Effect of Intravenous Amino Acids on Protein Metabolism of Preterm Infants during the First Three Days of Life

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ABSTRACT. Twenty-three preterm infants with respiratory distress syndrome (mean birth weight 1.07 kg, SD 0.24 kg) were randomly assigned to receive glucose alone or glucose with amino acids ($1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) i.v. beginning on the 1st d of life. Blood ammonia and serum urea, CO_2 content, sodium, potassium, chloride, and ionized calcium concentrations were normal and did not differ between treatment groups. Nitrogen balance was significantly greater in the group that received amino acids [$88 \text{ (SD } 54) \text{ versus } -135 \text{ (SD } 45) \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$]. In 12 infants (seven, glucose-only; five, glucose and amino acids), leucine kinetic studies were also performed on the 3rd d of life. These 12 infants received a 4-h primed constant infusion of L-[1- ^{13}C] leucine. Blood and breath were collected and analyzed for [1- ^{13}C]ketoisocaproate and $^{13}\text{CO}_2$, respectively. Leucine turnover and oxidation were calculated. Both leucine turnover and oxidation were significantly higher in the group receiving amino acids than in the glucose-only group [$241 \text{ (SD } 38) \text{ versus } 164 \text{ (SD } 25) \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and $71 \text{ (SD } 22) \text{ versus } 40 \text{ (SD } 17) \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively]. In addition, the calculated rate of protein synthesis was higher in the group receiving amino acids [$6.9 \text{ (SD } 1.1) \text{ versus } 5.0 \text{ (SD } 1.2) \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$]. These data indicate that the i.v. administration of amino acids ($1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) to ill preterm infants beginning on the 1st d of life improves whole-body protein balance as a result of increased protein synthesis. (*Pediatr Res* 33: 106–111, 1993)

Abbreviations

KIC, ketoisocaproate

Recent advances in the treatment of respiratory distress syndrome have dramatically improved the survival of very low birth weight infants, necessitating improvements in early nutritional support given their limited metabolic reserves (1). In the past, the unstable clinical condition and apparently immature metabolic pathways of the newly born preterm infant resulted in the reluctance of physicians to administer i.v. amino acids during the 1st wk of life. However, the i.v. infusion of amino acids beginning on the 1st d of life may limit the endogenous nitrogen losses occurring during the 1st wk and stimulate a greater intake of energy (2).

Data on the safety and efficacy of intravenously infusing amino

acids in low birth weight infants during the first 3 d of life are limited (2, 3). Utilization of intravenously infused amino acids has been studied in older and more stable infants by nitrogen balance (4, 5). However, interpretation of data obtained by the nitrogen balance technique is limited by errors inherent to the method (6).

In vivo kinetics of individual amino acids have been studied to understand the dynamics of protein metabolism (7–9). Leucine, an essential amino acid catabolized mainly in muscle, the body's major protein store, is uniquely suited as a representative amino acid whose kinetics appear to reflect those of whole-body protein turnover, breakdown, and synthesis (8).

Thus, to provide information on the safety and efficacy of i.v. administration of crystalline amino acids to low birth weight infants during the first 3 d of life, we determined leucine kinetics, nitrogen balance, and various other biochemical indices in these infants.

MATERIALS AND METHODS

Twenty-three preterm infants (Table 1) with respiratory distress syndrome who were less than 24 h old and required mechanically assisted ventilation and indwelling arterial catheters were enrolled into a controlled trial comparing parenteral glucose *versus* glucose plus amino acids during the first 3 d of life. The birth weights of all infants were appropriate for gestational age as calculated from the 1st d of the mother's last menstrual period or, when dates were uncertain, by the method of Ballard *et al.* (10). The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects, and written consent was obtained for each subject from one or both parents.

Patients were assigned by envelope randomization to receive a solution containing glucose alone or glucose and amino acids ($1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, Aminosyn PF, Abbott Laboratories, North Chicago, IL) i.v. within 24 h of birth; infusion of the assigned solution was therefore begun on d 1 of life. The amino acid solution used did not contain premixed cysteine because of poor long-term solubility. A cysteine hydrochloride additive (Cysteine Hydrochloride Injection, Abbott Laboratories) is available for use with this solution. This cysteine additive was omitted inadvertently from the nutrient solutions administered to the first four infants assigned to receive glucose and amino acids. The subsequent seven patients received the amino acid solution plus the cysteine additive (0.5 g cysteine hydrochloride monohydrate/ 12.5 g of other amino acids).

No infant received enteral feedings or parenteral lipid emulsion. The amino acid infusions were started at a mean age of 15 h (SD 3). Nutrient solutions were infused i.v. with the use of mechanical pumps. Thermal support was provided with radiant warmers. Weight was determined daily; fluid intake was recorded, and urine volume was measured and recorded. The

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Table 1. *Clinical characteristics*

	Group	
	Glucose	Glucose and amino acids
No.	12	11
Birth wt (kg)*	1.09 (0.24)	1.05 (0.25)
Gestational age (wk)*	28.5 (1.8)	28.5 (1.8)
Sex (M:F)	6:6	3:8

* Mean (SD).

intakes of water, glucose, and electrolytes were ordered by the attending physicians and were not dictated by the experimental protocol. Glucose intake was typically adjusted according to chemical estimates (Chemstrip bG, Boehringer Mannheim Diagnostics, Indianapolis, IN) of blood glucose concentration performed at the bedside every 2 to 4 h.

Biochemical indices. Blood for ammonia nitrogen and ionized calcium was obtained on admission to the study and on the 3rd d of life. Serum sodium, potassium, chloride, carbon dioxide (CO_2 content), and urea levels were obtained from clinically indicated monitoring data when available. These analyses, including ammonia and ionized calcium, were performed in the hospital clinical chemistry laboratory using standard methods.

C-peptide. C-peptide concentrations in urine were determined by RIA, and C-peptide excretion was expressed as pmol/mg creatinine/24 h.

Nitrogen balance. In boys, urine was collected directly into 15-mL test tubes, whereas in girls a specially constructed metabolic mattress was used. Immediately after each voiding, urine was transferred to a refrigerated storage vessel which contained 0.5 mL of 9 N HCl. At the end of the collection period, the urine was measured in a graduated cylinder and frozen at -20°C .

Urine was digested by the micro-Kjeldahl method, and total nitrogen was determined by microdiffusion (11).

Urine was collected between 48 and 72 h of age. In 15 infants, urine collections were of 24 h duration. In eight infants, urine collections were of less than 24 h (6–17 h) duration for various technical reasons. Even though some infants had urine collections of less than 24 h, collections of 6 to 12 h have been shown to be accurate estimates of 24-h collections in parenterally fed infants (12, 13).

Nitrogen intake was calculated from the amount of amino acids infused between 48 and 72 h of life using the weight ratio of nitrogen to amino acids; this ratio was 0.1617 and 0.1595 for the infants receiving the amino acid solution without and with cysteine, respectively. We calculated nitrogen balance as intake minus urinary excretion. Fecal nitrogen losses were assumed to be negligible because most infants did not produce feces during the period of the balance study. In another study (14) of somewhat older preterm infants (aged 2–5 d) who were not fed enterally and who received greater parenteral intakes of amino acids than did our infants, mean fecal excretion of nitrogen was reported to be $12 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Fecal nitrogen excretion by our infants was probably even less.

Leucine metabolism. To assess whole-body amino acid and protein kinetics, a 4-h leucine tracer study was performed between 68 and 76 h of age while the infant continued to receive the initially assigned glucose or glucose and amino acid solution. We adapted for use in sick preterm infants the method of leucine kinetics as described by Matthews *et al.* (15). Our subjects were mechanically ventilated via endotracheal tubes and had indwelling arterial catheters and therefore provided ready access to expired air and blood without additional placement of intravascular catheters.

Leucine kinetic studies were conducted in 16 patients. In all 16 studies, an isotopic plateau of $[1-^{13}\text{C}]$ leucine in plasma was achieved. In four of the initial five patients, enrichments of $^{13}\text{CO}_2$ in breath could not be assessed for technical reasons. We report

here results of leucine kinetic studies from 12 infants. These 12 infants were given a priming dose of $[1-^{13}\text{C}]$ leucine ($4.0 \mu\text{mol} \cdot \text{kg}^{-1}$) at time zero, followed by a continuous i.v. infusion of $[1-^{13}\text{C}]$ leucine [mean $6.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (SD 0.5)] for 240 min using a calibrated syringe pump (model 975, Harvard Apparatus, Inc., Millis, MA). The last seven infants studied also received a priming dose of $[^{13}\text{C}]$ bicarbonate ($0.15 \text{ mg} \cdot \text{kg}^{-1}$) in addition to the priming dose of $[1-^{13}\text{C}]$ leucine. $[1-^{13}\text{C}]$ leucine (99.0% ^{13}C) and sodium $[^{13}\text{C}]$ bicarbonate (99.0% ^{13}C) were obtained from Tracer Technologies (Somerville, MA).

CO_2 output was continuously measured before and during the 4-h leucine balance study using a closed-circuit computerized indirect calorimeter (MGM/jr, Utah Medical Products, Midvale, UT) (16). Leucine metabolism was determined only in infants without evidence of endotracheal tube leak. Endotracheal tube leak was determined audibly and by measuring the CO_2 concentration in the oropharynx. Infants were excluded if the oropharyngeal CO_2 concentration exceeded 0.03%.

Blood was obtained from a indwelling arterial catheter and quickly placed into a cold vacuum tube (Vacutainer, Becton Dickinson, Rutherford, NJ) containing dry sodium heparin. The blood was immediately centrifuged and the plasma stored at -60°C for later analysis. Blood samples were obtained before administration of the isotope and also 210 and 240 min after the beginning of the $[1-^{13}\text{C}]$ leucine infusion to determine the plateau plasma enrichment of $[1-^{13}\text{C}]$ leucine and $[1-^{13}\text{C}]$ KIC. In four infants, blood samples were also obtained at 120 and 180 min to further confirm that $[1-^{13}\text{C}]$ leucine and $[1-^{13}\text{C}]$ KIC were at plateau values. Samples of expired air downstream from a nonbreathing valve and mixing chamber were collected by vacuum into 20-mL tubes (Venoject, Terumo Medical Corp., Elkton, MD). $[1-^{13}\text{C}]$ leucine and $[1-^{13}\text{C}]$ KIC in plasma and $^{13}\text{CO}_2$ in breath were measured as previously described (17–19). In the first five of the 12 infants for whom leucine kinetic data are reported, expired air was collected before administration of isotope and 120, 180, 210, and 240 min after beginning the $[1-^{13}\text{C}]$ leucine infusion. To assure that a plateau enrichment of carbon-13 in breath had been reached by 180 min, a 360-min infusion of $[1-^{13}\text{C}]$ leucine was performed in one of these five infants. In this infant, a plateau of carbon-13 in breath was apparent by 180 min after the start of the $[1-^{13}\text{C}]$ leucine infusion. In the remaining seven infants, a priming dose of $[^{13}\text{C}]$ bicarbonate was administered i.v. to more quickly achieve a plateau of carbon-13 in breath, and samples of breath were collected every 20 min. For the five infants that did not receive bicarbonate priming, the plateau value of carbon-13 in breath CO_2 was calculated as the mean of values between 180 and 240 min after beginning $[1-^{13}\text{C}]$ leucine infusion. For the other seven infants, those receiving a priming dose of bicarbonate, the plateau value of $^{13}\text{CO}_2$ in breath was calculated as the mean of values between 120 and 240 min after beginning the $[1-^{13}\text{C}]$ leucine infusion.

To estimate more accurately the whole-body turnover rate of leucine, the plasma enrichment of $[1-^{13}\text{C}]$ KIC was used as an index of leucine tracer dilution throughout the entire system (17, 20).

The model for leucine metabolism used here has been used by previous investigators (15, 21). The model generates the mass equation

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

where Q is the rate of leucine turnover (or rate of appearance or disappearance); S, the rate of leucine incorporation into protein; O, the rate of leucine oxidation; B, the rate of leucine release from protein; and I, the rate of influx of exogenous leucine—in this case, zero for the infants infused only with glucose, and the rate of nontracer leucine intake in the infants infused with glucose and amino acids.

Whole-body leucine turnover was calculated from the dilution

of the infused L-[1-¹³C]leucine at isotopic steady state:

$$Q = i \cdot \left(\frac{E_i}{E_p} - 1 \right) \quad (2)$$

where i is the L-[1-¹³C]leucine infusion rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$); E_i , the enrichment of the L-[1-¹³C]leucine infused (atoms % excess); and E_p , the [1-¹³C]KIC enrichment in plasma at isotopic plateau (atoms excess). Plasma KIC enrichment is taken to reflect the dilution of the leucine tracer intracellularly and therefore to represent the appearance rate of unlabeled leucine into the whole-body system (17, 20).

The rate of ¹³CO₂ excretion ($F^{13}\text{CO}_2$) was calculated using the following formula:

$$F^{13}\text{CO}_2 = \frac{\text{FCO}_2 \cdot \text{ECO}_2}{W \cdot \text{CF}} \quad (3)$$

where FCO_2 is the CO₂ production rate ($\mu\text{mol} \cdot \text{h}^{-1}$); ECO_2 , the enrichment of exhaled ¹³CO₂ (atom % excess expressed as a fraction); W , the body weight (kg); and CF , the correction factor that accounts for the percentage of ¹³CO₂ temporarily retained by the body. CF was assumed to be 0.98, the mean value reported by Bresson *et al.* (22) for parenterally fed infants.

Leucine oxidation (L_{ox}) was then determined from the rate of excretion of ¹³CO₂:

$$L_{\text{ox}} = F^{13}\text{CO}_2 \cdot \left(\frac{1}{E_p} - \frac{1}{E_i} \right) \cdot 100 \quad (4)$$

where E_p is the plasma enrichment of [1-¹³C]KIC (atoms % excess), because KIC is the direct precursor source of the carbon atom that is first lost during oxidation, and E_i is the initial enrichment of L-[1-¹³C]leucine.

From the leucine indices Q , S , O , and B in equation 1, estimates of whole-body protein turnover, synthesis, and breakdown in $\text{g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ were obtained by multiplying the indices of leucine (in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) by the constants 24 h/d and 1 g protein/590 μmol leucine. The factor of 590 μmol leucine/g protein assumes an average leucine content in protein of 7.8% (23, 24). From these values, we calculated net protein balance as synthesis minus breakdown.

Statistical analysis. Biochemical and energy intake data were analyzed for within-group differences by the two-tailed t test and for differences between groups by one-way analysis of variance. C-peptide data were analyzed by the Wilcoxon rank sum test. Leucine kinetics and nitrogen balance data were analyzed for within-group differences by one-way analysis of variance, and significant differences were determined *post hoc* by the t test. p values less than 0.05 were considered significant.

RESULTS

The daily energy intakes for the glucose alone and the glucose and amino acid groups differed significantly on d 2 and 3 (Table 2). Infants infused i.v. with glucose alone had a stable intake of energy over the 3-d study period. However, infants given glucose and amino acids had significantly greater intakes of energy on the 2nd and 3rd d of life; the higher energy intakes on d 2 and 3 were entirely due to higher glucose intakes than on d 1. The glucose intake in both groups was not dictated by experimental protocol but was adjusted by the treating physicians based on chemical estimates of blood glucose according to standard nursing procedure.

Therefore, we presume that the higher glucose energy intake on d 2 and 3 in the glucose and amino acid group was required to prevent hypoglycemia.

Plasma ammonia concentrations on the 3rd d of life were similar and ranged from 45 to 90 $\mu\text{mol/L}$ and from 46 to 82 $\mu\text{mol/L}$ for the infants infused i.v. with glucose alone and with glucose and amino acids, respectively (Table 3).

Serum urea concentrations were similar on d 1 and d 3 in infants given glucose alone (Table 3). Serum urea concentration increased significantly in the infants infused i.v. with a cysteine-free amino acid solution, from 3.8 mmol/L (SD 1.6) on d 1 to 7.4 mmol/L (SD 2.2) on d 3. The serum urea concentrations for infants infused with a cysteine-containing amino acid solution were similar on d 1 and 3, 5.3 mmol/L (SD 2.1) and 5.3 mmol/L (SD 2.8), respectively.

The mean CO₂ concentrations were similar between groups (Table 3), as were the ionized calcium, sodium, potassium, and chloride concentrations.

The mean excretion of C-peptide was 17.6 pmol/mg creatinine/24 h (SD 11.8) and 30.0 pmol/mg creatinine/24 h (SD 22.3) for the glucose alone and the glucose and amino acid groups, respectively. This difference was not statistically significant.

The infants that received glucose and amino acids were in positive nitrogen balance (Table 4) and retained a mean of 0.6 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (SD 0.3) of protein (amino acids) (88 mg nitrogen $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), whereas the infants infused with glucose alone had a mean loss of 0.8 g protein $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (SD 0.3) (−135 mg nitrogen $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), assuming that nitrogen represents 0.160 g/g of protein.

Nitrogen balance in infants infused i.v. with the cysteine-containing amino acid solution was significantly higher (115 mg $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, SD 41) than in those infused with the cysteine-free amino acid solution (42 mg $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, SD 42).

Background (baseline) carbon-13 abundance in expired CO₂ was not significantly different between the groups; it was −14.12 (SD 0.36) δ/mil in the infants receiving glucose alone and −14.82 (SD 0.75) δ/mil in the infants receiving both glucose and amino acids. The plateau enrichments of plasma [1-¹³C]KIC and breath ¹³CO₂ and the rates of L-[1-¹³C]leucine infusion and ¹³CO₂ excretion in breath were determined for each infant (Table 5). From these data, leucine turnover and oxidation and percentage of leucine turnover oxidized were calculated (Table 6). The mean [1-¹³C]leucine enrichments 210 and 240 min after the start of the infusion were 3.55 atom % excess (SD 0.87) and 3.58 atoms % excess (SD 0.85), respectively. The mean enrichments of [1-¹³C]KIC 210 and 240 min after the beginning of the i.v. infusion of L-[1-¹³C]leucine were 3.38 atoms % excess (SD 1.05) and 3.34 atoms % excess (SD 0.81), respectively.

Leucine turnover was significantly greater in the infants given glucose and amino acids than in those who received only glucose. The mean rate of leucine oxidation was also significantly greater in the infants infused i.v. with glucose and amino acids than in those infants infused i.v. with glucose alone. The percentage of leucine turnover oxidized was similar in the two groups.

The estimated whole-body protein turnover was significantly greater in the infants infused with both glucose and amino acids (9.8 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, SD 1.5) than in those infused with glucose alone (6.7 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, SD 0.9). Protein synthesis was significantly increased in the infants infused with glucose and amino acids, whereas protein breakdown and net balance were similar (Table 7).

DISCUSSION

The i.v. infusion of amino acids (1.5 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) in sick low birth weight infants during the first 3 d of life appears to be safe based on the biochemical indices we measured. In addition, we previously (3) found no abnormal elevations of plasma amino acids in preterm infants given this regimen of early amino acid infusion. Low or normal concentrations of all measured plasma amino acids were found despite amino acid supplementation (3).

Table 2. Energy intakes in preterm infants ($\text{kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)*

Group	n	d 1	d 2	d 3
Glucose	12	29 (7)	32 (10)	35 (12)
Glucose and amino acids	11	35 (8)	50 (19)†	54 (11)†

* Mean (SD).

† $p < 0.05$ as compared with glucose-only group.

Table 3. *Biochemical indices**

	Group							
	Glucose				Glucose and amino acids			
	<i>n</i>	d 1	<i>n</i>	d 3	<i>n</i>	d 1	<i>n</i>	d 3
Plasma ammonia ($\mu\text{mol/L}$)	6	65 (20)	8	65 (14)	5	83 (29)	5	59 (14)
Serum urea (mmol/L)	12	4.6 (1.4)	12	4.6 (1.4)	11	5.0 (2.1)	11	6.1 (2.9)
CO_2 (mmol/L)	11	22 (2)	12	23 (5)	11	22 (2)	11	24 (4)

* Mean (SD).

Table 4. *Nitrogen balance data ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)*

Group	<i>n</i>	Nitrogen intake*	Urine nitrogen*	Nitrogen balance*
Glucose	12	0 (0)	135 (45)	-135 (45)
Glucose and amino acids	11	250 (8)	156 (52)	88 (54)†

* Mean (SD).

† $p < 0.05$ as compared with glucose-only group.

The lack of elevated plasma amino acid concentrations lends further support to the safety of amino acids administered i.v. beginning on the 1st d of life.

It is well known that sufficient nonprotein energy must be consumed to efficiently utilize ingested protein. Although not as well recognized, amino acids modify energy intake by releasing insulin (25, 26), which creates a need for more exogenous glucose energy to prevent hypoglycemia. The early infusion of amino acids in preterm infants appears to have altered glucose metabolism, increasing their need for glucose. Previous investigators (26, 27) have shown that preterm infants infused i.v. with amino acids have greater serum insulin concentrations than those infused with glucose alone. Although not statistically significant, urinary C-peptide excretion (a marker for insulin secretion) tended to be greater in our infants receiving glucose and amino acids. Additional work is needed to quantitate the effects of amino acids on insulin secretion in the critically ill low birth weight infant.

The i.v. administration of similar amounts of amino acids ($1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) to ill preterm infants has been shown previously (4, 5) to result in positive nitrogen balance despite inadequate energy intake. Our demonstration of positive nitrogen balance in infants infused with amino acids should be interpreted with caution because of the short period of study and the low nonprotein energy intake (6, 28). The metabolic fate of the infused amino

acids, moreover, cannot be determined on the basis of nitrogen balance.

Infants infused with cysteine-containing amino acid solution retained a significantly greater amount of infused nitrogen than did infants receiving cysteine-free solution. Moreover, the serum concentration of urea nitrogen increased significantly during the 3-d study period only in the infants who did not receive cysteine. Bodwell (29) has summarized evidence indicating that when proteins of different quality are fed to provide similar nitrogen intakes, animals consuming the protein of lower quality demonstrate higher serum levels of urea nitrogen. Serum concentration of urea nitrogen has been used as an index of the adequacy of the amino acid intake in studies of rats (30) and human adults (31) and infants (32–34) during steady state conditions.

Hepatic cystathionase activity is extremely low in early fetal life and increases slowly as the fetus reaches term (35). Snyderman (36) suggested that cystine may be an essential amino acid for the preterm infant, but her data were not presented in sufficient detail to permit thorough review. Previous studies in parenterally fed infants have failed to demonstrate a significant effect of cysteine on nitrogen balance or growth (37, 38). These studies examined larger and older preterm infants than those in our study.

We speculate that, in infants of less than 32 wk gestation, cystathionase is not present in sufficient quantity during the first 3 d of life to permit adequate conversion of methionine to cysteine. However, sufficient activity may appear with increased gestational age or postnatal age so that exogenous cysteine is no longer essential (39).

Leucine turnover and oxidation have been studied in term infants (40, 41) with results similar to those in our study of preterm infants infused with glucose alone for 3 d. Frazer and Bier (40) studied four term infants at 4 to 8 h of age, whose mean rate of leucine turnover was $151 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (SD 28). Denne and Kalhan (41) measured leucine kinetics in 12 term infants

Table 5. *Isotopic enrichment of plasma KIC and expired CO_2 at plateau**

Subject	Group	Infusion rate of L-[1- ^{13}C] leucine ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)	Enrichment of plasma [1- ^{13}C] KIC (atoms % excess)†	F^{13}CO_2 ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)	^{13}C enrichment in breath CO_2 (atoms % excess $\times 10^3$)‡
A	G	6.50	3.39 (0.64)	0.78	5.38 (0.54)
B	G	6.46	5.00 (0.18)	2.25	8.11 (0.27)
C	G	7.09	4.50 (0.00)	2.95	5.34 (0.54)
D	G	6.71	3.27 (0.25)	1.43	5.79 (0.69)
E	G	6.28	3.40 (0.02)	3.13	8.57 (0.90)
F	G	6.66	4.12 (0.03)	1.79	8.21 (0.78)
G	G	6.67	3.95 (0.04)	2.11	7.63 (1.33)
H	GAA‡	6.69	2.12 (0.01)	2.38	10.31 (0.99)
I	GAA	5.69	2.55 (1.09)	1.75	9.85 (0.38)
J	GAA	6.39	2.71 (0.05)	2.13	9.70 (0.43)
K	GAA	6.71	2.65 (0.06)	2.97	8.16 (0.04)
L	GAA	7.16	3.22 (0.15)	1.97	7.86 (0.42)

* G, glucose-only group; GAA, glucose and amino acid group; F^{13}CO_2 , rate of $^{13}\text{CO}_2$ excretion in breath.

† Mean of two to four samples for plasma and two to eight samples for breath (SD).

‡ Subject H did not receive cysteine hydrochloride additive; the other four GAA subjects did.

Table 6. Leucine metabolism ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)*

Group	n	Turnover	Oxidation	Leucine turnover oxidized (%)
Glucose	7	164 (25)	40 (17)	25 (10)
Glucose and amino acids	5	241 (38)†	71 (22)†	29 (7)

* Mean (SD).

† $p < 0.05$ as compared with glucose-only group.Table 7. Effect of amino acid supplementation on protein synthesis, breakdown, and net tissue gain ($\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$)*

Group	n	Synthesis	Breakdown	Synthesis minus breakdown
Glucose	7	5.0 (1.2)	6.7 (1.0)	-1.6 (0.7)
Glucose and amino acids	5	6.9 (1.1)†	7.4 (1.5)	-0.5 (0.9)

* Mean (SD).

† $p < 0.05$ as compared with glucose-only group.

between 1 and 3 d of age who were fasted for 6 to 9 h. Mean leucine turnover was $164 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 28) in their 12 term infants, the same as the mean leucine turnover value in our seven preterm infants infused with glucose alone for 3 d, $164 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 25). Leucine oxidation in the term infants studied by Denne and Kalhan (41) was $34 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 11), as compared with $40 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 21) in our preterm infants receiving glucose alone.

Increased rates of leucine turnover have been shown with enterally fed preterm infants in both the fed and postabsorptive states (42, 43). de Benoist *et al.* (42) studied leucine kinetics in seven continuously fed preterm infants weighing a mean of 1.73 kg with a mean age of 22 d. Leucine turnover averaged $333 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 44); the mean rate of leucine oxidation was $42 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 17). Denne (43) studied leucine kinetics in seven postabsorptive preterm infants (mean 7.7 d of age); their leucine turnover and oxidation rates were $233 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 58) and $28 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively. Leucine turnover in our five preterm infants receiving amino acids i.v., $241 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 38), was similar to that in postabsorptive preterm infants (43) but lower than in fed infants (42). The rate of leucine oxidation in our infants receiving amino acids, $71 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (SD 27), was higher than the rate of oxidation found in the infants studied by de Benoist *et al.* (42) and Denne (43). These rates of leucine oxidation most likely reflect the overall clinical and nutritional status of these infants. An alternative explanation might be that parenterally delivered amino acids are disposed of more rapidly than are those fed enterally.

Our infants infused i.v. with glucose alone had protein turnover rates ($6.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) that were similar to those of the term infants studied by Frazer and Bier (40) and Denne and Kalhan (41) ($6.1 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ and $6.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, respectively). This similarity between term and preterm infants contrasts with the data of Nissim *et al.* (44), who found higher protein turnover rates in younger and less mature infants. Our results suggest that protein turnover in infants is influenced by energy and nutrient intake. Despite the period of negative energy balance in our glucose-alone group, protein turnover was high as compared with the fasted adult (15) and was consistent with the findings of Denne and Kalhan (41).

The rate of protein synthesis was higher in the infants infused i.v. with glucose and amino acids than in the glucose-alone group. Further, the increased rate of protein synthesis coupled with an unchanged rate of protein breakdown resulted in improved protein balance in the infants administered glucose and amino acids. However, the increased rate of protein synthesis seen in the infants infused with glucose and amino acids cannot be attributed

solely to the intake of amino acids but may reflect the interactions of increased insulin secretion (45) and glucose intake (46) or the difference in energy intake on d 2 and 3 (Table 2).

Whole-body protein balance was assessed with leucine kinetics. Infants infused with glucose alone had a mean loss of $1.6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ of endogenous body protein, representing a 5% loss of total body protein during the 3-d study period assuming the same daily loss and assuming that the protein content of a 1-kg infant is 94 g (47). Alternatively, in infants infused with glucose and amino acids ($1.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$), net protein balance was $-0.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, which represents a 2% loss of total body protein during the 3-d study period. These estimates differ from the data obtained by the nitrogen balance technique. Using urinary nitrogen excretion to estimate protein balance, a 3% loss and a 2% gain of total body protein would have been inferred in our infants infused i.v. with glucose alone and with glucose and amino acids, respectively. This discrepancy is probably due, at least in part, to the tendency of the nitrogen balance technique to overestimate nitrogen retention (6, 28).

The effects of infused amino acids on protein synthesis and breakdown found in our study using L-[1- ^{13}C]leucine (Table 7) are very similar to the results of a similar study recently reported by van Lingen *et al.* (48) using [^{15}N]glycine.

In summary, early amino acid administration ($1.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) appears to be biochemically safe. An increased rate of whole-body protein synthesis coupled with an unchanged rate of protein breakdown is seen with the administration of amino acids, resulting in improved protein balance. The early administration of amino acids was associated with a greater intake of energy, perhaps as a result of increased insulin secretion. Additional study of the safety and efficacy of infused amino acids in the first days of life is warranted.

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