasia and died at age 7 mo. No. 20 was transferred d 25 in 30% O_2 . No. 23 had positive blood culture d 0. Treated with i.v. antibiotics but died on d 10. No. 30 was transferred d 38 in 30% O_2 . No. 35 had raised C-reactive protein the day before study commenced, but negative infection screen. Treated with i.v. antibiotics d 1-15.

† Small for gestational age.

Table 3. TPN regimen for both groups of infants

Study day	Composition (/kg/d)		
	N (mg)	Nonprotein kcal	Fat (g)
1		30	
2	140	30	
3	210	45	1
4	280	60	2
5	430	90	3
8	430	90	3

N intakes (mg/kg/h)*		
Group A	Group B	
2	6.0 ± 0.6 (5.4–6.7)	5.7 ± 0.3 (5.0–6.1)
3	8.4 ± 1.2 (5.4–9.4)	9.2 ± 0.6 (8.7–10.6)
4	12.4 ± 2 (10.1–16.4)	11.8 ± 0.4 (11.0–12.5)
5	17.4 ± 0.8 (16.5–19.6)	17.3 ± 1.3 (15.1–19.7)

Energy intakes (kcal/kg/d)*		
1	31 ± 3.1 (27–33)	30 ± 3.2 (25–33)
2	32 ± 3.4 (26–40)	31 ± 4.5 (26–39)
3	49 ± 10.5 (36–69)	46 ± 3.5 (43–52)
4	58 ± 7 (49–65)	59 ± 3.3 (52–62)
5	83 ± 7.7 (66–92)	88 ± 6.3 (76–95)

* Mean ± SD (range).

of measurement of whole body turnover rates with [$1\text{-}^{13}\text{C}$]leucine have been described previously (17). Leucine enters the body free leucine pool from the diet (I) and from the degradation of whole body protein (D), whereas leucine exit is by protein synthesis (S) and leucine oxidation (O). It is assumed that the body pool of leucine remains constant in size during the infusion; this seems reasonable because TPN was infused at a constant rate and no infant was studied until at least 10 h after the last TPN infusion was changed. Hence,

$$Q = I + D = S + O \quad (1)$$

where Q is the total turnover rate of the free leucine pool, termed the leucine flux (17). Q is calculated from the isotopic enrichment of the free leucine in plasma after a plateau value (E_p , atom % excess) has been reached, using the mean plateau ^{13}C enrichment value. Baseline [$1\text{-}^{13}\text{C}$]leucine enrichment measurements were obtained daily, before the start of each infusion, and these were subtracted from the plateau value.

$$Q = i \times \left(\frac{E_i}{E_p} - 1 \right) \mu\text{mol/kg/h} \quad (2)$$

where i is the L-[$1\text{-}^{13}\text{C}$]leucine infusion rate ($\mu\text{mol/kg/h}$) and E_i is the enrichment of L-[$1\text{-}^{13}\text{C}$]leucine infused (atom % excess). The rate of inflow of leucine from body protein degradation (D) is then calculated from the flux and the amount of leucine infused per h in TPN using equation 1 and is expressed as μmol leucine/kg/h. In the absence of direct measurements of leucine oxidation by collection of expired $^{13}\text{CO}_2$, the rates of leucine oxidation (O) and incorporation of leucine by whole body protein synthesis were calculated as follows: the rate of N excretion was measured over the 12-h period and a value (N_u), corrected for changes in the size of the body urea pool, calculated as described previously (13). From the rate of N intake in TPN (N_i), the apparent rate of N retention (N_r) was calculated.

$$N_r = N_i - N_u \quad (3)$$

This was not corrected for insensible losses. The rate of leucine retention in body protein (leu_r) was then calculated on the assumption that leucine composes 8% of body protein [3.817 mmol leucine/g N (18)]; thus,

$$\text{Leu}_r = N_r \times 3.817 \quad (4)$$

Leucine oxidation (O) was calculated as leucine intake minus leucine retention, and synthesis (in units of $\mu\text{mol/kg/h}$ leucine) was calculated from

$$S = Q - O \quad (5)$$

Statistical analysis was performed by analysis of variance for repeated measures and t test for paired and grouped data with results expressed as mean ± SD.

RESULTS

There was no difference between the two groups of infants in gestational age or birth weight (Table 2), but there was a significant difference in postnatal age between the two groups at the start of the study (d 1) [37 ± 15 and 54 ± 18 h, groups A and B, respectively ($p = 0.013$)]. However, this difference was not apparent in any of the other measurements. On d 1, N excretion (5.8 ± 3.6 and 5.7 ± 2.3 mg/kg/h), rates of leucine incorporation into protein synthesis (129 ± 26.5 and 120 ± 36.3 $\mu\text{mol/kg/h}$), and leucine release by protein degradation (153 ± 33 and 142 ± 39 $\mu\text{mol/kg/h}$, for groups A and B, respectively) were not significantly different.

By 2 h of infusion, plasma leucine enrichments were at a plateau, as shown by mean values (atom % excess) of 4.5 ± 1 , 4.4 ± 0.9 , and 4.4 ± 1 for d 1 and 2.7 ± 0.4 , 2.6 ± 0.4 , and 2.6 ± 0.4 for d 5 at 2, 4, and 6 h, respectively. The mean baseline [$1\text{-}^{13}\text{C}$]leucine rose from zero on the first study day to 0.41 ± 0.12 atom % excess on study d 5. The mean coefficient of variation for the plateaus were $6.4 \pm 2.8\%$ and $5.2 \pm 3.3\%$, for d 1 and 5, respectively.

Data for individual infants in the two groups over the study period d 1–5 are shown in Tables 4–8, and the combined responses of all infants are illustrated in Figure 1. As indicated in the table footnotes, where there were missing data these were calculated from the values on other days using a regression equation, before analysis of variance. These did not compose more than 10% of the total number of values so that the demonstration of significance was not distorted by the derived values. So that this limit was not exceeded, infant no. 28 was excluded from the final analysis. However, this infant was included in the analysis of those who had successful studies on d 5 and 8. Taking all the infants together, N excretion remained almost steady, but net protein gain increased significantly throughout the study (Tables 4 and 5, $p < 0.001$). Whole body leucine flux (Fig. 1) increased in all infants throughout the study period ($p < 0.001$). Moreover, protein synthesis and breakdown rates (as indicated by leucine incorporation into, and release from, whole body protein, Fig. 1) increased progressively on each day ($p < 0.001$) as the amount of i.v. nutrition increased daily, from d 2 through d 5.

When the two groups were analyzed separately, there were significant increases in rates of flux and protein synthesis as the study progressed for both group A and B ($p < 0.001$; tables 6 and 7). The increase in protein breakdown (Table 8), although smaller, was significant for both groups ($p = 0.003$ and $p = 0.005$, groups A and B, respectively). N intake was the same for the two groups throughout the study, and each group showed a significant increase in net protein gain (Table 5) as nutritional intake increased ($p < 0.001$).

Comparison of the changes in rates of protein synthesis, breakdown, and net protein gain over the 5 study d showed no differences between groups A and B. The percentage of N intake that was retained (or the efficiency of N retention) was also similar ($60 \pm 16\%$ and $67 \pm 11\%$, groups A and B, respectively, on d 5). There was no correlation for rates of protein synthesis, breakdown, or N retention with gestational age, birth weight, or postnatal age.

Six infants were studied again on d 8 (infants no. 10–12, 20,

Table 4. *N* excretion (mg/kg/h) on each study day

Patient no.	Study day				
	1	2	3	4	5
Group A*					
9	8.5	7.6	11.7	4.2	6.4
10	6.5	5.7	6.1	5.2	4.7
11	4.1	9.1	4.7	7.6	8.8
12	4.4	7.1	4.9	3.4	7.4
13	4.5	3.8	3.8	5.4	9.7
22	1.3	2.2	1.2	0.3	1.1
25	1.8	2.0	1.3	5.3	9.4
27	6.3	7.8	7.5	5.3	6.7
28	8.7	14.6	9.9	12.7	10.7
30	6.5	8.0	6.7	3.6	5.1
34	14.0	16.7	14.5	5.5	6.9
Mean $\pm$ SD	5.8 $\pm$ 3.6	7.0 $\pm$ 4.2	6.2 $\pm$ 4.2	4.6 $\pm$ 1.9	6.6 $\pm$ 2.6
Group B					
8	5.1	2.2	4.9	4.9	4.7
17	4.1	4.1	1.6	5.4	5.0
18	3.9	5.0	4.3	4.2	4.2
20	7.3	5.3	6.3	4.0	4.2
21	2.7	6.3	6.3	8.3	8.4
23	4.6	3.7	8.1	7.6	8.7
29	5.8	6.6	7.1	4.5	6.4
32	9.9	5.0	2.2	2.3	3.1
35	7.9	4.3	6.8	6.2	6.8
Mean $\pm$ SD	5.7 $\pm$ 2.3	4.7 $\pm$ 1.3	5.3 $\pm$ 2.2	5.3 $\pm$ 1.9	5.7 $\pm$ 2

* The means for group A exclude infant no. 28 because this infant was not included in the 5-d analysis. The N data for no. 28 remain in the table because the infant was included in the analysis of study d 5 and 8 (see Results).

Table 5. *N* retention (mg/kg/h) on each study day*

Patient no.	Study day				
	1	2	3	4	5
Group A†					
9	-8.5	-1.5	-2.3	9.0	10.9
10	-6.5	0.4	2.4	6.7	12.5
11	-4.1	-3.0	4.2	4.5	8.5
12	-4.4	-1.2	4.4	8.6	9.1
13	-4.5	2.9	4.5	6.4	7.5
22	-1.3	3.5	7.5	11.1	16.5
25	-1.8	3.8	4.1	11.1	10.2
27	-6.3	-2.1	1.1	4.8	10.6
28	-8.7	-7.3	-1.9	-0.9	5.6
30	-6.5	-2.4	2.6	8.4	11.7
34	-14.0	-10.7	-5.6	5.7	10.6
Mean $\pm$ SD	-5.8 $\pm$ 3.6	-1.0 $\pm$ 4.2	2.3 $\pm$ 3.8	7.6 $\pm$ 2.4	10.8 $\pm$ 2.5
Group B					
8	-5.1	3.4	3.8	6.8	13.0
17	-4.1	1.8	7.3	6.3	13.2
18	-3.9	1.0	4.9	7.5	12.6
20	-7.3	0.3	2.7	7.0	13.0
21	-2.7	-0.3	2.8	3.5	9.2
23	-4.6	2.0	1.5	4.3	8.6
29	-5.8	-1.8	1.8	6.7	11.2
32	-9.9	0.4	8.4	10.0	12.2
35	-7.9	1.2	1.7	6.3	12.9
Mean $\pm$ SD	-5.7 $\pm$ 2.3	0.9 $\pm$ 1.5	3.9 $\pm$ 2.5	6.5 $\pm$ 1.9	11.8 $\pm$ 1.7

* Increase in net protein gain over study period, $p < 0.001$, for groups A and B separately. Change in rate of net protein gain not different between groups.

† The means for group A exclude infant no. 28 because this infant was not included in the 5-d analysis. The N data for no. 28 remain in the table because this infant was included in the analysis of study d 5 and 8 (see Results).

21, and 28), when nutritional intake had been maintained at a constant level since d 5, and the results were compared with those from d 5 (Table 9, Fig. 2). Protein synthesis increased significantly from 222 ± 20 μ mol/kg/h on d 5 to 258 ± 29 μ mol/

kg/h on d 8 (mean increase, 36 ± 33 μ mol/kg/h, $p = 0.04$, paired t test). Protein breakdown also increased from d 5 to d 8 [185 ± 28 and 219 ± 23 μ mol/kg/h, respectively ($p < 0.05$)], but net protein gain was unchanged at 9.6 ± 3.0 mg/kg/h on both days.

Table 6. Whole body leucine flux ($\mu\text{mol/kg/h}$) on study d 1-5*

Patient no.	Study day				
	1	2	3	4	5
Group A					
9	168	227	268	228	294
10	186	165	205	232	259
11	119	171	183	232	280
12	119	151	210	243†	276
13	119	143	175	234	279
22	168	171	178	206	264
25	135†	194	185	261	302
27	213†	223	291	278	310
30	142	216	264†	314	340
34	202	233†	251	320†	376
Mean $\pm$ SD	157 $\pm$ 36	189 $\pm$ 33	221 $\pm$ 43	255 $\pm$ 38	298 $\pm$ 36
Group B					
8	123	139	157	211	217
17	109	149	138	219	207
18	129	161	178	185	234
20	234	196	208	226	246
21	117	153	199	205	252
23	145	177	186	212	291
29	121	150†	181	204	239
32	139	153	168	255	327†
35	136	191	196†	195	260
Mean $\pm$ SD	139 $\pm$ 37	163 $\pm$ 20	179 $\pm$ 22	212 $\pm$ 20	253 $\pm$ 37

* Increase in whole body leucine flux over study period, $p < 0.001$, for groups A and B separately. Change in rate of flux over study period not different between groups.

† Missing values introduced for analysis of variance (see Results).

Table 7. Leucine incorporation into body protein ($\mu\text{mol/kg/h}$) on study d 1-5*

Patient no.	Study day				
	1	2	3	4	5
Group A					
9	135	186	206	187	236
10	161	131	166	190	208
11	103	124	148	179	213
12	102	113	173	207†	216
13	101	115	145	190	209
22	163	151	157	183	226
25	146†	175	170	209	228
27	179†	182	246	238	251
30	117	175	221†	277	288
34	149	173†	179	256†	315
Mean $\pm$ SD	136 $\pm$ 28	152 $\pm$ 29	181 $\pm$ 33	212 $\pm$ 34	239 $\pm$ 36
Group B					
8	104	128	134	187	190
17	94	131	128	192	179
18	114	139	157	164	210
20	206	173	179	205	221
21	107	126	170	167	211
23	127	160	150	177	249
29	99	123†	149	182	206
32	101	132	154	240	267†
35	105	172	166†	166	225
Mean $\pm$ SD	117 $\pm$ 35	143 $\pm$ 20	154 $\pm$ 16	187 $\pm$ 24	217 $\pm$ 27

* Increase in rates over study period, $p < 0.001$, for groups A and B separately. Change in rates over study period not different between groups.

† Missing values introduced for analysis of variance (see Results).

DISCUSSION

This study of low birth weight infants shows that, during the first 4 d of i.v. nutrition, the retention of protein passed from negative to positive (Fig. 1). This was accompanied by a progressive increase in rates of whole body protein synthesis, with a much smaller increase in protein degradation (Fig. 1). By d 5,

the mean value of protein synthesis for all the infants had risen to $224 \mu\text{mol/kg/h}$, which corresponds to 8.8 g/kg/d (Fig. 1). This value is the same as that reported previously for parenterally fed LBWN (9) and is about one third lower than that found in enterally fed premature infants (10, 19-21).

The method used in the present study was a modification of the original leucine infusion method (12) using urinary N meas-

Table 8. Leucine release from body protein ($\mu\text{mol/kg/h}$) on study d 1–5*

Patient no.	Study day				
	1	2	3	4	5
Group A					
9	168	192	214	152	194
10	175	130	157	158	160
11	119	135	132	162	180
12	119	117	156	168†	181
13	118	104	127	165	180
22	168	138	128	140	164
25	145†	163	154	167	190
27	205†	190	241	220	211
30	143	183	213†	244	243
34	202	207†	200	243†	274
Mean $\pm$ SD	156 $\pm$ 33	156 $\pm$ 36	172 $\pm$ 41	182 $\pm$ 39	198 $\pm$ 36
Group B					
8	124	115	120	161	140
17	109	123	100	168	128
18	129	135	138	135	162
20	234	172	169	179	171
21	117	127	159	154	176
23	145	153	145	160	216
29	122	133†	142	157	163
32	139	130	122	202	193†
35	124	167	152†	141	175
Mean $\pm$ SD	138 $\pm$ 37	139 $\pm$ 20	139 $\pm$ 21	162 $\pm$ 20	169 $\pm$ 26

* Increase in rates over study period, $p = 0.003$ and $p = 0.005$, groups A and B, respectively. Change in rates over study period not different between groups.

† Missing values introduced for analysis of variance (see Results).

Fig. 1. Changes in rates of protein synthesis, breakdown, and retention over study d 1–5 for group A and B infants together. *, $p < 0.001$ for increases in protein synthesis, breakdown, and retention throughout study.

urements to estimate oxidation. We have previously used this method to avoid $^{13}\text{CO}_2$ collection, which is difficult in LBWN (13). It has previously been shown that leucine oxidation correlates well with N excretion (19). The method of calculation requires that the contribution of leucine oxidation to N excretion is known, and this was taken to be 3.817 mmol leucine/g N (18, 22) when the infants were receiving i.v. glucose. However, when amino acid mixtures of differing composition were given on study d 2–5, the contribution of leucine to N excretion would be different from its contribution to either body protein or the amino acids given. Indeed, this factor would vary between infants, depending on the proportion of dietary N that was retained. The method of calculating protein synthesis and leucine oxidation via leucine retention (from N retention; see Materials and Methods) was adopted to avoid this problem, inasmuch as knowledge of the factor for the contribution of leucine to N excretion is not needed, only its contribution to body protein, which should be the same whatever the composition of the diet. Although we have no way of knowing if the value chosen for the proportion of leucine in whole body protein is appropriate for LBWN, its actual magnitude has little influence on the conclusions reached. When a value 20% higher was substituted for 3.817 mmol

Table 9. Rates of leucine incorporation into, and release from, body protein, and protein retention on study d 5 compared to study d 8*

Infant no.	Leucine incorporation ($\mu\text{mol/kg/h}$)		Leucine release ($\mu\text{mol/kg/h}$)		Protein retention (mg/kg/h)	
	Day 5	Day 8	Day 5	Day 8	Day 5	Day 8
10	208	267	160	215	12.5	13.1
11	213	216	180	200	8.5	4.2
12	216	276	181	221	9.1	9.8
20	221	226	171	192	13.0	9.8
21	211	288	176	256	9.2	8.4
28	262	274	240	227	5.6	12.3
Mean $\pm$ SD	222 $\pm$ 20	258 $\pm$ 29†	185 $\pm$ 28	218 $\pm$ 23†	9.6 $\pm$ 2.7	9.6 $\pm$ 3.2

* Maximum i.v. nutrition maintained from study d 5 through study d 8 ($n = 6$).

† Significantly different from d 5, $p < 0.05$.

Fig. 2. Rates of protein synthesis, breakdown, and retention on study d 5 compared to study d 8. Maximum i.v. nutrition maintained from study d 5 through study d 8. *, Significantly different from d 5, $p < 0.05$.

leucine/g N, the calculated rates of protein synthesis and leucine oxidation changed, but the differences between groups A and B remained the same.

There are several possible reasons for the progressive increase in protein synthesis rates, but we think that these changes reflect the progressive increase in dietary intake. Indeed, the results showing that increases in dietary intake were accompanied by increases in synthesis and much smaller increases in protein degradation are very similar to those reported by Golden *et al.* (23) in children recovering from malnutrition. Moreover, in growing pigs and steers, there are similar relationships between total intake and protein synthesis and degradation (24, 25), and in these studies increases with dietary intake could be separated from effects of increasing age by use of a randomized cross-over design. There was however, a further increase in protein synthesis in the six infants who were studied again after 3 d on maximum i.v. nutrition (study d 8, Table 9, Fig. 2), with a similar increase in breakdown, but there was no change in net protein gain. This increase in protein turnover may have been the result of other factors such as the improving clinical and nutritional status of the infants, which may also have contributed to the increases in protein turnover seen on d 1–5.

There was no apparent decline in the rates of increase of protein synthesis and retention at the higher intakes, suggesting that the infants were well able to tolerate the higher intakes of energy and protein on d 5. No measurements of energy expenditure were made to see whether the dietary energy intake was adequate. However, only 60% of the N given on d 5 and 8 was retained, making it unlikely that total N intake was insufficient. Indeed, greater N retention has been seen in parenterally fed LBWN receiving similar N and energy intakes (9). However, other factors, such as the severity of illness in the present patients, would influence N retention. Moreover, it was noted that the variability in N excretion (Table 4) coincided with episodes when the infants were judged clinically to be "sicker." It is notable that in the infants studied here, positive protein retention (*i.e.* growth) was in some cases accompanied by a loss in body weight. This is most likely to have resulted from changes in hydration (26).

Over the study period, there was no difference between the patterns of N retention, protein synthesis, and breakdown in the two groups, implying that there is no difference in efficacy between the two amino acid solutions. Dietary factors that might have limited protein utilization, other than the amino acid composition, were standardized for both groups so that any effect of the different constituent amino acids of the two solutions could be examined. The quantities of essential amino N in the two solutions are very similar, but the concentrations of the individual essential amino acids differed (Table 1). Specific amino acids, such as leucine, might have the capacity to enhance protein retention (27, 28). Moreover, the concentration of phenylalanine, lowered in Vamin Infant because of concern over

potentially neurotoxic levels found in the plasma of infants receiving Vamin 9 glucose (1–4), could have become limiting for protein retention. However, it seems that in practice the differences in amino acid composition between the two mixtures did not influence the retention or turnover of protein, with the result that no differences between infants given the two solutions were apparent.

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