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## Whole Body Protein Synthesis and Energy Expenditure in Very Low Birth Weight Infants

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**ABSTRACT.** The aim of the present work was to study whole body protein synthesis and breakdown, as well as energy metabolism, in very low birth weight premature infants (<1500 g) during their rapid growth phase. Ten very low birth weight infants were studied during their first and second months of life. They received a mean energy intake of 114 kcal/kg·day and 3 g protein/kg·day as breast milk or milk formula. The average weight gain was 15 g/kg·day. The apparent energy digestibility was 88%, i.e. 99 kcal/kg·day. Their resting postprandial energy expenditure was 58 kcal/kg·day, indicating that 41 kcal/kg·day was retained. The apparent protein digestibility was 89%, i.e. 2.65 g/kg·day. Their rate of protein oxidation was 0.88 g/kg·day so that protein retention was 1.76 g/kg·day. There was a linear relationship between N retention and N intake ( $r = 0.78$ ,  $p < 0.001$ ). The slope of the regression line indicates a net efficiency of N utilization of 67%. Estimates of body composition from the energy balance,

coupled with N balance method, showed that 25% of the gain was fat and 75% was lean tissue. Whole body protein synthesis and breakdown were determined using repeated oral administration of <sup>15</sup>N glycine for 60–72 h, and <sup>15</sup>N enrichment in urinary urea was measured. Protein synthesis averaged 11.2 g/kg·day and protein breakdown 9.4 g/kg·day. Muscular protein breakdown, as estimated by 3-methylhistidine excretion, contributed to 12% of the total protein breakdown. There was a positive correlation ( $r = 0.68$ ,  $p < 0.05$ ) between protein synthesis and protein gain, as well as between resting energy expenditure and protein gain ( $r = 0.58$ ,  $p < 0.01$ ). The slope of the regression line indicated that 1 g of protein gain required the synthesis of five times more protein (5 g) and resulted in an extra energy expenditure of 10 kcal. Thus, the net cost of protein synthesis in these very low birth weight infants was 2 kcal/g. (*Pediatr Res* 19: 679–687, 1985)

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### Abbreviations

3-MHis, 3-methylhistidine

Very low birth weight infants (birth weight < 1500 g) represent a veritable challenge to neonatal medicine and nutrition. In addition to common early medical complications (*i.e.* respiratory distress) very low birth weight infants present gastrointestinal immaturity and limited protein and energy reserves. In an attempt to achieve growth rate similar to that obtained in intra-uterine infants, very low birth weight infants are fed a substantial amount of food energy. The disposal of energy intake in these infants has been the object of a limited number of investigations (1, 2). These studies have shown that during the rapid growth period, the energy expenditure per kilogram of body weight is elevated, as compared to values published for full term infants (1-3). The question arises whether this high metabolic rate is due to the energy cost of growth, particularly the cost of protein synthesis.

Dynamic aspects of protein metabolism can be studied by using nonradioactive, stable  $^{15}\text{N}$  labeled amino acids as biological markers (4-7). Whole body protein synthesis and protein breakdown in very low birth weight infants reared either on human milk (5), various "humanized" cow milk (6, 7), or given total parenteral nutrition (4) were found to be elevated in all situations. Since protein synthesis is known to entail a considerable amount of energy expenditure for the formation of peptide bonds, it is of interest to determine whether there is a relationship between the rate of protein synthesis and the amount of energy expended by very low birth weight infants. This approach can also be used to obtain the energetic cost of protein synthesis in these infants.

#### MATERIALS AND METHODS

**Infants.** Ten very low birth weight infants admitted to the neonatal intensive care unit of the University Hospital were studied. Their birth weight ranged from 950-1420 g and their gestational age between 28-31 wk, confirmed by Dubowitz score (8). All were appropriate weight for gestational age and were free of any major medical problem at the time of the study. The clinical and growth characteristics of the infants are given in Table 1. The purpose and the procedures of the study were carefully explained to the parents and their agreement obtained. The experimental protocol was submitted to and accepted by the Ethical Committee of the University Hospital.

**Assessment of growth.** The following measurements were made during the first 8 wk of life. Weight was measured daily to the nearest 5 g. The weekly weight gain was calculated from the linear regression line of daily weight over time. This procedure was used to avoid imprecision in assessing weight gain due to day-to-day fluctuations in body weight. Weight gain at the time of each metabolic balance was assessed in the same way, the 3-day balance being situated in the middle of the week considered.

Crown-to-heel length was measured weekly using a stadiometer. Increments in crown-to-heel length over the whole period, expressed as centimeters gained per week, were calculated from the regression line of weekly length over time. Head circumference was measured weekly with a cloth tape, and increment was similarly calculated. A cerebral ultrasound (performed in eight infants) showed that all infants but two, identified in Table 1, were free from cerebral ventricular dilatation during the period of the study.

**Diet.** Four infants received their mother's milk: expressed breast milk was stored in a refrigerator and given to the infant over the following 48 h. The others were fed an infant formula (Alprena MCT, Nestlé Ltd, Vevey, Switzerland) prepared from whole milk, whey, medium-chain triglycerides, lactalbumin, and vegetable oils. Details of the diet composition are given in Table 2. During the 3-day balance,  $^{15}\text{N}$ -glycine was diluted in deionized water, the resulting solution was sterilized through a  $0.2\ \mu$  filter (Nalgene, Sybron, NY), and then was added to the daily feeding. The preparation was thoroughly mixed, an aliquot taken for analysis and the remainder distributed in equal portions corre-

Table 2. Diet

Infant	Type	Gross energy intake (kcal/kg·day)	Protein intake (g/kg·day)	Protein energy (%)
MS	EBM*	115	2.83	14
PG	EBM†	110	3.15	16
FM <sub>1</sub>	EBM†	112	3.21	16
FM <sub>2</sub>	EBM†	109	2.7	14
FM <sub>3</sub>	EBM†	106	2.39	13
YR <sub>1</sub>	EBM*	132	3.18	14
YR <sub>2</sub>	Milk formula	123	3.50	16
YR <sub>3</sub>	Milk formula‡	105	2.85	15
LH <sub>1</sub>	Milk formula	126	3.51	16
LH <sub>2</sub>	Milk formula	107	2.97	16
CV <sub>1</sub>	Milk formula§	86	1.61	10
CV <sub>2</sub>	Milk formula	120	3.3	15
JNN	Milk formula	123	3.67	17
AG	Milk formula	109	2.95	15
MC	Milk formula	122	3.45	16
MD	Milk formula	122	3.3	15
Mean ±		114	3.04	15
SD		11	0.51	1.7

\* EBM = expressed breast milk from their own mother supplemented with other mothers' milk and † from their own mother only.

‡ Received a formula (Nau) similar in protein and amino acid content.

§ With 10% glucose iv (*i.e.* + 21 kcal/kg·day glucose energy).

Table 1. Clinical and growth characteristics

Infant	Sex	Gestational age (wk)	Birth wt (g)	Wt gain* (g/kg·day)	Birth length (cm)	Length increment (cm/wk)	Head circumference	
							at birth (cm)	increments (cm/wk)
MS	F	28	950	20	37	1.07	24.5	1.11
PG	M	30	1250	13	38	0.98	26	0.95†
FM	M	30	1100	14	39	0.87	26	0.89
YR	M	30	1150	13	38	0.90	25.3	0.99
LH	F	31	1360	17	41	0.65	29.5	0.93
CV	F	30	1070	18	39	0.42	26	0.62
JNN	M	29	1420	14	40	0.89	28	1.05†
AG	M	28	1130	16	43	0.99	28	0.91
MC	F	31	1410	14	40.5	0.85	28.5	0.83
MD	F	30	1390	15	42	0.50	27.8	1.07
Mean ±		30	1223	15	39.8	0.81	27	0.94
SD		1	166	2	2	0.22	1.6	0.14

\* 1st wk postnatal excluded.

† Ultrasound examination showed a moderate ventricular dilatation, which arrested spontaneously.

sponding to the number of feeds. The amount of  $^{15}\text{N}$ -glycine (95% enrichment, CEA, Saclay, Gif-sur-Yvette, France) was calculated to provide about 5 mg  $^{15}\text{N}$  per kilogram per day. Every 24-h milk samples were analyzed for total nitrogen,  $^{15}\text{N}$ , and energy content.

**Metabolic balance techniques.** Energy and nitrogen balances and whole body protein turnover studies were performed in each infant. The metabolic balance was initiated during the period of weight gain only, and as soon as the infant's respiratory status was stable. Furthermore the balance was performed after at least 3 days adaptation to the diet. The balance lasted between 60 to 72 h and energy expenditure was measured on the last 24 h. The infants were nursed in incubators and were fed at 2 or 3 hourly intervals via a nasogastric tube. The volume ingested was measured using a 10-ml syringe, and was recorded. Residual milk in the syringe was repeatedly measured and found to be much less than 0.5 ml and was therefore neglected. When the nasogastric tube had to be changed, the residual milk it contained was subtracted from the amount given. Any regurgitation (subject AG) was collected to a cellulose paper placed between the infant and the bedding. Urine was collected by continuous aspiration from an adhesive plastic bag into a chilled cylinder (9) which was changed before each meal, *i.e.* every 2–3 h. Using this technique, it was possible to study the infants in their usual prone position.

**Analytical techniques.** Stools were scrapped from the cellulose paper, homogenized with mortar and pestle, and lyophilized. Cellulose paper containing residual feces were dried in a vacuum oven at 40° C, then weighed, and the amount of dry residual feces were taken as the difference between the dry dirty and the dry clean papers. Analyses performed on five studies have shown little difference between the energy density of residual fecal content and that of the scraped feces. Therefore the total amount of nitrogen and fecal energy was calculated from the concentrations found in scraped homogenized feces. Nitrogen content of milk, stools, and urine were determined by the Kjeldahl method. Protein content of milk was calculated by multiplying total nitrogen by 6.38. Milk powder, samples of breast milk, and stools were burned in an adiabatic bomb calorimeter (PARR, model 1241, Moline, IL) to determine their gross energy content. Urinary energy loss was calculated from total urinary nitrogen (g)  $\times$  7.9 kcal/g N or 1.24 kcal/g oxidized protein (10). A constant percentage of each 2- or 3-h urine sample was taken to reconstitute a 24-h urine pool. The latter was analyzed for total nitrogen, urea, ammonia, creatinine, and amino acids. Creatinine was determined by the Jaffé reaction in an Autoanalyzer (Technicon, Tarry Town, NY). In order to determine 3-methylhistidine excretion, urinary amino acids were analyzed by liquid phase chromatography (Biotronik, Frankfurt, BRD). Urea was titrated with urease in an automatic analyzer (Greiner, Langenthal, Switzerland) and ammonia by the Berthelot method (Roche Diagnostica, Basel, Switzerland). In each fractional urine sample, ammonia was separated by 1-h aeration after urine alkalinization with 12.5 N NaOH, and trapped in 0.01 N HCl. Then the sample was further brought to a pH equal to 6.1, urease (Bittmann, 5 U/mg) was added and incubated for 2 h at 30° C, and after realkalinization ammonia was again trapped in 0.01 N HCl. The  $^{15}\text{N}$  enrichment of urea and ammonia was subsequently determined automatically by emission spectrometry (Isonitromat 5201 VEB, Statron, DDR) from the ammonium chloride salt. The  $^{15}\text{N}$  enrichment in stools was determined in the same way from ammonium chloride resulting from Kjeldahl digestion. The net  $^{15}\text{N}$  enrichment was calculated as the difference between the total  $^{15}\text{N}$  enrichment minus the natural abundance of  $^{15}\text{N}$ , determined to be 0.37 atoms %.

**Measurement of energy expenditure.** Total daily energy expenditure was measured by open circuit calorimetry as previously described (1). A tight perspex box containing the whole infant was placed inside the incubator. During the measurement, the infant's thermoneutrality was continuously monitored via a ther-

mistance placed on the abdominal skin. Activity was checked every minute using Brück's activity scale (11). The latter varies from a -4 level (eyes closed, no movement) to a +5 level (crying, eyes open or closed). Total energy expenditure was taken as the mean of the whole period of measurement whereas energy expenditure in resting state was assessed when the child had an activity score of -4 or -3 (eyes closed, facial movements). The measurements were taken after completion of the last meal over a period of 1 to 3 h (mean 1 h 35 min). Our previous study (1) has shown that the pattern of energy expenditure in very low birth weight infants fed at constant intermittent intervals is stable (range of intraindividual coefficient of variation = 3.5–6.6%) so that the measurements may be extrapolated to 24 h with relatively little error.

**Composition of weight gain.** Assuming that energy retained is mainly stored in the form of lipids and proteins and knowing that the gross energy content of protein is 5.6 kcal/g, an estimation of the composition of weight gain was obtained as follows:

$$\text{energy stored in lipids} = \text{energy balance} - (\text{protein retained} \times 5.6)$$

where energy balance and energy stored are in kcal/kg·day and protein retained in g/kg·day.

Knowing that the energy density of lipids is 9.3 kcal/g an estimate of the quantity of lipids stored (g/kg·d) was obtained as follows:

$$\text{quantity of lipids stored} = \frac{\text{energy stored in lipids}}{9.3}$$

**Protein turnover.** Whole-body protein synthesis and breakdown were estimated by the method of Picou and Taylor-Roberts (12), as subsequently modified by Steffee *et al.* (13) using  $^{15}\text{N}$ -glycine.

The assumptions involved with this method have been discussed previously (12, 14). Three methods were used to estimate the enrichment in  $^{15}\text{N}$  and products at plateau: 1) simple visual inspection, 2) exponential curve fit, and 3) polynomial curve fit.

Using visual inspection, the starting point of the plateau was taken as the first value which is followed at some time later by a lower one (14) and the plateau was calculated from the arithmetic means of this point and the following ones. The second approach assumes that the time course of the labeling follows a single exponential of the form  $y = y_0(1 - e^{-\lambda t})$  (13). The third approach assumes that the response follows a second degree polynomial regression and the plateau is defined when the function attains its maximum.

**Muscle protein breakdown.** Muscle protein breakdown was calculated from the measurement of 24-h urinary excretion of 3-MHis, using the values of Lunyong and Friedman (15) for the 3-MHis content of skeletal muscle in premature infants between 26 and 38 wk gestation.

$$\text{muscle protein breakdown} = \frac{\text{3-MHis excretion}}{\text{skeletal muscle 3-MHis content}}$$

Lunyong and Friedman (15) found a 3MHis content of 2.64  $\mu\text{mol/g}$  protein at 26–32 wk and of 3.28  $\mu\text{mol/g}$  protein at 37–38 wk of gestation. Assuming that the increment in 3-MHis content is essentially linear, the average change in 3-MHis per week gestation was calculated from a linear interpolation between these values.

## RESULTS

**Composition and apparent digestibility of the diet.** Details of the diet composition and of the energy and protein digestibility are given in Tables 2 and 3, respectively. The protein energy to total energy ratio averaged 15% and was not different in infants receiving breast milk compared with those receiving formula milk. Mean gross energy intake was 114 kcal/kg·day and the apparent energy digestibility was 88%. Mean nitrogen intake was

Table 3. Apparent digestible energy and protein

Infant	Gross energy intake (kcal/kg·day)	Fecal energy (kcal/kg·day)	Apparent energy digestibility (%)	Gross N intake (g/kg·day)	Fecal N (g/kg·day)	Apparent N digestibility (%)
MS	115	26	77	0.444	0.140	69
PG	110			0.494		
FM <sub>1</sub>	112	28	75	0.503	0.058	89
FM <sub>2</sub>	109			0.424		
FM <sub>3</sub>	106	11	90	0.375	0.043	89
YR <sub>1</sub>	132	26	80	0.498	0.051	90
YR <sub>2</sub>	123	17	87	0.548	0.042	92
YR <sub>3</sub>	105			0.447		
LH <sub>1</sub>	126	19	85	0.551	0.061	89
LH <sub>2</sub>	107	5	95	0.466	0.038	92
CV <sub>1</sub>	86	2	96	0.253	0.011	96
CV <sub>2</sub>	120	11	91	0.520	0.050	90
JNN	123	13	90	0.576	0.070	88
AG	109	8	93	0.463	0.052	89
MC	122	4	96	0.540	0.025	95
MD	122			0.521		
Mean ±	114	14	88	0.476	0.053	89
SD	11.1	9.0	7	0.080	0.032	7

Table 4. Energy and nitrogen balance

Infant	Age at study (days)	Wt at study (g)	Wt gain (g/kg·day)	Metabolizable energy* (kcal/kg·day)	Energy expenditure (kcal/kg·day)	Energy balance (kcal/kg·day)	N absorbed (g/kg·day)	Urinary N (g/kg·day)	N balance* (g/kg·day)
MS	26	1310	15.7	88	55	33	0.304	0.111	0.193
PG	6	1110	12.8	96†			0.440‡	0.164	0.276
FM <sub>1</sub>	11	1080	9.3	83	58	25	0.460	0.093	0.352
FM <sub>2</sub>	24	1340	12.3	96†	52	44	0.377‡	0.090	0.287
FM <sub>3</sub>	46	1820	14.7	95	59	36	0.332	0.091	0.241
YR <sub>1</sub>	19	1250	11.9	105	53	52	0.447	0.098	0.349
YR <sub>2</sub>	40	1720	17.0	105	59	46	0.506	0.194	0.312
YR <sub>3</sub>	62	2390	16.6	92†	61	31	0.398‡	0.106	0.292
LH <sub>1</sub>	8	1240	18.4	107	70	37	0.490	0.116	0.374
LH <sub>2</sub>	30	1810	14.4	101	61	40	0.428	0.186	0.242
CV <sub>1</sub>	4	1020	7.2	83	43	40	0.242	0.125	0.117
CV <sub>2</sub>	15	1200	20.8	107	62	45	0.470	0.164	0.306
JNN	39	2140	22.3	109	68	41	0.506	0.174	0.332
AG	51	2230	16.5	100	58	42	0.411	0.117	0.294
MC	21	1680	11.5	116	53	63	0.515	0.268	0.247
MD	20	1430	19.7	107†			0.464‡	0.090	0.374
Mean ±	26	1548	15	99	58	41	0.424	0.137	0.287
SD	17	108	4	9.6	6.8	9.3	0.078	0.050	0.068

\* Metabolizable energy = gross energy intake - (fecal energy + urinary energy); N Balance = gross N intake - (fecal N + urinary N) = N absorbed - urinary N.

† Assuming 12% energy loss in feces.

‡ Assuming 11% loss in feces.

0.476 g/kg·day and the apparent nitrogen digestibility was 89%.

**Energy balance (Table 4).** Metabolizable energy was calculated from gross energy of the diet minus fecal and urinary energy losses. The mean metabolizable energy was 99 kcal/kg·day, giving a mean positive energy balance of 41 kcal/kg·day. No relationship was found between gross energy intake and energy balance, whereas a significant positive relationship ( $r = 0.75$ ,  $p < 0.01$ ) was found between metabolizable energy intake and energy balance (Fig. 1). The slope of the regression indicates that approximately 70% of metabolizable energy given above maintenance requirement is stored and the remaining 30% is oxidized. The intercept at zero energy balance (not shown in Fig. 1) indicates that 40 kcal/kg·day of metabolizable energy are required to maintain energy balance.

Mean resting energy expenditure ( $y$ , in kcal/kg·day) augmented with increasing weight gain ( $x$ , in g/kg·day) and the regression analysis equation is  $y = 39.1 + 1.2x$  ( $r = 0.81$ ;  $n = 14$ ;  $p < 0.001$ ). At zero weight gain resting energy expenditure is approximately 40 kcal/kg·day. The slope of the regression line shows that 1.2 kcal are expended for each additional gram of weight gain; thus for infants growing at a rate of 15 g/kg·day and having a resting energy expenditure of 57 kcal/kg·day, the cost of new tissue synthesis is 18 kcal or 32% of their daily resting energy expenditure.

**Nitrogen balance (Table 4).** Since obligatory nitrogen losses from skin and sweat were not measured, "crude" nitrogen balance only is presented. Urinary nitrogen loss represented about one-third of nitrogen apparently absorbed, and the average nitro-

gen balance was 0.287 g/kg·day. Figure 2 shows the positive, statistically significant relationship ( $r = 0.78$ ;  $p < 0.001$ ) between nitrogen intake and nitrogen balance. The linear regression line extrapolated to zero nitrogen intake (not shown in Fig. 2) gives a value of  $-0.032$  g/kg·day which is an estimate of apparent

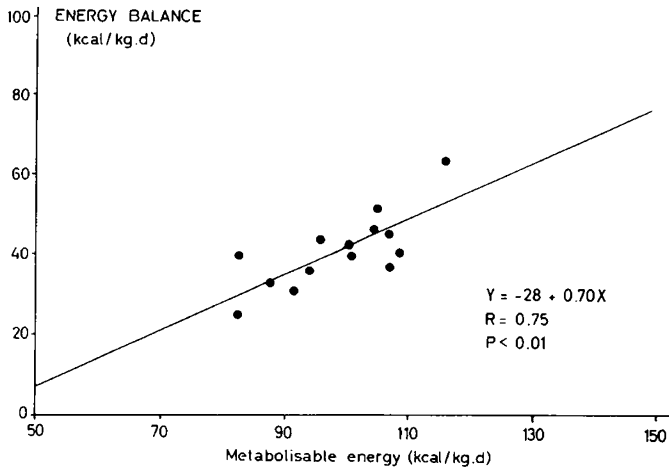


Fig. 1. Relationship between metabolizable energy intake and energy balance.

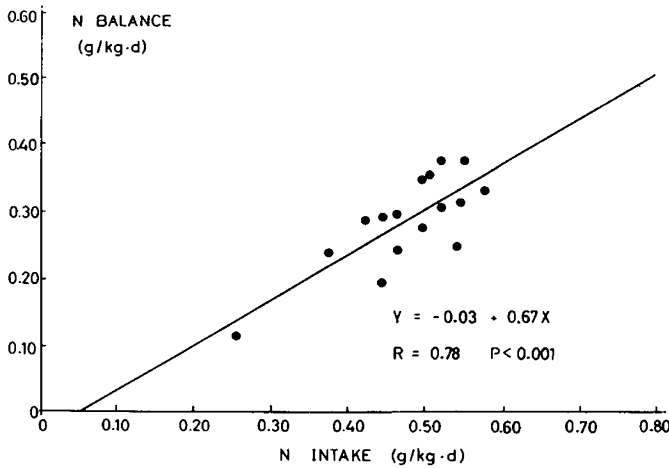


Fig. 2. Relationship between nitrogen intake and nitrogen balance.

obligatory nitrogen losses. At zero N balance ( $y = 0$ ), an estimate of 0.048 g/kg·day was found for the nitrogen intake required to maintain constant body N. The slope of the regression line, *i.e.* the coefficient of net protein utilization, was 0.67.

*Weight gain and estimate of the composition of weight gain* (Table 5). The average weight gain during the study was 15 g/kg·day. The mean lipid gain calculated from energy and N balances was 3.4 g/kg·day, thus giving a mean fat free mass gain of 11.6 g/kg·day; *i.e.* 75% of weight gain. The mean ratio of protein gain to fat free mass gain was 17%.

*Total body protein turnover.* Urinary ammonia showed  $^{15}\text{N}$  enrichment in all studies, but in five studies urea failed to be enriched at all. Using the visual approach, ammonia and urea plateau were reached by 30 to 54 h (mean 36 h for ammonia and 41 h for urea, Fig. 3). Nevertheless in half of the cases a pseudo-plateau was observed with ammonia before 30 h. No matter which method was used to assess the plateau, protein synthesis obtained from ammonia enrichment was significantly lower ( $p < 0.001$ ) than that calculated from urea enrichment.

Mean protein synthesis ( $\pm$ SD) calculated by these three methods is:

- 1) Using ammonia as end-product: visual method :  $6.24 \pm 1.61$  g/kg·day; exponential method :  $6.03 \pm 1.70$  g/kg·day; polynomial method :  $5.83 \pm 1.51$  g/kg·day. No significant

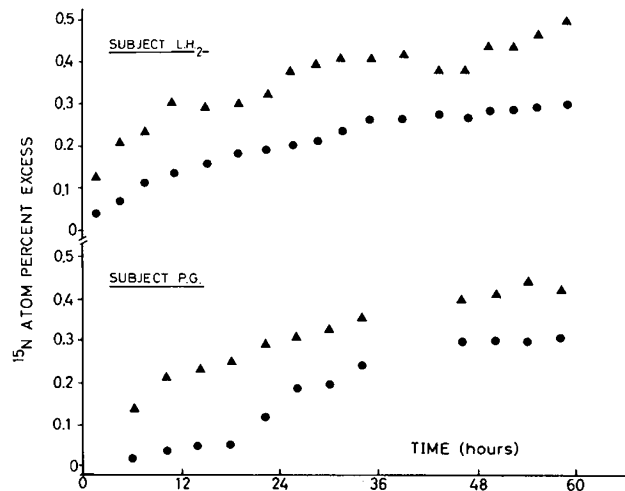


Fig. 3. Time-course of  $^{15}\text{N}$ -enrichment in urea (●) and ammonia (▲) following administration of  $^{15}\text{N}$ -glycine in two infants.

Table 5. Composition of wt gain

Infant	Wt gain (g/kg·day)	Protein gain (g/kg·day)	Energy stored in protein (kcal/kg·day)	Energy stored in lipid (kcal/kg·day)	Lipid gain (g/kg·day)	Fat free mass (g/kg·day)	Fat free mass (%)	Protein
								Fat free mass (%)
MS	15.7	1.21	6.8	26.2	2.8	12.9	82	9
FM	9.3	2.2	12.3	12.7	1.4	7.9	85	28
FM	12.3	1.79	10.0	34	3.7	8.6	70	21
FM	14.7	1.51	8.5	27.5	3.0	11.7	80	13
YR	11.9	2.18	12.2	39.8	4.3	7.6	64	29
YR	17.0	1.95	10.9	35.1	3.8	13.2	78	15
YR	16.6	1.83	10.3	20.7	2.2	14.4	87	13
LH	18.4	2.34	13.1	23.9	2.6	15.8	86	15
LH	14.4	1.51	8.5	31.5	3.4	11	76	14
CV	7.2	0.73	4.1	35.9	3.9	3.3	46	22
CV	20.8	1.91	10.7	34.3	3.7	17.1	82	11
JNN	22.3	2.08	11.6	29.4	3.2	19.1	85	11
AG	16.5	1.84	10.3	31.7	3.4	13.1	79	14
MC	11.5	1.54	8.6	54.4	5.8	5.7	49	27
Mean $\pm$	15	1.76	9.85	31.2	3.4	11.5	75	17.3
SD	4.2	0.43	2.40	9.7	1.0	4.5	13.2	6.8

difference in protein synthesis was found among the exponential, the visual, or polynomial methods. However, the polynomial *versus* the visual method gave significantly different results ( $p < 0.001$ ). 2) Using urea as end-product: visual method : :  $12.6 \pm 4.86$  g/kg·day; exponential method : :  $8.63 \pm 2.32$  g/kg·day; polynomial method : :  $11.2 \pm 3.55$  g/kg·day. There was a significant difference between the exponential *versus* the polynomial method ( $p < 0.005$ ), as well as between the exponential and polynomial *versus* the visual ( $p < 0.001$  and  $< 0.05$ , respectively).

However, there was a direct relationship between protein synthesis calculated from visual and exponential method ( $p < 0.001$ ), as well as between the visual and polynomial method ( $p < 0.001$ ). As suggested by Waterlow *et al.* (14), we used the visual method as reference and compared the two other methods against it: the regression line that was the nearest to the identity line (*i.e.* slope = 1) and had the highest correlation coefficient was the polynomial method. The equations giving protein synthesis calculated by the visual approach (y) from that calculated by the polynomial approach (x) are: Urea:  $y = 0.18 + 1.065 x$ ;  $r = 0.962$ ;  $n = 10$ . Ammonia:  $y = 0.008 + 1.077 x$ ;  $r = 0.976$ ;  $n = 14$ . Therefore for reasons outlined in the discussion, protein synthesis and breakdown presented in this report are based on the polynomial method (Table 6).

Protein synthesis calculated using any of the end-products was significantly correlated with protein gain. Figure 4 gives the linear correlation between protein synthesis calculated from enrichment in urea and protein gain. The slope of the regression indicates that for each gram of protein gained there is an increase in protein synthesis of about 5 g (accompanied by a simultaneous breakdown of 4 g).

*Relationship between energy expenditure, protein gain, and protein synthesis.* Figure 5 shows the relationship between resting energy expenditure and net protein gain ( $r = 0.65$ ,  $p < 0.01$ ). From the slope of the regression line one can infer that for each gram of protein gain, there is a simultaneous increase of approximately 10 kcal of energy expenditure. The intercept at zero protein gain indicates that about 40 kcal/kg·day are expended when nitrogen balance is equilibrated and this corresponds to

the estimate of metabolizable energy which was necessary to maintain zero energy balance (Fig. 1). There was a positive relationship between resting energy expenditure and protein synthesis but the relationship was on the borderline of statistical significance ( $r = 0.56$ ;  $n = 9$ ;  $p < 0.07$ ), so that the energy cost of protein synthesis cannot be calculated directly. However, as 5 g of protein are synthesized for each gram of protein gain (Fig. 4), with an extra energy expenditure of 10 kcal, an indirect estimate of the cost of protein synthesis would be 2 kcal/g synthesized.

*Muscle protein breakdown* (Table 7). Mean muscle protein breakdown was found to be about 1 g/kg·day, a value representing 13% of total protein breakdown.

## DISCUSSION

*Clinical course and growth.* The infants grew without clinical complications and their average weight gain, length, and head

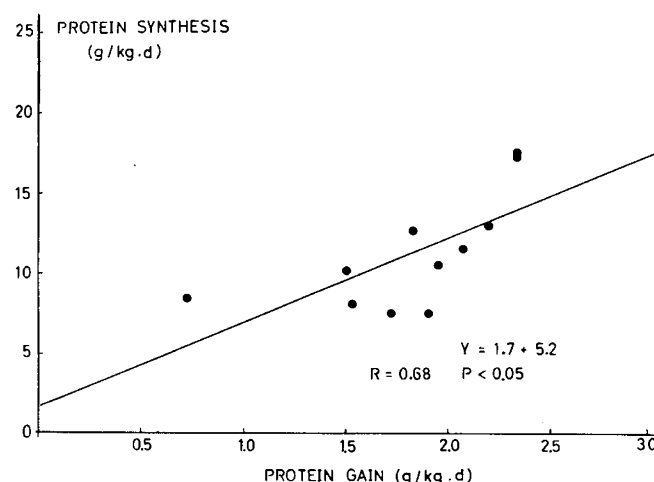


Fig. 4. Relationship between protein gain and protein synthesis.

Table 6. Total body nitrogen turnover

Infant	Urea					NH <sub>3</sub>				
	N flux Q̇ (g/kg·day)	Protein synthesis* (g/kg·day)	Protein breakdown* (g/kg·day)	Ṡ/Q̇ (%)	Ḃ/Q̇ (%)	N flux Q̇ (g/kg·day)	Protein synthesis* (g/kg·day)	Protein breakdown* (g/kg·day)	Ṡ/Q̇ (%)	Ḃ/Q̇ (%)
MS	†	†	†	†	†	0.80	4.3	3.1	86	62
PG	1.35	7.4	5.7	88	68	1.02	5.3	3.6	84	57
FM <sub>1</sub>	2.16	12.9	10.7	96	79	1.08	6.2	4.0	91	59
FM <sub>2</sub>	†	†	†	†	†	1.56	9.2	7.4	94	76
FM <sub>3</sub>	†	†	†	†	†	0.69	3.8	2.3	87	52
YR <sub>1</sub>	†	†	†	†	†	1.30	7.5	5.3	92	66
YR <sub>2</sub>	1.86	10.4	8.4	90	73	1.06	5.4	3.5	82	52
YR <sub>3</sub>	†	†	†	†	†	0.73	3.9	2.1	85	45
LH <sub>1</sub>	2.91	17.4	15.1	96	83	1.26	7.1	4.8	91	61
LH <sub>2</sub>	1.81	10.2	8.7	90	76	1.17	6.1	4.6	84	63
CV <sub>1</sub>	1.47	8.4	7.7	92	84	0.78	4.1	3.4	84	68
CV <sub>2</sub>	1.36	7.4	5.5	88	65	1.04	5.5	3.6	84	55
JNN	2.01	11.5	9.4	91	75	1.20	6.4	4.3	85	58
AG	2.15	12.7	10.9	95	81	1.01	5.6	3.7	88	59
MC	1.58	8.2	6.6	83	69	‡	‡	‡	‡	‡
MD	2.83	17.1	14.8	97	84	1.25	7.2	4.9	93	68
Mean ±	1.95	11.2	9.4	91	76	1.06	5.8	4.0	87	60
SD	0.54	3.5	3.3	4	7	0.24	1.5	1.3	4	8

\* (N × 6.25).

† No enrichment in urea.

‡ No data.

circumference were the same as these found by Largo *et al.* (16) and other authors (3, 17) for very low birth weight infants receiving a similar type of diet.

**Digestibility of the diet.** Throughout the study, all infants tolerated their diet well. The mean apparent energy and nitrogen digestibility (Table 3) are both similar to that obtained in very low birth weight infants fed their own mother's milk or an adapted infant formula (18, 19). However, the apparent digestibility appears to be greater than that observed in premature infants fed pooled breast milk (18) or given a very high amount of energy intake (20). In the present study no attempt was made to compare breast-fed and formula-fed infants because of the small number of infants in the breast-fed group, and because some infants were repeatedly studied in each group.

**Energy balance.** The fact that energy balance correlated well with metabolizable energy, but not with gross energy intake, demonstrates that differences in energy digestibility play an important role in the energy balance of these infants. Seventy percent of metabolizable energy given above maintenance requirement is stored, a value nearly as high as that calculated

from the results of Spady *et al.* (21) in children recovering from malnutrition (80%). At zero energy balance, the intercept of the regression line shows that maintenance energy is about 40 kcal/kg·day; this estimate is similar to the estimation of energy expenditure at zero weight gain (the extrapolation of the regression line between weight gain and energy expenditure) and to the energy expenditure of nongrowing very low birth weight infants previously studied under similar experimental conditions (1). It does not differ substantially from the amount of metabolizable energy which permits term infants to maintain an equilibrated energy balance (22).

**Nitrogen balance.** The relationship between nitrogen intake and nitrogen balance found in the present study (Fig. 2) indicates a maintenance nitrogen requirement of 48 mg/kg·day. However, this estimate does not include the insensible nitrogen losses that were not measured in the present study. Pencharz *et al.* (6) calculated that insensible nitrogen loss is 15.2 mg/kg·day in premature infants. Using the value given by these investigators, the corrected maintenance nitrogen requirement was found to be 70 mg/kg·day, a value close to that found by Jackson *et al.* (5). The net efficiency of protein utilization (67%) is also similar to those reported by these authors. The infants' birth weights were between the 10th and the 50th percentile of Lubchenko standards (23) and the daily nitrogen retention of infants receiving around 500 mg N/kg·day was close to the nitrogen retention calculated by Jackson *et al.* (5) for fetuses of the same gestational age growing along the 50th percentile. According to Figure 2, a daily intake of 500–550 mg/kg·day of nitrogen (3.2–3.5 g of protein) would be sufficient to allow nitrogen retention at the same rate as that occurring *in utero*.

**Composition of weight gain.** Data of Widdowson (24) indicate that fetuses of the same weight and gestational age as in the present study would have the following composition of weight gain: fat, 18%; protein, 13%; fat free mass, 82%; and the ratio of protein to fat free mass would be about 16%. The fat free mass gain of our infants, expressed as a fraction of weight gain, was 75% (range 46–87%), thus slightly less than that occurring *in utero*. The mean ratio of protein to fat free mass of 16% (range 9–27%) found in the present study is similar to that given by Widdowson (24). The large variability in the amount of fat free mass and of the percentage of protein in fat free mass explain

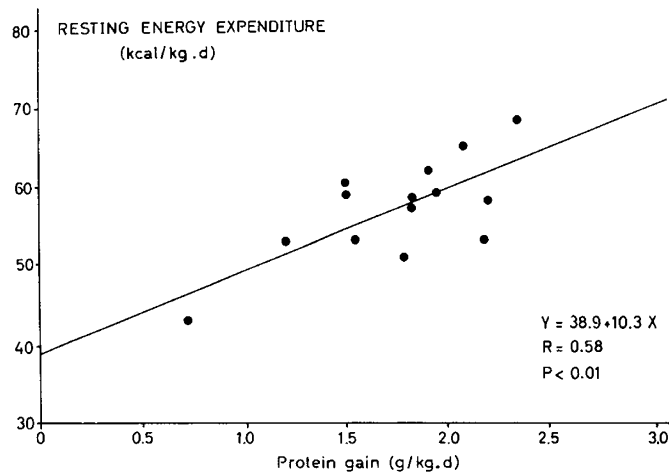


Fig. 5. Relationship between resting energy expenditure and protein gain.

Table 7. Skeletal muscle breakdown

Infant	3-mHis excretion (μmol/kg·day)	Muscle protein breakdown (g/kg·day)	Total protein* breakdown (g/kg·day)	Muscle protein breakdown	
				Total protein breakdown (%)	Creatinine excretion (mg/kg·day)
MS	3.1	1.1	*	*	10.33
PG	3.4	1.3	5.7	21	8.94
FM <sub>1</sub>	2.3	0.8	10.7	7	10.65
FM <sub>2</sub>	2.9	1.0	*	*	10.61
FM <sub>3</sub>	2.9	0.9	*	*	13.00
YR <sub>1</sub>	2.4	0.8	*	*	9.53
YR <sub>2</sub>	2.7	0.9	8.4	10	11.13
YR <sub>3</sub>	1.8	0.6	*	*	11.05
LH <sub>1</sub>	2.4	0.9	15.1	6	10.12
LH <sub>2</sub>	3.6	1.2	8.7	13	12.76
CV <sub>1</sub>	4.3	1.6	7.7	20	13.16
CV <sub>2</sub>	3.3	1.1	5.5	21	14.67
JNN	3.4	1.1	9.4	12	9.0
AG	2.7	0.9	10.9	8	10.36
MC	2.4	0.8	6.6	12	11.79
MD	2.9	1.0	14.8	7	16.36
Mean ±	2.9	1.0	9.4	12	11.47
SD	0.61	0.24	3.3	6	2.06

\* (N × 6.25).

why it is fallacious to attempt to predict protein (or lean tissue) gain simply from weight gain in these infants.

The infants of the present study had a smaller fat retention than that found by Reichman *et al.* (19). The infants of the latter study were gaining 5.4 g/kg·day of fat, or 32% of their daily weight gain, versus 3.4 g/kg·day, or 25% in the present study, but they were also receiving a higher metabolizable energy: 130 versus 99 kcal/kg·day in the present study. Further the method used to assess fat retention was not the same. However, the fat retention of both studies still appears to be greater than that occurring in the fetus, and the possible reasons and consequences for this have been discussed previously (19).

**Protein turnover.** Some of the difficulties encountered when assessing the plateau of either urea or ammonia enrichment are illustrated by the examples shown in Figure 3. Using visual inspection, it was difficult in some cases to determine the plateau (*e.g.* infant LH). The exponential fit sometimes gives unreliable values; in infant PG the plateau predicted would be more than three times higher than the visual plateau. Waterlow *et al.* (14) have already discussed these issues in detail and concluded that the visual inspection was the best approach. Assessing the plateau with a second degree polynomial regression has no theoretical background (in contrast to the exponential method), but the estimates proved to be closer to the visual plateau than that computed with the exponential method. Since the second degree polynomial regression provides a more reproducible assessment than the visual inspection, the former approach was used in this study.

When considering whole body protein synthesis, there is still some debate about which of the precursor pools of ammonia or urea represents the best precursor pool for protein synthesis. Some experimental evidence suggests that in addition to glutamine, glycine participates to a greater extent to ammonia formation than the other amino acids (25). This could explain, at least partially, why protein synthesis calculated from ammonia enrichment is 50% lower than that calculated from urea enrichment. Furthermore, unless the tracer is given with the diet at least at hourly intervals, there may be large fluctuations in ammonia labeling (26) and these fluctuations lead to substantial difficulties in the determination of true plateau. Therefore, the ammonia enrichment method for assessing protein synthesis needs further evaluation; the discussion of the results of this study will be limited to those obtained from urea enrichment.

It should be noted that in five of our studies, urea failed to be enriched; this is in agreement with the results of Jackson *et al.* (5) who studied very low birth weight infants fed pooled breast milk. Considering the fact that very low birth weight infants did not retain nitrogen as efficiently as *in utero*, Jackson *et al.* (5) postulated that glycine availability might be insufficient to meet the high needs for growth. We observed that infants in whom urea failed to be enriched were among those receiving the smallest amount of nitrogen (*i.e.* < 500 mg N/kg·day). The total amount of glycine received by the infants (tracer included) ranged from 53 mg/kg·day (infant CV<sub>1</sub>) to 97 mg/kg·day in formula-fed infants. The infant YR<sub>3</sub>, in whom urea failed to be enriched, received 80 mg/kg·day of glycine and 447 mg N/kg·day. In this infant, glycine availability does not explain the absence of <sup>15</sup>N enrichment in urea; further studies are needed to explain this phenomenon.

With the <sup>15</sup>N urea enrichment method, the mean rate of protein synthesis was 11.2 g/kg·day, a value similar to those found in very low birth weight infants by other authors (4–5, 7). The relationship between protein synthesis and protein gain (Fig. 4) shows that for each additional gram of protein gained, there is a large increase in protein synthesis, *i.e.* approximately 5 g. The extrapolation of the line to zero protein synthesis, however, may not be valid since the range of measurements was not extended to values below 0.7 g of protein gain. For comparison, infants recovering from protein-energy malnutrition, ingesting

various levels of energy (60–270 kcal/kg·day and 9.2% protein energy), exhibited a mean protein synthesis rate of 7 g/kg·day (27), and an increase of 1.4 g of synthesis for each gram of protein gained; these values are substantially lower than our respective estimates. The lower “efficiency” of protein gain (*i.e.* the amount of protein gained per gram of protein synthesis) in very low birth weight infants, as compared to infants recovering from protein-energy deprivation, remains to be investigated.

Muscle protein breakdown accounted for 13% of total protein breakdown. Duffy *et al.* (4) found a similar percentage in very low birth weight infants receiving total parenteral nutrition during the first week of life. If we assume that the creatinine excretion rate in these infants reflects the same amount of muscle protein in the body as found in older children (28) (*i.e.* 1 g of muscle protein is associated with a creatinine excretion of 0.25 mg/day), the amount of muscle protein of these very low birth weight infants is about 45 g/kg (calculated from Table 7), and their total amount of body protein is approximately 100 g (24). Thus the contribution of muscle to total protein breakdown (13%) is much less than the proportion of muscle protein to total body protein (45%), indicating that the rapid protein turnover is mainly due to other rapidly growing organs, such as the brain, liver, and viscera.

**Relationship between energy expenditure, protein gain, and protein synthesis.** In these rapidly growing infants, the total cost of growth (*i.e.* the energy retained plus the energy cost of new tissue synthesis) represents about half the metabolizable energy, confirming previous results (2). The cost of new tissue synthesis has been estimated to be 19 or 13% (1, 29) of the daily energy expenditure. In the present study it represented a far greater fraction, *i.e.* 32% of the resting energy expenditure. From biochemical considerations, the synthesis of tissue protein entails a considerable amount of energy. Therefore, we attempted to see whether the daily resting energy expenditure of these infants was related to their rate of protein synthesis.

The positive relationship existing between resting energy expenditure and protein synthesis was on the borderline of significance ( $r = 0.53$ ;  $p < 0.07$ ), probably due to the small number of observations which could be used for this analysis. However, it is also possible to estimate the cost of protein synthesis in an indirect manner. The relationship between protein gain and resting energy expenditure indicates that for each additional gram of protein gained, there is an increase in energy expenditure of 10 kcal (Fig. 5). On the other hand, for each gram of additional protein gain, there is an increase in protein synthesis of 5 g (Fig. 4). If we assume that all the extra energy expenditure is due to protein synthesis, the cost of protein synthesis would be 2 kcal/g (10 kcal/5 g). This cost is twice as great as the theoretical cost of protein synthesis calculated from the number of ATP necessary to form peptide bonds (~1 kcal/g protein) (30). However, the cost calculated from stoichiometry is minimal. Furthermore, other energy dissipative mechanisms may be involved during growth which are not yet known.

The present study indicates that increase in protein gain in very low birth weight infants is accompanied by a considerable increase in both protein synthesis and breakdown, confirming previous studies (4–7). The substantial increase in protein turnover may be viewed as a physiological phenomenon which allows fast remodeling of body protein during rapid growth. This energetically wasteful process is accompanied by an increase in energy expenditure estimated to be approximately 2 kcal/g protein synthesized or 10 kcal/g protein retained. Further studies are needed to understand the mechanisms of these energy dissipative processes.

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## The Development of Antioxidant Enzymatic Defense in the Perinatal Rat Lung: Activities of Superoxide Dismutase, Glutathione Peroxidase, and Catalase

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**ABSTRACT.** This report describes the development of superoxide dismutase, glutathione peroxidase, and catalase activities in fetal (days 16-22) and neonatal (day 2 postpartum) lungs of normal rats. Each of the enzymes showed

an individual pattern of development in the perinatal period. Glutathione peroxidase activity increased by 135% ( $p < 0.001$ ) during the last 3 days before birth, catalase activity by 105% ( $p < 0.01$ ) during the first 2 postnatal days, and the activity of superoxide dismutase by 52% ( $p < 0.05$ ) from gestational day 19 to 2 days after birth. Contamination by superoxide dismutase from blood in the lung samples accounted for less than 2% of the activity. In contrast, not less than 10-30% of glutathione peroxidase

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