Whole Body Protein Turnover Measured with ^{13}C -Leucine and Energy Expenditure in Preterm Infants

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ABSTRACT. Our study was undertaken in preterm infants to examine the relationship of whole body protein kinetics with protein intake and energy expenditure. Leucine kinetics were determined in seven low birth wt preterm infants fed human milk or human milk enriched with protein (2.5 to 4.3 g protein/kg·d). The infants received a short (4-h) constant infusion of L-[^{13}C]leucine and leucine turnover and oxidation were calculated from ^{13}C -plasma leucine and expired $^{13}\text{CO}_2$ enrichments measured by mass spectrometry. Energy expenditure was measured by indirect calorimetry. Nonoxidative leucine disposal (an estimate of protein synthesis) and leucine derived from protein (an estimate of protein breakdown) were, respectively, 2.98 ± 0.82 and 2.06 ± 0.74 $\mu\text{mol/kg}\cdot\text{min}$. Whole body protein turnover and deposition, derived from leucine kinetics, were 8.22 ± 2.31 and 2.17 ± 0.50 g/kg·d, whereas energy expenditure was 56.3 kcal/kg·day. Protein turnover was correlated with protein intake but not with protein deposition. Energy expenditure was correlated with protein turnover, synthesis, and breakdown but not with protein deposition. These data are in agreement with the fact that protein deposition depends upon protein intake, but they also suggest that an elevated protein deposition is not necessarily the result of a rapid protein turnover or associated with an elevated energy expenditure. (*Pediatr Res* 28: 147–152, 1990)

Abbreviations

AGA-PT, appropriate for gestational age preterm
GC-MS, gas chromatography-mass spectrometry
KIC, ketosisacproate

In premature infants, protein deposition represents approximately 12% of the body wt gain (1). Protein intake and energy balance are the main determinants of protein deposition, which is the difference between protein synthesis and protein breakdown. Both of these processes require energy, although breakdown requires less of it than synthesis. An increase in protein deposition may be the result of an increased protein synthesis and/or decreased protein breakdown. A separate measurement of these two components is therefore required to better understand the mechanism by which protein deposition is modified.

Such measurements rely on the use of stable isotope labeled tracers. Basically, two methods are available. The "end product method" uses ^{15}N -labeled amino acids (usually glycine) or yeast

protein and has been used in babies (2–9) because it requires neither infusion nor blood sampling. However, this method is time consuming (at least 12 h and up to 70 h) and relies on assumptions that might not be verifiable, particularly in the preterm infant. It is currently infrequently used in adults because of the practical advantages of the so-called "precursor method," which is a constant short infusion of a radioactive or stable isotope-labeled essential amino acid, such as leucine (10, 11) (see Calculations). To our knowledge, the latter method has been used only once in term infants (12) and only once, in modified form, in preterm infants (13).

Therefore, our study was undertaken in AGA-PT infants to determine in this age group: 1) the relationship between protein metabolism and energy expenditure, 2) the influence of protein intake on protein metabolism.

MATERIALS AND METHODS

Patients. Seven otherwise healthy AGA-PT infants were studied (Table 1). At the time of study, they had been gaining wt steadily for at least 1 wk. The study protocol was approved by the Ethical Committee of University Claude Bernard and a written informed parental consent was obtained for each patient.

Feeding. At the time of study, the infants had been fed for at least 1 wk with 165 mL/kg·d of either human milk (patients 1–4) or protein-enriched human milk (patients 5–7). Depending on the availability of breast milk from mother, either pooled human milk or the patient's mother's own milk was used. Protein supplementation was obtained by adding cow's milk casein hydrolyzate to human milk to raise the protein intake by 0.9 g/kg·d.

Study protocol. As described in Figure 1, milk was infused through a nasogastric tube at a constant rate of 6.1 mL/kg·h over 8 h. During this time, the infants were in incubators in a thermoneutral environment and indirect calorimetry was performed using an open circuit system that allowed continuous measurement of O_2 consumption and CO_2 production as previously described (14). After 4 h of feeding, a primed (11 $\mu\text{mol/kg}$), continuous (0.184 $\mu\text{mol/kg}\cdot\text{min}$) infusion of L-[^{13}C]leucine was given through a 23-gauge i.v. needle by means of a carefully calibrated syringe pump (Infu 362, Datex, Uhewiesen, Switzerland) and continued for 4 h. L-[^{13}C]leucine (CEA, Gif-sur-Yvette, France) was tested sterile and pyrogen-free and dissolved in normal saline to be infused at a final rate of 2 mL/h. Blood was sampled from an i.v. line inserted into a hand vein and kept patent with normal saline before the initiation of the tracer infusion (baseline) and every 15 min over the last hour of infusion (T 180, 195, 210, 225, 240). The total amount of blood withdrawn over the study was 4 mL. Blood was centrifuged immediately and plasma stored at -80°C for further analysis.

Expired air was continuously collected at the exhaust of the hood into Douglas bags at baseline and during four periods of

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15 min over the last hour of study. Samples of air were then transferred from the Douglas bags in 250-mL evacuated glass containers for subsequent ^{13}C analysis. Urine was collected over 8 h for determination of nitrogen excretion.

Analytical methods. Plasma ^{13}C -leucine enrichments were determined by GC-MS using 0.3-mL plasma samples. Neutralized perchloric acid extracts of plasma were acidified ($\text{pH} \approx 1$) and poured on a cation exchange resin (Dowex AG 50 $\times$ 8, H⁺ form, Richmond, VA). The columns were rinsed with 0.01 N HCl and the amino acids were eluted with NH_4OH 4 N, taken to dryness and derivatized as their tert-butyl dimethyl silyl derivative (MTBSTFA, Pierce, Rockford, IL) (15). Two μL of the samples were then injected in duplicate into a 25-m fused capillary silica column coated with OV 1701 (Chrompack, Middleburg, Netherlands), kept at 185°C in a gas chromatograph (Fractovap 4160, Carlo Erba, Milano, Italy), and leucine was separated from other amino acids. The gas chromatograph was coupled with a quadrupole mass spectrometer (Nermag R 10/10, Rueil Malmaison, France) working in the electronic ionization mode and monitoring the ions m/z 302 and 303, representing leucine (M) and ^{13}C -enriched leucine (M + 1), respectively. The ^{13}C -leucine enrichment (mole percent excess) (16) in the samples was then calculated by comparison with standard curves of known ^{13}C -leucine mole percent excess analyzed under the same conditions. Leucine concentrations were determined on the same GC-MS run, using norleucine as internal standard.

$^{13}\text{CO}_2$ was purified from the air samples by cryogenic distillation (17) and analyzed for $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio on a dual inlet isotope ratio mass spectrometer (Sira 12, VG Isogas Instruments, Middlewich, UK). ^{13}C isotopic abundance was expressed as the ‰ difference versus a Pee Dee Belemnite reference standard (^{13}C Pee Dee Belemnite ‰). Although the CO_2 analyzed at the exhaust of the hood came mostly from the infant, there was a small contamination by the CO_2 present in the room air flowing through the calorimeter. Appropriate corrections were made to take into account this contamination, knowing the airflow through the calorimeter and the $^{13}\text{CO}_2$ room air content. The magnitude of these corrections was usually 3% and never exceeded 7%.

The actual protein content of the milks was determined by two independent methods. First, milk leucine concentrations were measured by GC-MS as described above, after complete hydrolysis of the milk proteins with 6 N HCl during 24 h at 110°C. The obtained numbers were used for leucine kinetics calculations. Then a protein content was derived from leucine concentration, assuming a 9.2% (wt/wt) leucine content in milk protein (18). Total milk protein content was also checked by determining the nitrogen content by pyrochemiluminescence (ANTEK 703C, Sopares, Paris) assuming that 6.25 g of protein equals 1 g of nitrogen. There was an excellent agreement between the two methods ($r = 0.991$). Urinary nitrogen was also determined by chemiluminescence.

Calculations. O_2 consumption, CO_2 production, respiratory quotient, and energy expenditure were calculated as described by Jéquier (19). Classic isotopic equations for stable isotopes were used for determination of leucine kinetics (10). At near

steady state for leucine concentrations, ^{13}C -leucine enrichment, and $^{13}\text{CO}_2$ enrichment, leucine turnover rate (Q) ($\mu\text{mol/kg} \cdot \text{min}$) is determined by the following equation:

$$Q = \frac{F \times E_i}{\text{Leu MPE}} - F$$

where F is the ^{13}C -leucine infusion rate ($\mu\text{mol/kg} \cdot \text{min}$), E_i is the isotopic enrichment of the infusate, and Leu MPE is the plasma ^{13}C -leucine enrichment. E_i was checked by GC-MS and was 99%.

Leucine oxidation (Ox) ($\mu\text{mol/kg} \cdot \text{min}$) is:

$$\text{Ox} = \frac{\text{VCO}_2 \times ^{13}\text{CO}_2 \text{ APE}}{\text{Leu MPE}} \times \frac{1}{0.8}$$

where VCO_2 is the amount of expired CO_2 in μmol of $\text{CO}_2/\text{kg} \cdot \text{min}$, $^{13}\text{CO}_2$ APE is the expired CO_2 ^{13}C enrichment, and 0.8 is a factor correcting for incomplete recovery of labeled bicarbonate. Previously published (20) recoveries of ^{13}C -bicarbonate in newborns ranged from 0.70 to 0.84. Assuming recoveries at either of these extremes modified estimates of nonoxidative leucine disposal and net leucine balance (see below) by less than 4%.

The amount of dietary leucine (D) received was obtained by multiplying the milk infusion rate by milk leucine concentration. Inasmuch as leucine is an essential amino acid, without *de novo* synthesis, the two sources for leucine appearance in plasma are dietary leucine and leucine produced from protein breakdown (8). Conversely, leucine disappearing from plasma is either irreversibly oxidized to CO_2 (Ox) or incorporated into protein (Non-oxidative leucine disposal = S). At steady state:

$$Q = D + B = S + \text{Ox}$$

Knowing Q, D, and Ox, B (an index of protein breakdown) and S (an index of protein synthesis) can be determined. Net leucine balance, an index of protein deposition, is calculated as $S - B$.

Whole body protein breakdown, synthesis, and deposition ($\text{g/kg} \cdot \text{d}$) can then be extrapolated from leucine kinetics assuming an average leucine content of whole body protein of 8% (wt/wt) (10).

Statistics. All values are expressed as means $\pm$ SD. Correlations were determined by the least square regression method, assuming a normal distribution, and by a nonparametric test (Spearman rank correlation test).

RESULTS

Near steady state conditions were obtained during the 4th hour of tracer infusion for plasma leucine concentrations and both ^{13}C -leucine and $^{13}\text{CO}_2$ enrichments, therefore allowing leucine kinetics calculations (Figure 2). Coefficient of variations for leucine concentrations and enrichments were 9.0 and 5.0%, respectively. Plasma leucine concentrations were not different in infants fed human milk ($105 \pm 51 \mu\text{mol/L}$) or enriched human milk ($146 \pm 64 \mu\text{mol/L}$).

Leucine intakes and kinetics are presented on Table 2. As shown on the first column, leucine (and protein) intakes were

Table 1. Clinical characteristics of patients studied

	Patient	Birth wt (g)	Gestational age (wk)	Postnatal age at study (wk)	Wt at study (g)	Daily wt gain over 7 d (g/kg·d)
Human milk	1	1200	27.5	4.0	1650	15.5
	2	1030	28.0	6.0	1900	13.5
	3	1320	29.0	4.5	1860	16.3
	4	1670	32.0	3.5	1780	8.3
Protein-enriched human milk	5	1140	32.0	4.0	1740	23.0
	6	1570	32.5	2.5	1800	19.5
	7	1300	28.0	5.0	1840	22.6

Fig. 1. Study protocol.

Fig. 2. Plasma leucine concentrations, ¹³C-leucine enrichments, and ¹³CO₂ enrichment during the last hour of a 4-h infusion of L-[1-¹³C]leucine in fed preterm infants.

very variable. The ratio between net leucine balance and non-oxidative leucine disposal was 30.9%. Estimates of protein kinetics derived from leucine kinetics are shown on Table 3. When expressed per kg of metabolic body wt ($W^{0.75}$) (12) instead of kg of body wt, protein turnover became 9.51 ± 2.68 g/kg^{0.75}·d.

Leucine turnover (Fig. 3), leucine flux from proteins, and nonoxidative leucine disposal were correlated with leucine intake, whichever test was used ($p < 0.01$ parametric test, and $p < 0.01$, 0.02, and 0.01, respectively, nonparametric test). Leucine intake and leucine balance and oxidation were loosely correlated ($p = < 0.05$, Fig. 3), but when a nonparametric test was used, these two correlations were not significant ($p = 0.22$ and 0.07, respectively) due to the small number of patients and probably to the lack of patients with a leucine intake in the 1.23 to 1.58 μmol/kg·min range.

On the other hand, leucine turnover or nonoxidative leucine disposal were not correlated with net leucine balance ($r = 0.42$ and 0.49, respectively). The mean leucine turnover and nonoxi-

dative leucine disposal were not significantly higher in the infants fed with enriched human milk than in infants fed with unenriched milk.

Energy expenditure was correlated with leucine turnover (Fig. 3), leucine flux from proteins, and nonoxidative leucine disposal (Fig. 3) ($p < 0.01$ parametric test, and $p < 0.02$, 0.02, and 0.052, respectively, nonparametric test). No relationship could be found between energy expenditure and net leucine balance ($r = 0.16$).

All these correlations also existed for estimates of protein kinetics derived from leucine kinetics.

DISCUSSION

Kinetics of protein metabolism remain difficult to estimate in adults or children. Nitrogen balance yields no information on protein synthesis and catabolism and studies with ¹⁵N tracers are time-consuming. For these reasons, short infusions of labeled leucine (or other essential amino acids) have been extensively used in adults. In fasting term infants, leucine turnover was measured by a method similar to ours and was found to be 2.2 μmol/kg·min (21) and 2.7 μmol/kg·min with 22% of the flux being oxidized (12). In fed term infants, the same group (22) reported leucine turnover of 3.35 μmol/kg·min, of which 15% was oxidized. The only ¹³C-leucine study available (13) in preterm infants uses a modified method, i.e. intragastric administration of the tracer and sampling of the urinary free leucine pool for measurement of the ¹³C-leucine enrichment, assuming that the latter is representative of plasma enrichment. However, 12 h of tracer administration were needed to reach a steady state. The authors found a mean leucine flux of 5.55 μmol/kg·min (with a protein intake of 2.9 g/kg·d), but this value might have been overestimated by 10 to 30% because in human adults (23) or rats (24), after oral administration of a tracer, a 10–30% fraction of the tracer does not reach the plasma compartment due to a first-pass splanchnic extraction.

Our choice of studying the infants in the fed state was dictated by the need to obtain near steady state for leucine concentrations and ¹³C-leucine and ¹³CO₂ enrichments, which is a prerequisite for the calculations. In preliminary experiments (data not shown), L-[1-¹³C]leucine was added to the bottle of three preterm infants fed every 4 h. ¹³C-leucine enrichment and leucine concentrations were determined at regular intervals during the 4 h until the next meal. It appeared that 1) leucine concentrations are not at steady state between two meals and 2) ¹³C-leucine from the milk still appears in the plasma 3 h after a meal, at a rate very variable from one infant to another, because at this time, dietary leucine represented 7 to 28% of the total leucine flux. Therefore, we chose to study the infants in the fed state during a constant nasogastric milk infusion, which permitted us to obtain a reasonable steady state. In the fed state, dietary leucine was subtracted from total leucine turnover to calculate leucine produced from protein breakdown. We assumed that all the dietary proteins were hydrolyzed in the gut and absorbed. Actually, 83 to 86% of human milk proteins (1, 7) are usually absorbed in preterm infants. Applying such a correction factor to our data increases leucine produced from protein breakdown by 11% and decreases net leucine balance by 24%. However, although the absolute numbers are modified, the various relationships between leucine intake, kinetics, and energy expenditure are not modified.

An important point in the measurement of whole body leucine turnover is the choice of the precursor enrichment for calculations of turnover and oxidation. It has been demonstrated repeatedly (25, 26) that the use of the plasma ¹³C-KIC enrichment gives more reliable results than ¹³C-leucine enrichment. KIC is the product of the intracellular transamination of leucine and its plasma enrichment during an infusion of labeled leucine is very close to the intracellular ¹³C-leucine enrichment (27, 28). Consistently, the ratio of plasma ¹³C-KIC to plasma ¹³C-leucine enrichments is approximately 0.8 (25–29). Because of the small

Table 2. Leucine kinetics ($\mu\text{mol/kg}\cdot\text{min}$) and energy expenditure ($\text{kcal/kg}\cdot\text{min}$) in preterm infants fed either human milk (patients 1-4) or protein-enriched human milk (patients 5-7)

Patient	Dietary leucine	Leucine turnover	Leucine oxidized	Plasma leucine derived from protein	Nonoxidative leucine disappearance from plasma	Net leucine balance	Energy expenditure
1	1.58	2.98	0.53	1.40	2.45	1.05	47.6
2	1.23	2.53	0.47	1.30	2.06	0.76	51.0
3	1.86	3.91	0.70	2.05	3.21	1.16	52.0
4	1.23	3.09	0.71	1.86	2.38	0.52	52.3
Mean (patients 1-4)	1.48	3.13	0.60	1.65	2.53	0.87	50.7
95% confidence interval	1.00-1.96	2.22-4.04	0.41-0.79	1.08-2.22	1.76-3.30	0.41-1.33	47.3-54.1
5	1.87	4.34	0.90	2.47	3.44	0.97	64.1
6	2.08	5.56	1.08	3.48	4.48	1.00	73.0
7	1.61	3.45	0.63	1.84	2.82	0.98	54.1
Mean (patients 5-7)	1.85	4.45	0.87	2.60	3.58	0.98	63.7
95% confidence interval	1.27-2.43	1.83-7.07	0.31-1.43	0.55-4.65	1.50-5.66	0.95-1.02	40.3-87.1
Mean (total)	1.64	3.69	0.72	2.06	2.98	0.92	56.3
SD	0.33	1.02	0.21	0.74	0.82	0.21	9.0

Table 3. Whole body protein kinetics ($\text{g/kg}\cdot\text{d}$) in preterm infants fed human milk (patients 1-4) or protein-enriched human milk (patients 5-7)

Patient	Dietary protein	Protein turnover	Protein synthesis	Protein breakdown	Net protein gain
1	3.24	6.54	5.78	3.30	2.47
2	2.53	5.60	4.86	3.07	1.79
3	3.82	8.66	7.58	4.84	2.74
4	2.53	6.92	5.62	4.39	1.23
Mean (patients 1-4)	3.03	6.93	5.96	3.90	2.08
5	3.84	9.66	8.12	5.82	2.30
6	4.27	12.48	10.57	8.21	2.36
7	3.31	7.65	6.66	4.34	2.32
Mean (patients 5-7)	3.81	9.93	8.45	6.12	2.33
Mean (total)	3.36	8.22	7.02	4.85	2.17
SD	0.66	2.31	1.93	1.75	0.50

amount of plasma available, KIC enrichments could not be measured in our patients. If such a correction factor is applied to our numbers, nonoxidative leucine disposal becomes $3.72 \mu\text{mol/kg}\cdot\text{min}$ (instead of 2.98), leucine derived from protein breakdown becomes $2.98 \mu\text{mol/kg}\cdot\text{min}$ (instead of 2.06) and net leucine balance therefore becomes $0.74 \mu\text{mol/kg}\cdot\text{min}$ (instead of 0.92).

When whole body protein kinetics are extrapolated from leucine kinetics, we obtained a mean protein gain of approximately $2 \text{ g/kg}\cdot\text{d}$ (i.e. 320 mg of nitrogen), a value in reasonable agreement with those obtained during nitrogen balances by our group (1) or others (7, 9, 13, 30) in preterm infants receiving human milk either alone or protein enriched. However, our values of protein turnover are somewhat lower than most of the values obtained with ^{15}N -glycine, which range from 10 to more than $20 \text{ g/kg}\cdot\text{d}$ (9). This could be due to the peculiarities of glycine metabolism in the preterm infants (7), inasmuch as quite similar results are obtained in adults with ^{15}N -glycine and ^{13}C -leucine (31). Moreover, when expressed per kg of metabolic wt ($\text{W}^{0.75}$) as advocated by some authors (12), protein turnover in the fed state is similar in preterm infants ($9.52 \text{ g/kg}^{0.75}\cdot\text{d}$) (our results), full term babies ($10.8 \text{ g/kg}^{0.75}\cdot\text{d}$) (22), and adults ($13.7 \text{ g/kg}^{0.75}\cdot\text{d}$) (10).

In any case, more important than the absolute numbers are the qualitative variations of leucine kinetics observed in relation to variable leucine (protein) intake and energy metabolism. Leucine levels were not different whether human milk was enriched or not, which is likely due to the wide variance of the human milk protein content. Nitrogen balance is known to become more positive when nitrogen intake increases (1, 7, 32, 33) despite increased nitrogen excretion (1, 32). This is consistent with improved leucine balance when leucine intake increases despite increased leucine oxidation, although the small number of patients studied does not permit us to establish a strong correlation between those parameters. Leucine turnover (and estimates of protein synthesis and catabolism) is more rapid when leucine (protein) intakes increase, which is well known in adults (10). On the other hand, we could not find any significant relationship between net leucine balance and leucine turnover or nonoxidative leucine disposal, inasmuch as high leucine balance could be obtained with a rather low leucine turnover, such as that in patients 1 and 3. This suggests that a rapid protein synthesis rate can be inefficient in terms of protein gain because of a concomitant elevated protein breakdown. This is supported by recent data from Stack *et al.* (9) but stands in contrast with

Fig. 3. Relationships between whole body leucine intake, leucine turnover, nonoxidative leucine disposal, leucine oxidation, net leucine balance (all in $\mu\text{mol/kg} \cdot \text{min}$), and energy expenditure ($\text{kcal/kg} \cdot \text{min}$) in preterm infants.

data from Catzeflis *et al.* (7), who found a positive relationship between protein gain and synthesis.

There was a good correlation between leucine turnover (and nonoxidative leucine disposal) and energy expenditure, reflecting the fact that protein synthesis is an energy requiring process. Contrary to previous reports (7), energy expenditure was not correlated with net leucine balance, which again might be due to the small number of patients but might also suggest that a high turnover requiring much energy can have a poor yield in terms of leucine balance. It is of interest to note that patients 1 and 3 had the best leucine balance with a low energy expenditure despite a moderate protein intake, suggesting an optimal use of the human milk proteins. Although the actual energy content of the milks was not determined, it is possible that the higher protein deposition resulted from an elevated energy content (33). Another possibility is that human milk proteins are better used than proteins from other sources.

In conclusion, whole body leucine kinetics were determined in preterm infants by using a short (4-h) constant infusion of ^{13}C -labeled leucine. Estimates of protein kinetics were derived from leucine kinetics. The average protein turnover derived from leucine kinetics in AGA-PT infants was $8.2 \text{ g/kg} \cdot \text{d}$, which is lower than most previously published values. In these patients, rapid leucine (or protein) turnover was associated with high protein intake and with high energy expenditure but not necessarily with high protein deposition.

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REFERENCES

- Putet G, Senterre J, Rigo J, Salle B 1984 Nutrient balance, energy utilization and composition of weight gain in very low birth weight infants fed pooled human milk or a preterm formula. *J Pediatr* 105:79-85
- Nicholson JF 1970 Rate of protein synthesis in premature infants. *Pediatr Res* 4:389-397
- Pencharz PB, Hasson M, Desgranges F, Papageorgian A 1981 Total body protein turnover in human premature neonates: effects of birth weight, intrauterine nutritional status, and diet. *Clin Sci* 61:207-215
- Duffy B, Gunn T, Collinge J, Pencharz P 1981 The effect of varying protein quality and energy intake on the nitrogen metabolism of parenterally fed very low birthweight (<1600 g) infants. *Pediatr Res* 15:1040-1044
- Jackson AA, Shaw JCL, Barber A, Golden MHN 1981 Nitrogen metabolism in preterm infants fed human donor breast milk: the possible essentiality of glycine. *Pediatr Res* 15:1454-1461
- Pencharz PB, Farr L, Papageorgian A 1983 The effects of human milk and low protein formulae on the rates of total body protein turnover and urinary 3 methyl histidine excretion of preterm infants. *Clin Sci* 64:611-616
- Catzeflis C, Schutz Y, Michele JL, Welsch C, Arnaud MJ, Jéquier E 1985 Whole body protein synthesis and energy expenditure in very low birth weight infants. *Pediatr Res* 19:679-687
- Plath C, Meine W, Wutzke KD, Krienke L, Towe J, Massute G, Windischmann C 1987 ^{15}N tracer kinetic studies on the validity of various ^{15}N tracer substances for determining whole body protein turnover. *J Pediatr Gastroenterol Nutr* 6:400-408
- Stack T, Reeds PJ, Preston T, May S, Lloyd DJ, Aggett PJ 1989 ^{15}N tracer studies of protein metabolism in low birth weight preterm infants: a comparison of ^{15}N glycine and ^{15}N yeast protein hydrolysate and of human milk and formula fed babies. *Pediatr Res* 25:167-172
- Motil KJ, Bier DM, Matthews DE, Burke JF, Young VR 1981 Whole body leucine and lysine metabolism: response to dietary protein intake in young men. *Am J Physiol* 240:E712-E721

11. Bier DM, Matthews DE, Young VR 1985 Interpretation of amino acid kinetic studies in the context of whole body protein metabolism. In: Garrow JS, Halliday D (ed) *Substrate and Energy Metabolism*. John Libbey, London, pp 27-36
12. Denne SC, Kahlan SC 1987 Leucine metabolism in human newborns. *Am J Physiol* 253:E608-E615
13. De Benoist B, Abdulzarrak Y, Brooke OG, Halliday D, Millward DJ 1984 The measurement of whole body protein turnover in the preterm infant with intragastric infusion of L-[1-¹³C]leucine and sampling of the urinary pool. *Clin Sci* 66:155-164
14. Putet G, Senterre J, Rigo J, Salle B 1987 Energy balance and composition of body weight. *Biol Neonate* 52:17-24
15. Schwenk WF, Berg PJ, Beaufre B, Miles JM, Haymond MW 1984 Use of test-butylmethyl silylation in GC/MS analysis of physiologic compounds found in plasma using electron impact ionization. *Anal Biochem* 141:101-109
16. Cobelli C, Toffolo G, Bier DM, Nosadini R 1987 Models to interpret kinetic data in stable isotope tracer studies. *Am J Physiol* 253:E551-E564
17. Schoeller DA, Schneider JF, Solomans NW, Watkins JB, Klein PD 1977 Clinical diagnosis with the stable isotope ¹³C in CO₂ breath tests: methodology and fundamental considerations. *J Lab Clin Med* 90:412-421
18. *Tables Scientifiques Geigy* 1963. JR Geigy SA, Basel, p 515
19. Jéquier E 1980 Métabolisme énergétique. *Encycl Med Chir Paris Nutr* 10371(A10):1-15
20. Van Aerde JII, Sauer PJJ, Pencharz BP, Canagarayar U, Beesley T, Smith JM, Swyer PR 1985 The effect of energy intake and expenditure on the recovery of ¹³CO₂ in the parenterally fed neonate during a four hours primed constant infusion of NaH ¹³CO₃. *Pediatr Res* 19:806-810
21. Frazer TE, Bier DM 1980 Essential amino acid turnover in the human newborn. *Pediatr Res* 14:571A(abstr)
22. Denne SC, Rossi EM, Kahlan SC 1989 Leucine and protein metabolism in human newborns: effect of feeding. *Pediatr Res* 25:287A(abstr)
23. Hoerr RA, Matthews DE, Bier DM, Blackburn GL, Young VR 1984 Splanchnic bed metabolism in man measured by simultaneous intravenous and intragastric infusion of stable isotope tracers of leucine. *Am J Clin Nutr* 36:687A(abstr)
24. Karlstad MD, De Michele SJ, Istfan N, Blackburn GL, Bistrian BR 1984 Effect of burn and first pass splanchnic leucine extraction on protein kinetics in rats. *Am J Physiol* 255:R303-R309
25. Matthews DE, Schwarz JP, Xang RC, Motil KJ, Young VR, Bier DM 1982 Relationship of plasma leucine and α ketoisocaproate during a L-[1-¹³C] leucine infusion in man: a method for measuring intracellular tracer enrichment. *Metabolism* 31:1105-1112
26. Schwenk WF, Beaufre B, Haymond MW 1985 Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am J Physiol* 249:E646-E650
27. Laurent BC, Moldawer LL, Young VR, Bistrian BR, Blackburn GL 1984 Whole body leucine and muscle protein kinetics in rats fed varying protein intakes. *Am J Physiol* 246:E444-E451
28. Vazquez JA, Paul HS, Abidi SA 1986 Relation between plasma and tissue parameters of leucine metabolism in fed and starved rats. *Am J Physiol* 250:E615-E621
29. Beaufre B, Horber FF, Schwenk WF, Marsh HM, Matthews D, Gerich JE, Haymond MW 1989 Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. *Am J Physiol* 20:E712-E721
30. Reichmann BL, Chessex P, Putet G 1982 Partition of energy metabolism and energy cost of growth in the very low birth weight infant. *Pediatrics* 69:446-451
31. Waterlow JC, Garlick PJ, Millward DJ 1978 Protein turnover in mammalian tissues and in the whole body. North-Holland, Amsterdam, pp 335-337
32. Snyderman S, Boyer A, Kogut M 1969 The protein requirement of the premature infant. The effect of protein intake on the retention of nitrogen. *J Pediatr* 74:872-876
33. Senterre J 1979 Nitrogen balance and protein requirements of preterm infants. In: Visser HKA (ed) *Nutrition and Metabolism of the Fetus and Infant*. Martinus Nijhoff, London, pp 195-212