Protein balance in the first week of life in ventilated neonates receiving parenteral nutrition


ABSTRACT

Background: Protein intake is frequently delayed in ill neonates because of concerns about their ability to metabolize substrates.

Objective: We aimed to determine the factors affecting protein balance in ventilated, parenterally fed newborns during the first week of life.

Design: Leucine kinetic studies were performed in 19 neonates by using the [1-13C]leucine tracer technique after 24 h of a stable total parenteral nutrition (TPN) regimen. TPN intakes were prescribed by rotating attending physicians, enabling assessment of protein metabolism over a range of clinically used nutrient intakes.

Results: Mean (±SD) birth weight was 1.497 ± 0.779 kg, gestational age at birth was 30.3 ± 4.0 wk, and age at study was 3.9 ± 1.4 d. Amino acid intakes (AAIs) ranged from 0.0 to 2.9 g·kg⁻¹·d⁻¹. Based on leucine kinetic data, protein balance was calculated as the difference between protein synthesis and catabolism. By multiple regression analysis, AAI was the only predictor associated independently with protein balance (P < 0.01); energy intake, lipid intake, glucose intake, birth weight, and gestational age were not. Both leucine oxidation and nonoxidative leucine disposal rates were significantly correlated with leucine intake (P < 0.0005 and P < 0.01, respectively). Of the 12 infants with AAIs > 1 g·kg⁻¹·d⁻¹, only 1 infant was significantly catabolic (protein balance < −1 g·kg⁻¹·d⁻¹). There was no evidence of protein intolerance as determined by elevated creatinine (69 ± 31 μmol/L), plasma urea nitrogen (6.7 ± 2.53 mmol/L), or metabolic acidosis (pH: 7.36 ± 0.05).

Conclusions: Ill neonates can achieve a positive protein balance in the first days of life without laboratory evidence of protein toxicity.


KEY WORDS Protein balance, protein synthesis, protein catabolism, neonates, leucine kinetics, total parenteral nutrition, amino acid intake, low birth weight

INTRODUCTION

Although it is agreed that optimal nutrition is a primary goal of neonatal care, the nutrient administration strategies needed to achieve this goal are not completely understood. Thus, nutritional regimens may vary greatly from one nursery to another, particularly for the feeding of ill neonates or extremely low-birth-weight (ELBW) infants in the first days of life. In these infants, parenteral nutrition is often gradually advanced over the first 1–2 wk of life because of concerns that the infants may be intolerant of substrate administration shortly after birth. This inability to metabolize nutrients is often attributed to the stress of the birth process, immature metabolic pathways in preterm infants, and the various pathophysiologic processes associated with different disease states, such as infection.

ELBW infants who receive only supplemental intravenous glucose lose >1% of their total protein stores each day (1), but the addition of intravenous amino acids can reverse the degree of protein catabolism (1–5). Nevertheless, the introduction of amino acids in the first days of life in ill and premature infants is often limited because of concerns over these infants’ inability to metabolize specific amino acids, which can result in hyperaminoacidemia, uremia, and metabolic acidosis (6).

We hypothesized that in a group of neonates who were considered to be relatively well based on the need for mechanical ventilation, many of the infants could achieve a positive protein balance in the first days of life with parenteral amino acids intakes ≥1–2 g·kg⁻¹·d⁻¹, irrespective of energy intake or gestational age, and that the most important determinant of protein balance would be amino acid intake. We studied infants in a single intensive care nursery where parenteral nutrition was prescribed by rotating physicians with different nutrient intake strategies; thus, the study population received a range of amino acid and energy intakes during the first week of life.

SUBJECTS AND METHODS

Subjects

Nineteen newborn infants admitted to the Neonatal Intensive Care Unit at the University of Colorado Health Sciences Center were studied during the first week of life. Enrollment criteria

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included mechanical ventilation, central venous and arterial access, exclusive parenteral nutrition, and relative clinical stability as assessed by the attending neonatologist. Written parental consent for infant participation was obtained before the study. The protocol was approved by the Institutional Review Board of the University of Colorado Health Sciences Center.

Protocol

Birth weight, gestational age, weight on the day of study, study age, medications at the time of study, and nutrient intake for 24 h before each study were recorded for each infant. Severity of illness at the time of the study was determined by calculating the ventilatory index, which is mean airway pressure × intermittent mandatory ventilation rate, and by calculating 2 different severity-of-illness scores that are used for neonates: the score for neonatal acute physiology (7) and the neonatal therapeutic intervention scoring system (8).

Total parenteral nutrition (TPN) intakes were prescribed at the discretion of the attending physician. Glucose intakes were titrated to maintain a serum glucose concentration of 4.5–8.0 mmol/L. The amino acid solution (Trophamine; McGaw, Inc, Irvine, CA) was supplemented with 40 mg cysteine hydrochloride/g amino acids (Abbott Laboratories, Abbott Park, IL), and lipid was supplied as a 20% solution (Intralipid; Clintec Nutrition, Deerfield, IL). Minerals, trace elements, and vitamins were provided according to recommended intakes (Neotrace-4: Fujisawa USA, Inc, Deerfield, IL; and MVI: Astra USA, Inc, Westborough, MA). Heparin (1 U·kg⁻¹·h⁻¹) was administered in the TPN solution.

Each infant was maintained on the same TPN solution for ≥24–48 h before being studied to ensure a nutritional steady state. For the study, the body bicarbonate pool was primed with 6.9 μmol [¹³C]bicarbonate/kg as described by Van Aerde et al (9), administered through either a central or a peripheral vein. Simultaneously, a priming infusion of [¹³C]leucine (7.6 μmol/kg) was administered over 5 min, followed by a 4–5-h constant infusion of [¹³C]leucine (7.6 μmol·kg⁻¹·h⁻¹). The same infusion pump (model 1001; MedFusion Systems, Inc, Norcross, GA) was used in all studies. Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA). Blood samples were drawn from an indwelling arterial or venous line (different from the isotope infusion catheter). Blood samples were drawn before giving the isotope priming infusions and then at 2–4 steady state time points. Steady state enrichment was considered to be the average of the enrichment values. Because of concern about the volume of blood withdrawal, a limited number of blood samples were available for determining steady state, particularly in extremely preterm infants (n = 2 time points).

Indirect calorimetry to determine carbon dioxide production was performed for 4–6 h with a calorimeter designed specifically for use in small infants (10). Mixed expired air was collected from the ventilator circuit before the isotope infusions were started and at time points coinciding with the steady state blood sampling for assessment of [¹³CO₂] enrichment. In most infants, additional steady state breath samples were collected. Plasma creatinine, urea nitrogen, and glucose concentrations were measured in all infants before the start of the isotope study. Urine for determination of urinary nitrogen excretion was collected beginning at the time of the isotope study.

Sample analysis

Immediately after sampling, blood specimens were placed in heparin-coated tubes, centrifuged at 4°C at 1000 × g for 15 min, and stored at −20°C until analyzed. Because of the restricted volume of blood collected from ELBW infants, leucine but not ketonecarboxylic acid enrichment and concentration values were measured. Plasma leucine enrichment (ie, mole percent excess, or MPE) was determined as described previously (11). Gas chromatography–mass spectrometry analysis was performed on a 5970 mass selective detector interfaced to a 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). A 15 m × 0.25 mm × 0.25 μm DB1 capillary column (Jones Chromatography, Lakewood, CO) was used. Multiple ion selection was performed on derivatized samples. Concentration and enrichment were determined simultaneously by using norleucine as the internal standard. The concentration and enrichment of the infusate were also measured. The actual infusion rate was determined by weighing the infusion syringe and tubing before and after the study and was confirmed by calculating the regression of multiple time point determinations of infused volume versus time of infusion.

Breath [¹³CO₂] was purified from ventilator-expired gas samples by cryogenic distillation under a vacuum; the isotope ratio of [¹³CO₂]/[¹²CO₂] was analyzed on a dual-inlet gas isotope ratio mass spectrometer (model 252; Finnigan MAT, San Jose, CA). Plasma glucose was measured periodically during the study in 25-μL samples by the glucose oxidase method with a glucose analyzer.

Calculations

Nitrogen balance was calculated as nitrogen intake minus urinary nitrogen excretion, expressed as g·kg⁻¹·d⁻¹. Urinary nitrogen collections ranged from 6 to 24 h. In infants receiving parenteral nutrition, collections of 6–12-h duration accurately reflect 24-h collections (12, 13). Nitrogen intake was calculated from amino acid intake during the 24 h before the start of the study (ie, g nitrogen intake = 0.156 × g amino acid intake). Both cutaneous and stool nitrogen losses were considered to be negligible because it is known that newborn infants receiving parenteral intakes only have minimal sweat and stool nitrogen losses. Protein balance (g·kg⁻¹·d⁻¹) was then calculated as nitrogen balance (g·kg⁻¹·d⁻¹) × 6.25 g protein/g nitrogen.

The model for determining various aspects of leucine metabolism is based on the following mass equation (14):

\[ Q_{\text{lec}} = NOD_{\text{lec}} \times Ox_{\text{lec}} + Ox_{\text{lec}} = RP_{\text{lec}} \times \text{Int}_{\text{lec}} \]  

where \( Q_{\text{lec}} \) is the leucine flux or turnover rate, \( NOD_{\text{lec}} \) is the rate of nonoxidative leucine disposal or the rate of incorporation of leucine into protein (which can be used to estimate protein synthesis), \( Ox_{\text{lec}} \) is the rate of leucine oxidation, \( RP_{\text{lec}} \) is the rate of leucine release from protein breakdown or the endogenous leucine appearance rate (which can be used to estimate protein breakdown), and \( \text{Int}_{\text{lec}} \) is leucine intake (dietary leucine plus the infused leucine tracer).

The whole-body leucine turnover rate (\( Q_{\text{lec}} \), μmol·kg⁻¹·h⁻¹) was calculated from the steady state dilution of the infused leucine tracer as

\[ Q_{\text{lec}} = \text{Inf}_{l} \times (\text{MPE}_{\text{inf}}/\text{MPE}_{p} - 1) \]

where \( \text{Inf}_{l} \) is the infusion rate of the labeled leucine (μmol·kg⁻¹·h⁻¹), \( \text{MPE}_{\text{inf}} \) is the leucine enrichment of the infusate, and \( \text{MPE}_{p} \) is the plasma enrichment of the leucine at steady state.

From exhaled breath measurements, the leucine oxidation rate (\( Ox_{\text{lec}} \), μmol·kg⁻¹·h⁻¹) was calculated as
\[
\text{Ox leuc} = \frac{(\dot{V}CO_2 \times ^{13}CO_2 \text{MPE})}{(\text{MPE}_{P} \times 0.8)}
\]

where \(\dot{V}CO_2\) is the rate of carbon dioxide production (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)), \(^{13}CO_2\text{MPE}\) is the \(^{13}\text{C}\) enrichment of expired carbon dioxide at steady state, and 0.8 is a constant representing the fractional recovery of \(^{13}\text{CO}_2\) obtained during \(^{13}\text{C}\)bicarbonate infusions (15), which corrects for the loss of the \(^{13}\text{C}\) label. In addition, leucine oxidation was calculated by the method of Van Aerde et al (9), whereby the percentage recovery of \(^{13}\text{CO}_2\) in neonates is equal to 64.2 + 0.1667 \(x\), where \(x\) is energy intake in kcal \(\cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) (9).

NOD leuc and RP leuc can then be determined by using equation 1.

Because leucine is an essential amino acid, and with the assumption that the average leucine content of protein is 590 \(\mu\text{mol/g protein}\) (16), the whole-body protein synthesis rate (\(S_{pro}\)) and catabolism rate (\(C_{pro}\)) can be extrapolated from leucine kinetic data. Whole-body protein balance (\(\text{Bal}_{pro}\)) is then estimated as

\[
\text{Bal}_{pro} = S_{pro} - C_{pro}
\]

On the basis of the methodologic errors inherent in these techniques, protein balance was considered to be positive or anabolic if > 1 g \(\cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) and negative or catabolic if < –1 g \(\cdot \text{kg}^{-1} \cdot \text{d}^{-1}\); values between these were considered to indicate infants in zero protein balance.

### Statistical Analysis

Both simple and multiple regression analyses were performed to assess the effects of nutrient intakes (protein, glucose, lipid, and total nonprotein energy intakes), demographic variables (gestational age at birth, birth weight, and chronologic age at study), and severity of illness (assessed by the ventilatory index, score for neonatal acute physiology, and the neonatal therapeutic intervention scoring system) on protein balance. Analyses were performed with STATVIEW (Abacus Concepts, Inc, Berkeley, CA). Average values are expressed as means ± SDs.

### RESULTS

The clinical characteristics and diagnoses of the 19 infants studied are outlined in Table 1 (infants are ordered by protein balance from most positive to most negative, top to bottom). As would be expected, mean birth weight (1.497 ± 0.779 kg) and mean study weight (1.513 ± 0.794 kg) were similar because the infants were <1 wk old when the isotope study was performed. All infants were the appropriate size for their gestational age. All
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Infants are ordered from most positive to most negative protein balance, top to bottom. Int\textsubscript{leuc}, leucine intake from the diet plus leucine from the isotope infusion; Q\textsubscript{leuc}, leucine flux; O\textsubscript{leuc}, leucine oxidation rate; NOD\textsubscript{leuc}, leucine nonoxidative disposal or rate of incorporation of leucine into protein; RP\textsubscript{leuc}, leucine released from protein breakdown; Bal\textsubscript{leuc}, leucine balance; Bal\textsubscript{pro}, protein balance.
but 3 infants were receiving antibiotics at the time of the study. Five infants received sodium bicarbonate as a therapy for metabolic acidosis, but only one infant received it on the day of study (infant 12, in whom bicarbonate was administered before the study and in whom metabolic acidosis was presumed to be secondary to a symptomatic patent ductus arteriosus). Because both steroids and catecholamines may affect protein metabolism, exposure to these drugs was determined. The mothers of 10 infants received prenatal steroids, although the number of doses and administration time before delivery were highly variable. The average age at study of infants exposed to steroids prenatally was 3.5 ± 1.4 d (range: 2.5–7.0 d). Only 1 infant received postnatal steroids, and this was a single dose of methylprednisolone of 30 mg/kg administered 2 d before the study for presumed septic shock (infant 19). Of the 9 infants who required pressor support since birth, 5 were still receiving dopamine infusions at the time of study, ranging from 2 to 10 μg·kg⁻¹·min⁻¹.

Laboratory values at the time of the study included a mean pH of 7.36 ± 0.05, mean plasma creatinine concentration of 69 ± 31 μmol/L [the mean (±SD) reference value for preterm infants is 99.1 ± 5.3 μmol/L], and mean plasma urea nitrogen concentration of 6.7 ± 2.53 mmol/L (normal range: 1.8–8.9 mmol/L). There was no correlation between amino acid intake and pH, creatinine, or plasma urea nitrogen. The mean plasma glucose concentration was 5.8 ± 2.0 mmol/L (range: 2.7–11.4 mmol/L). Plasma triacylglycerol concentrations were not measured as part of the study protocol because of the additional blood sampling that would have been required, but values available for 7 infants ranged from 0.26 to 1.06 mmol/L.

TPN composition and delivery rate were determined by the attending physician. Because nutritional practices varied among physicians, the study population received a wide range of protein and energy intakes. Nutrient intakes for the 24 h before the start of the study are shown in Table 2 (infants are ordered as in Table 1). Leucine and carbon dioxide steady state enrichment values are shown in Figure 1. Carbon dioxide production averaged 5.3 ± 0.8 mL·kg⁻¹·min⁻¹ (range: 4.4–7.4 mL·kg⁻¹·min⁻¹). Leucine kinetic measurements and calculated protein balance results are shown in Table 2. The leucine oxidation rate was calculated by 2 techniques used in neonatal studies: (1) a fixed ¹³C₂O₂ recovery fraction of 0.8 (17–21) and 2) the method of Van Aerde et al (9), in which the ¹³C₂O₂ recovery fraction is based on energy intake. The leucine oxidation rate increased by an average of 10.2 ± 3.9% according to the method of Van Aerde et al, but mean protein balance increased by only 0.22 ± 0.09 g·kg⁻¹·d⁻¹. The fixed-fraction recovery method was used for all subsequent calculations that
included a leucine oxidation rate value. The leucine oxidation rate was significantly correlated with leucine intake (Figure 2), as was the nonoxidative leucine disposal rate, which is the rate of incorporation of leucine into body proteins and which is thus used to calculate the rate of protein synthesis. Although there was a correlation between protein synthesis and amino acid intake, as shown in Figure 3, protein balance was not suggested by either leucine synthesis or the leucine breakdown rate alone. Rates of protein synthesis may be higher in catabolic infants than in anabolic infants, whereas rates of protein breakdown may be higher in anabolic than in catabolic infants. The most anabolic infant was the next-to-oldest infant and had the highest amino acid intake. The most catabolic infant was 7.0 d old, had received no lipid or amino acid intake, had been on pressor support previously, required buffer therapy for acidosis several days before the study, and was the only infant to receive postnatal steroids (a single, large dose 2 d before the study for presumed septic shock).

To determine which factors had the greatest effect on a positive protein balance, simple regression analyses were performed by using the previously described nutritional intakes, demographic factors, and severity of illness assessments. The following variables were univariately related to protein balance: amino acid intake \((P < 0.0001\); Figure 4\), lipid intake \((P < 0.005)\), and non-protein energy intake \((P < 0.005)\). However, when analyzed by multiple linear regression, amino acid intake was the only predictor that was independently associated with protein balance \((P < 0.01)\).

Of the 12 infants with amino acid intakes > 1 g · kg⁻¹ · d⁻¹, only 1 infant was significantly catabolic (protein balance < −1 g · kg⁻¹ · d⁻¹). To see whether these data supported the concern that extremely preterm infants may not be able to tolerate protein because of metabolic immaturity, the relation between amino acid intake and protein balance was analyzed separately for 9 infants weighing <1250 g at birth. There was a significant correlation between amino acid intake and protein balance for infants in this subpopulation \((P < 0.01)\), implying that the most fragile, immature infants can utilize parenteral protein for protein accretion.

Protein balance was also determined by the nitrogen balance technique. There was a good correlation between the nitrogen balance and the leucine kinetic methods for determining protein balance \((R^2 = 0.49, P < 0.001)\).

**DISCUSSION**

This study was done to determine factors that contribute to positive protein balance in a population of ventilated infants during...
the first days of life to learn how protein nutrition could be improved. Protein metabolism is known to be affected by various factors, including amino acid intake, energy intake, and underlying disease states. Several studies have shown the positive effect of amino acid intake on protein metabolism in exclusively parenterally fed neonates by using both nitrogen balance (2, 4, 22–24) and stable-isotope techniques (3, 25, 26). Use of stable-isotope methods allows for a more dynamic study of protein metabolism in newborns, and such studies have provided metabolic information in populations similar to ours (ie, ventilated neonates in the first week of life). van Toledo-Epping et al (26) measured leucine kinetics in 10 preterm infants at 6 d of age with a mean birth weight of 1275 g and parenteral intakes of 335 ± 105 kJ·kg\(^{-1}·d^{-1}\) and 1.8 g protein·kg\(^{-1}·d^{-1}\). As a group, these infants were minimally catabolic but had high protein synthetic and catabolic rates (11.8 ± 2.2 and 12.1 ± 2.1 g·kg\(^{-1}·d^{-1}\), respectively) compared with those reported in other studies (3, 27, 28). In a study of 23 ventilated infants with a mean weight of 1.07 kg and who were randomly assigned to receive parenteral glucose alone or glucose plus 1.5 g protein·kg\(^{-1}·d^{-1}\), Rivera et al (3) showed that protein administration significantly improved nitrogen balance on the third day of life (88 ± 54 mg·kg\(^{-1}·d^{-1}\)), although there was a negative protein balance in a subpopulation of infants studied by using leucine kinetics (−0.5 ± 0.9 g·kg\(^{-1}·d^{-1}\)). In our study, amino acid intakes as low as 1.0–1.5 g·kg\(^{-1}·d^{-1}\) could usually prevent net protein catabolism in most infants as determined by both stable-isotope and nitrogen balance techniques.

Hesitation to introduce amino acids during the early postnatal period, particularly in preterm infants, is the result of concerns that intolerance may produce hyperammonemia, azotemia, and metabolic acidosis. In a study by van Goudoever et al (5) of amino acid supplementation (1.15 g·kg\(^{-1}·d^{-1}\)) in preterm infants beginning on the first day of life, there were no significant differences in blood pH, base excess, or urea concentration compared with values in control infants who received glucose alone. In the study by Rivera et al (3), there were no significant differences in ammonia and urea concentrations over the first 3 d of life in ELBW infants receiving 1.5 g amino acids·kg\(^{-1}·d^{-1}\) compared with those in infants administered solely glucose. In the present study, urea, creatinine, and pH were within acceptable limits.

The amount and type of nutrient energy have been shown to affect protein balance in parenterally fed neonates (23, 24, 29, 30). In our study, although both total nonprotein energy and lipid intakes were correlated with protein balance by simple regression analysis, neither had a significant effect on protein balance when the relation was adjusted for amino acid intake. Because all infants received a relatively large intake of energy per gram protein, this study did not test either the protein-sparing effect of protein alone or the minimal energy intake above which protein balance was improved. Optimal glucose and lipid intakes that maximize protein accretion and growth have not yet been determined at all amino acid intakes in neonates, particularly in those who are ill or extremely preterm.

Medications administered at the time of the study that might have had an effect on protein metabolism were noted. As has been shown in numerous studies in animals and human adults given corticosteroids, dexamethasone administration causes protein wasting in neonates (31). Note that the most catabolic infant in our study had no amino acid intake, but was also the only infant to receive postnatal steroids. Increased protein breakdown was also reported in the first 24 h of life in preterm infants whose mothers received corticosteroids just before birth (5). The effect of steroids on neonatal protein metabolism in our study could not be determined because the number of maternal steroid doses, the time between maternal steroid administration and delivery, and the postnatal age of the infants at the time of the study were not controlled and were highly variable. It is likely that the steroid catabolic effect at the time of the study was less in our infants than in those studied by van Goudoever et al (5) because of the significantly greater postnatal age of our infants. There are no data in neonates on the effect of catecholamine pressor support, particularly at low doses, on protein metabolism. Nine infants received catecholamines during the postnatal period and they were distributed in all protein balance groups.

In adults, different disease and stress states have been shown to have negative effects on protein balance (32). However, little is known about specific nutrient requirements in different disease states in neonates, particularly ELBW infants (33). We chose to study protein synthesis and catabolism in a group of relatively ill neonates because nutritional strategies for these infants can vary widely as a result of concerns over substrate intolerance. Although several studies have examined the relation between energy expenditure and different measures of the severity of illness in neonates, this is the first neonatal study of which we are aware that examined the relation between severity of illness and protein balance. We were unable to show a correlation between degree of illness (as assessed by several indexes) and protein balance. We speculate that the stress response in neonates may be different from that in adults. We did show that most of the ill infants in this study (17 of 19) could be noncatabolic in the first week of life, and that the fragile population of ELBW infants does not appear to have a decreased ability to utilize amino acid intake (ie, lower protein balance at the same amino acid intake) compared with gestationally more mature neonates.

In conclusion, this study assessed protein metabolism in a population of ventilated neonates receiving a wide range of protein and energy intakes. We showed that parenteral nutrition could prevent a catabolic state in the first days of life in these infants, and that this could usually be achieved with as little as 1.0–1.5 g amino acids·kg\(^{-1}·d^{-1}\). Although the number of infants studied was small, we presented individual data for preterm infants who received higher amino acid intakes in the first days of life than has been reported previously and the protein appeared to be well tolerated. These data suggest that with use of the current pediatric amino acid solutions, which were developed in the late 1980s (34, 35), even sick and preterm newborns may be able to tolerate higher amino acid intakes than was thought previously. Further studies are needed to determine the efficacy and safety of nutritional regimens in producing a consistent, early anabolic state in newly born infants, particularly in those who are extremely preterm or who are critically ill.

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