Safety and Efficacy of Early Parenteral Lipid and High-Dose Amino Acid Administration to Very Low Birth Weight Infants

Hester Vlaardingerbroek, MD, PhD1, Marijn J. Vermeulen, MD, PhD1, Denise Rook, MD, PhD1, Chris H. P. van den Akker, MD, PhD1, Kristien Dorst1, Josias L. Wattimena2, Andras Vermes, PharmD, PhD3, Henk Schierbeek, PhD1,4,5, and Johannes B. van Goudoever, MD, PhD1,4,5

Objective To assess the efficacy and safety of early parenteral lipid and high-dose amino acid (AA) administration from birth onwards in very low birth weight (VLBW, birth weight <1500 g) infants.

Study design VLBW infants (n = 144; birth weight 862 ± 218 g; gestational age 27.4 ± 2.2 weeks) were randomized to receive 2.4 g of AA kg⁻¹·d⁻¹ (control group), or 2.4 g AA kg⁻¹·d⁻¹ plus 2-3 g lipids kg⁻¹·d⁻¹ (AA + lipid group), or 3.6 g AA kg⁻¹·d⁻¹ plus 2-3 g lipids kg⁻¹·d⁻¹ (high AA + lipid group) from birth onwards. The primary outcome was nitrogen balance. The secondary outcomes were biochemical variables, urea rate of appearance, growth rates, and clinical outcome.

Results The nitrogen balance on day 2 was significantly greater in both intervention groups compared with the control group. Greater amounts of AA administration did not further improve nitrogen balance compared with standard AA dose plus lipids and was associated with high plasma urea concentrations and high rates of urea appearance. No differences in other biochemical variables, growth, or clinical outcomes were observed.

Conclusions In VLBW infants, the administration of parenteral AA combined with lipids from birth onwards improved conditions for anabolism and growth, as shown by improved nitrogen balance. Greater levels of AA administration did not further improve the nitrogen balance but led to increased AA oxidation. Early lipid initiation and high-dose AA were well tolerated. (J Pediatr 2013;163:638-44).

See editorial, p 622

Malnutrition in humans during critical stages of development has long-lasting negative effects on both growth and neurodevelopment, at least through school age and possibly also into adulthood.¹ In recent decades, multiple studies have demonstrated that early parenteral amino acid (AA) administration (up to 3.0 g·kg⁻¹·d⁻¹) is safe, well tolerated, and results in an increased protein synthesis rate and positive nitrogen balance, indicating an anabolic state.²⁻⁸ However, lipid administration has not received similar attention. Lipids are not only fundamental to providing the essential n-6 and n-3 fatty acids necessary for central nervous system development but also supply the dietary energy necessary for the optimal use of AAs for protein synthesis. In most neonatal intensive care units (NICUs), lipids are introduced on the second or third day of life or in very low amounts (0.5-1 g·kg⁻¹·d⁻¹) from birth onwards because of concerns about lipid intolerance. However, meta-analyses of early lipid introduction (before day 2) have not shown increased risks of common neonatal morbidities.⁹,¹⁰

We describe neonatal growth, safety, and clinical course of very low birth weight (VLBW) infants subjected to nutritional regimens with different intravenous lipid and AA intakes starting soon after birth. We hypothesized that both lipids and greater levels of AA administration from birth onwards would be safe and well tolerated and would result in an improved nitrogen balance.

Methods

Between December 2008 and January 2012, we performed a randomized controlled trial at the NICU of the Erasmus MC–Sophia Children’s Hospital in Rotterdam, The Netherlands. The eligible patients were inborn VLBW infants (birth weight <1500 g) with a central venous catheter in place to allow for more concentrated glucose solutions and to restrict total fluid intake. Written informed consent was

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<thead>
<tr>
<th>AA</th>
<th>Amino acid</th>
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<tr>
<td>ECF</td>
<td>Ethyl chloroformate</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>ROP</td>
<td>Retinopathy of prematurity</td>
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<td>SGA</td>
<td>Small for gestational age</td>
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<td>TG</td>
<td>Triacylglycerol</td>
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<td>VLBW</td>
<td>Very low birth weight</td>
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From the ¹Division of Neonatology, Department of Pediatrics, Erasmus MC–Sophia Children’s Hospital; Departments of ²Internal Medicine and ³Hospital Pharmacy, Erasmus MC, Rotterdam, The Netherlands; ⁴Department of Pediatrics, Emma Children’s Hospital- AMC; and ⁵Department of Pediatrics, VU University Medical Center, Amsterdam, The Netherlands
The authors declare no conflicts of interest.
Registered with TrialRegister.nl: NTR1445.
obtained from the infants’ parents before they were included. Exclusion criteria were congenital anomalies, including chromosome defects and known metabolic diseases, or endocrine, renal, or hepatic disorders. The study protocol was approved by the institutional medical ethical review board.

The attending physician included infants within 6 hours of birth by opening a sealed, opaque randomization envelope stratified for weight (<1000 g or 1000-1499 g) and sex. The envelopes were created by a research pharmacist who was not involved in clinical care and were based on a computer-generated block randomization list with variable block sizes that was provided by a statistician. For logistic reasons, the study group randomization was open after inclusion; however, all technicians were blinded for study group randomization throughout the study and the analyses.

The infants received glucose (at least 4.0 mg·kg⁻¹·min⁻¹) and 2.4 g·kg⁻¹·d⁻¹ of AA (always on stock on the ward) as part of standard clinical care. Immediately after randomization to 1 of 3 groups, the experimental parenteral nutrition was substituted for all infants except those who were allocated to the control group.

The infants in the control group received glucose and AA (2.4 g·kg⁻¹·d⁻¹) during the first 2 days of life. Lipids were started on the second day of life at 1.4 g·kg⁻¹·d⁻¹ and were increased the following day to 2.8 g·kg⁻¹·d⁻¹.

The infants in the AA + lipid group received glucose and AA similar to the control infants (2.4 g·kg⁻¹·d⁻¹), but lipids were started soon after birth (starting dose of 2 g·kg⁻¹·d⁻¹, next day increased to 3 g·kg⁻¹·d⁻¹).

In addition to glucose from birth, the infants in the high AA + lipid group received both high-dose AA (3.6 g·kg⁻¹·d⁻¹ from birth onwards) and lipids (a starting dose of 2 g·kg⁻¹·d⁻¹, increased the next day to 3 g·kg⁻¹·d⁻¹).

All of the groups received the same AA product: Primene 10% (Baxter, Utrecht, The Netherlands). The infants in the control group received Intralipid 20% (Fresenius Kabi, Bad Homburg, Germany). The infants in the intervention groups were randomized to receive either Intralipid 20% or MOF lipid 20% (Fresenius Kabi). Because the lipid type did not have an effect on our primary outcome, lipid type was not included in the final analyses. In all groups, minimal enteral feeding was initiated on the day of birth and advanced to full enteral nutrition, according to the local protocol. After the third day of life, the nutritional regimen, including enteral feeding, was at the discretion of the attending physician.

Throughout the study, the local protocol was to temporarily lower the parenteral intake of AA when plasma urea concentrations were between 10 and 14 mmol/L (28-39 mg/dL) and to temporarily cease AA administration when plasma urea concentrations exceeded 14 mmol/L (39 mg/dL). Similarly, parenteral lipid intake was temporarily lowered when triacylglycerol (TG) concentrations were between 3 and 5 mmol/L (265-442 mg/dL) and temporarily stopped whenever TG concentrations exceeded 5 mmol/L (442 mg/dL). These guidelines were based on expert opinion.

According to the local protocol, repeated blood glucose concentrations >10 mmol/L (180 mg/dL) were treated with continuous intravenous insulin (starting dose 0.1 U·kg⁻¹·h⁻¹) if reducing the glucose infusion rate to a minimal intake of 4 mg·kg⁻¹·min⁻¹ was not effective. Baseline characteristics were recorded. The nutritional intake was recorded daily until the infants were on successful full enteral feeding (ie, no parenteral feeding for 2 consecutive days).

**Primary Outcome**

The efficacy of the intervention was analyzed by quantifying the nitrogen balance on postnatal days 2, 4, and 6. The nitrogen balance was calculated by subtracting the urinary nitrogen excretion from the recorded nutritional intakes (parenteral + enteral), under the assumption that 1 g of AA contains 160 mg of nitrogen. Nitrogen excretion was measured in urine with the use of a CHN elemental analyzer (ANA 1500; Carlo Erba Strumentazione, Milan, Italy). The urine was collected with gauze in the diaper during a 6- to 24-hour period on the study days. After centrifugation at 2800 g for 5 minutes, the urine samples were stored at −20°C until further analysis.

**Secondary Outcomes**

Hematology and biochemistry data were recorded during the first week of life. Hyperuricemia was defined as a urea concentration >10 mmol/L (28 mg/dL), and hypertriacylglycerolemia was defined as a TG concentration >3 mmol/L (265 mg/dL). In a subset of infants with an arterial catheter inserted for clinical reasons, a stable isotope study was performed on day 2 to measure the urea rate of appearance, reflecting the rate of urea synthesis (control group, n = 7; AA + lipid group, n = 9; high AA + lipid group, n = 12). During isotope infusion, no adjustments were made to the nutritional infusions. AA concentrations also were analyzed (control group, n = 11; AA + lipid group, n = 16; high AA + lipid group, n = 17) (Appendix; available at www.jpeds.com).

The time needed to regain birth weight, the growth rate during the first 28 days of life, the gain in lower leg length (knemometry)¹¹ during the first month of life, and growth until discharge home (or until 40 weeks corrected gestational age, whichever occurred first) were measured.¹² The safety of the intervention beyond the first week was monitored based on clinical outcome, that is, survival, duration of hospital stay, and neonatal morbidity. The definitions used for the clinical diagnosis are specified in the Appendix.

**Statistical Analyses**

Power calculation based on an expected increase of the nitrogen balance (primary outcome) by 75 ± 100 mg nitrogen kg⁻¹·d⁻¹ on day 2, ³ 30 infants per group were needed (α = 0.05 and β = 0.80). Accounting for expected losses to follow-up and practical limitations in blood and urine sampling, we included 50% more infants per group.

Linear regression analysis was used to calculate a mean length gain (mm/d) for each infant individually. Differences between groups were analyzed with χ² tests and a one-way ANOVA with Bonferroni correction for multiple testing, as
appropriate. Non-normally distributed data and differences between groups for urea synthesis rate and AA concentrations were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Mixed models and logistic regression analyses were used to test for significant changes in time and to correct for the potential influence of sex, gestational age, and small for gestational age (SGA). The values are expressed as the mean ± SD, number (percentage), or median (IQR). The significance level was set at \( P < .05 \). All statistical analyses were performed on an intention-to-treat basis using SPSS Version 20.0 (IBM SPSS Statistics, Somers, New York).

**Results**

We included 144 VLBW infants: 48 in the control group, 49 in the AA + lipid group, and 47 in the high AA + lipid group (Figure 1; available at www.jpeds.com). Baseline characteristics are in Table I. Parenteral AA and lipid intake (Figure 2; available at www.jpeds.com) and total parenteral + enteral protein and energy intake (Table II; available at www.jpeds.com) were in agreement with the study protocol. Compared with the targeted intake of 3.6 g AA/day, the cumulative parenteral AA deficit on day 7 was significantly lower in the high AA + lipid group than in the other 2 groups (control group: \(-13.3 ± 4.7 \) g/kg; AA + lipid group: \(-12.0 ± 2.8 \) g/kg; high AA + lipid group: \(-8.4 ± 4.8 \) g/kg). Compared with the targeted nonprotein energy intake of 90 kcal·kg\(^{-1}·d^{-1}\), the cumulative parenteral and enteral nonprotein energy deficit on day 7 was significantly lower in both intervention groups than in the control group (control group: \(-229 ± 71\) kcal/kg; AA + lipid group \(-178 ± 97\) kcal/kg; high AA + lipid group \(-181 ± 92\) kcal/kg).

According to local protocol, enteral feeding was gradually increased in all infants, starting with minimal enteral feeding. The mean enteral intake on day 2 was \(13 ± 10\) mL·kg\(^{-1}·d^{-1}\); on day 4, it was \(29 ± 22\) mL·kg\(^{-1}·d^{-1}\); and on day 6, it was \(43 ± 36\) mL·kg\(^{-1}·d^{-1}\) (no significant differences among groups). The infants received full enteral feeding after a median of 15 (IQR 12, 19) days in the control group, 15 (IQR 12, 19) days in the AA + lipid group, and also 15 (IQR 11, 17) days in the high AA + lipid group (no significant differences among groups).

In a significant number of infants, parenteral intake was adjusted because of hyperuremia (control group, \(n = 23\) [48%]; AA + lipid group, \(n = 19\) [39%]; high AA + lipid group, \(n = 38\) [81%], \(P < .001\)). In the high AA + lipid group, individual adjustments were made more frequently than in the control and AA + lipid groups (P-values of .002 and <.001, respectively). Adjustments were predominantly made during the first 2 days of life. Hypertriacylglycerolemia occurred frequently (controls 44%, AA + lipid group 27%, high AA + lipid group 45%), but we observed no differences between the groups that received lipid emulsions and the control group (\(P = .702\)). Neonatal morbidities, such as necrotizing enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity (ROP), and intraventricular hemorrhage, were not significantly more prevalent in the infants whose parenteral nutrient administration was adjusted (tested by multinominal regression with correction for gestational age, birth weight, and sex).

### Primary Outcome

Nitrogen intake, excretion, and balances on postnatal days 2, 4, and 6 are shown in Figure 3. On day 2, nitrogen balances were significantly greater in the AA + lipid groups compared with controls (\(P = .013\)), and they were greatest in the high AA + lipid group (\(P = .001\)), compared with controls. However, the nitrogen balance in the high AA + lipid group was not significantly greater compared with the AA + lipid group (\(P = 1.000\)).

### Secondary Outcomes

Plasma urea concentrations were significantly greater in the high AA + lipid group (Table III). On postnatal day 2, TG and glucose concentrations were significantly greater in the AA + lipid group compared with the control group. Aspartate amino transaminase was significantly lower on day 4 in the high AA + lipid group compared with the control group (Table III). Blood gas, platelet count, electrolytes, albumin, bilirubin, alanine amino transaminase, and cholesterol concentrations were not different among the groups. Hyperglycemia requiring insulin therapy occurred more frequently in both intervention groups than in the control group (24%, 21%, and 6%, respectively; \(P = .039\)). The urea rate of appearance on day 2 was greatest in the high AA + lipid group compared with the groups with standard AA intake. No difference was observed between the control and AA + lipid groups (Figure 4; available at www.jpeds.com).

Generally, the AA concentrations on day 2 were comparable between the control group and the high AA + lipid group and lower in the AA + lipid group (Figure 5). Most AA concentrations were within the reference range for term breastfed infants.

The median time needed to regain birth weight was 8 days (IQR 5, 12; no significant differences among the groups). Regression analysis showed no significant effect for sex, and SGA infants regained their birth weight 2 to 3 days later (\(P = .004\)). Weight gain, head circumference gain, and neurometry were not significantly different among the groups.

**Table I. Clinical characteristics**

<table>
<thead>
<tr>
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<th>Control group</th>
<th>AA + lipid group</th>
<th>High AA + lipid group</th>
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<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>27.8 ± 2.3</td>
<td>27.2 ± 2.2</td>
<td>27.2 ± 2.1</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>843 ± 224</td>
<td>876 ± 209</td>
<td>867 ± 223</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>(-2.6 ± 2.1)</td>
<td>(-1.7 ± 1.6)</td>
<td>(-1.9 ± 1.7)</td>
</tr>
<tr>
<td>SGA, n (%)</td>
<td>25 (52)</td>
<td>18 (37)</td>
<td>20 (43)</td>
</tr>
<tr>
<td>Prenatal steroids, n (%)</td>
<td>47 (98)</td>
<td>48 (98)</td>
<td>46 (98)</td>
</tr>
<tr>
<td>Apgar score at 5 minutes</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>CRIB score</td>
<td>5 ± 4</td>
<td>5 ± 3</td>
<td>5 ± 3</td>
</tr>
</tbody>
</table>

CRIB, Critical Risk Index for Babies; *Data are presented as the mean ± SD, unless otherwise indicated. †SGA birth weight z-score\(^2\) < -2.
(Table IV; available at www.jpeds.com). In all groups, weight and head circumference z-scores decreased significantly between birth and postnatal day 28 and improved between day 28 and discharge home. The weight and head circumference z-scores at discharge were not different from the z-scores at birth.

Clinical outcome, total duration of hospital stay, and peak plasma bilirubin concentrations were not different among the groups (Table V; available at www.jpeds.com). Mortality tended to be lower in the control group compared with both intervention groups, but this difference did not reach statistical significance. All deaths were related to respiratory and/or circulatory failure, sepsis, or cessation of treatment in the case of a poor neurologic prognosis. Regression analyses showed a significant effect of sex on the mortality rate, which was greater in boys in all groups. Correction for sex or being SGA at birth did not influence the differences in the clinical outcomes.

**Discussion**

We hypothesized that lipids would provide the energy required for energy-demanding protein synthesis, so that relatively fewer AA are oxidized and less nitrogen is excreted through urea synthesis and excretion. Indeed, we observed an increased nitrogen balance in the groups that received lipids. However, we could not demonstrate a decrease in the urea rate of appearance. In fact, the urea production rate increased significantly (Figure 4), suggesting that AA not used for protein synthesis were successfully oxidized, thereby preventing hyperaminoacidemia in the high AA + lipid group (Figure 5).

**Table III.** Serum glucose, urea, and TG concentrations during the first week of life

<table>
<thead>
<tr>
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<th>Control group</th>
<th>AA + lipid group</th>
<th>High AA + lipid group</th>
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<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 4</td>
<td>Day 7</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.5 ± 2.5^a</td>
<td>7.1 ± 2.6</td>
<td>5.9 ± 1.6</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>10.0 ± 4.3</td>
<td>8.2 ± 3.7</td>
<td>5.7 ± 2.8</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.8 ± 0.5</td>
<td>2.2 ± 1.4</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>Aspartate aminotransaminase, U/L</td>
<td>62.2 ± 88.3</td>
<td>30.5 ± 27.4</td>
<td>23.8 ± 11.5</td>
</tr>
</tbody>
</table>

*Data are presented as the mean ± SD.

^aSignificantly different from control group (ANOVA, P < .05).

^bSignificantly different from AA + lipid group (ANOVA, P < .05).
Several studies have shown that the early administration of AA can change a negative nitrogen balance into a positive balance, thus promoting anabolism. Although the actual protein intake showed a highly significant positive correlation with the nitrogen balance ($r = 0.761$, $P < .001$), in this study the administration of 50% additional AA combined with lipids (high AA + lipid group) did not further improve the nitrogen balance but lead to increased AA oxidation compared with standard AA plus lipids (AA + lipid group). A potential limitation in this study regarding the nitrogen balances is that these balances were calculated from the urinary nitrogen excretion, which may have underestimated the actual nitrogen excretion due to incomplete urine collections and nitrogen losses in stool, breath, and skin, although these latter losses are probably small and are relatively comparable between groups.

Growth was adequate in all groups, with a median of 8 days required to regain birth weight and an average in-hospital weight gain of 26 g·kg$^{-1}$·d$^{-1}$. Despite improved nitrogen balances, early lipids and greater levels of AA administration did not improve growth. Nonetheless, the cumulative difference in nitrogen intake and balance on days 2, 4, and 6 in between the control group and the high AA + lipid group equaled 310 and 160 mg/kg, meaning that approximately one-half of the extra administered AA were used for anabolism. The cumulative protein deficit in the high AA + lipid group after 7 days was approximately 5 g/kg less than that of the control group. If one-half of this amount is indeed used for anabolism, an extra 2.5 g protein/kg is deposited. Because neonatal tissue contains approximately 10% protein, this would amount to 25 g tissue growth per kg body weight in the first week, which is probably too small to be detected in practice.

**Figure 5.** Plasma concentrations of essential (top) and nonessential (bottom) AA on day 2 of life and reference values from healthy term breast-fed infants on postnatal day 11. Boxes and whiskers indicate the medians, IQR, and 2.5th and 97.5th percentiles. Ala, alanine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ileu, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.
In our trial, early lipid and high-dose AA administration seems safe based on hematology, biochemistry, plasma AA concentrations, and clinical outcome, although our study was not powered for differences in clinical outcomes. Hematology and biochemistry variables were not different between the treatment groups. However, infants in the early lipid groups required insulin more frequently, even though we would have hypothesized this to be less common in the high AA + lipid group. On the other hand, preterm infants are at high risk for disturbed glucose homeostasis resulting from limited substrate availability and potentially immature regulation of glucose metabolism. Parenteral lipid administration can contribute to hyperglycemia by competitively limiting glucose oxidation and by promoting gluconeogenesis.

Plasma AA concentrations were comparable between the control group and the high AA + lipid group, and they were lower in the AA + lipid group. Most AA concentrations were within previously reported ranges in healthy term breast-fed infants, in preterm infants, and in second and third trimester human fetuses in which cordocentesis was performed, although the appropriate and safe range of AA concentrations and the most suitable reference group for preterm infants are unknown.

Recent studies report on increased incidence of hypophosphatemia and hypokalemia with early and high-dose administration of AA and lipids. Despite greater nutritional intakes, in many NICUs electrolyte supplementations often is delayed until the second or third day of life. High AA intakes might induce progressive depletion of phosphate and potassium as the result of accelerated protein synthesis; for each gram of protein retained, 0.3 mmol phosphate is required for soft-tissue accretion. In case of limited phosphate supply endogenous phosphate is mobilized, demonstrated by hypophosphatemia, increased plasma calcium concentrations, diminished urinary phosphate excretion, and calcitriol. In our study, we did not measure phosphate concentrations. However, sodium, potassium, and calcium concentrations were not different between groups.

Most VLBW infants tolerate early lipid administration starting at birth, with no increased incidence of adverse events. Furthermore, early lipid administration improved nitrogen balance, thus creating conditions for anabolism and growth. Greater levels of AA administration combined with early lipid administration did not further improve the nitrogen balance, but it did lead to increased AA oxidation. We are awaiting the long-term follow-up results to determine whether this early nutritional intervention will have long-lasting effects on neurodevelopment and growth.

We thank Aimon Niklasson for calculating the growth z-scores and Vicky de Preter and Anja Luypaerts for help with the nitrogen excretion measurements.

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Appendix

Urea Synthesis Rate

Good manufacturing practice–tested $[^{15}\text{N}_2]$urea (98% enriched and tested for sterility and pyrogenicity) was purchased from Cambridge Isotope Laboratories (Andover, Massachusetts). Vials of $[^{15}\text{N}_2]$urea for the priming dose and continuous infusion were manufactured by the hospital pharmacy according to good manufacturing practice guidelines. Tests were performed to reassure the correct identity and concentration and a sterile and pyrogen-free product. Immediately before infusion, the $[^{15}\text{N}_2]$urea was dissolved in 0.9% saline. On the second day of life, the infants received a primed (50 μmol/kg), continuous (5.0 μmol·kg$^{-1}$·h$^{-1}$) infusion with $[^{15}\text{N}_2]$urea for 8 hours via the use of a Perfusor fm infusion pump (B.Braun Medical B.V., Oss, The Netherlands). After 6, 7, and 8 hours of infusion, 0.5 mL of blood was sampled from the arterial catheter. The total amount of blood sampled did not exceed 5% of the patient’s estimated total blood volume of 75 mL/kg. Immediately after collection in ethylenediaminetetraacetic acid-containing tubes, the samples were placed on melting ice and centrifuged (10 minutes, 3500 g). The plasma was stored at $-80^\circ$C until analysis. During isotope infusion, no adjustments were made to the nutritional infusions.

Plasma urea enrichment was determined on the basis of enrichment calibration curves. To each plasma sample (20 μL), 500 μL of internal standard ($[^{15}\text{N}_2,^{18}\text{O}]$urea in ethanol, 0.0644 μmol/mL) was added and used as an internal standard to calculate the concentration. After thorough mixing and centrifugation (3 minutes, 2800 g), 20 μL of the supernatant was isolated. To the supernatant, 30 μL of water, 50 μL of 0.3 M malonaldehyde bis(dimethyl acetal), and 80 μL of 37% HCl (12N) were added and thoroughly mixed. After 1 hour at room temperature, the acid was evaporated with a speedvac (GeneVac miVac; GeneVac Ltd, Ipswich, United Kingdom). After the samples were completely dry, they were derivatized by adding 50 μL of acetonitrile and 50 μL of N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide, left at room temperature for 15 minutes, and analyzed in triplicate on a gas chromatograph mass spectrometer (Agilent Technologies) on a VF-17 ms, 30 m (0.25 mm ID capillary column (Varian Inc, Middelburg, The Netherlands).

The urea rate of appearance was calculated as follows:

$$R_{\text{urea}} = \frac{1}{I} \times \left( \frac{E_t[^{15}\text{N}_2] \text{urea}}{E_{p[^{15}\text{N}_2] \text{urea}}} - 1 \right)$$

Where $I$ is the tracer infusion rate in μmol·kg$^{-1}$·h$^{-1}$, $E_t[^{15}\text{N}_2] \text{urea}$ is enrichment of the infused $[^{15}\text{N}_2] \text{urea}$ (measured as a batch) in mole percent excess, and $E_{p[^{15}\text{N}_2] \text{urea}}$ is the average $[^{15}\text{N}_2]$ plasma enrichment at steady state ($t = 6, 7, 8$ hours) in mole percent excess.

Plasma AA Concentrations

For each infant, the plasma AA concentrations were measured in 2 or 3 plasma samples withdrawn on day 2, and the concentrations of these samples were averaged. Plasma AA concentrations were analyzed with the following method: 50 μL of plasma was added to 20 μL of hydrochloric acid (pH < 3), 20 μL of internal standards mixture, and 200 μL of prewashed Dowex solution (Ag 50W-X8 H+, 200-400 mesh; Sigma Aldrich, Zwijndrecht, The Netherlands), and the samples were thoroughly shaken and centrifuged at 3000 g for 1 minute. The supernatant was discarded, and the pellet was washed twice with 1 mL of H$_2$O. After another centrifugation step, the AA were extracted from the remaining pellet using 0.5 mL of ammonia 6N and transferred to a new vial. The original vial was rinsed with 0.2 mL of ammonia 6N, and 0.2 mL of the supernatant was added to the new vial and evaporated with a speedvac (GeneVac miVac; GeneVac Ltd.). The samples were redissolved in 200 μL of H$_2$O and derivatized with ethyl chloroformate (ECF) by adding 140 μL of ethanol/pyridine (4:1) and 20 μL of ECF. The samples were left at room temperature for 5 minutes before extraction with 400 μL of hexane/dichloromethane/ECF (50:50:1). After centrifugation, 200 μL of the supernatant was transferred to a vial. The extraction step was repeated, and the second time 400 μL of the supernatant was added to the first portion. The combined solutions were evaporated under a stream of N$_2$ at room temperature, then redissolved in 50 μL of ethylacetate and analyzed in triplicate with a MSD 5975C Agilent gas chromatography mass spectrometer (Agilent Technologies) on a VF-17 ms, 30 m × 0.25 mm ID capillary column (Varian Inc). After this analysis was completed, the remaining samples were evaporated again under a stream of N$_2$ at room temperature, and 20 μL of pyridine and 50 μL of acetic anhydride was added to each sample. After 1 hour of derivatization at 60°C, the samples were evaporated under a stream of N$_2$ at room temperature and redissolved in 50 μL of ethylacetate. They were then reanalyzed in triplicate with the same gas chromatography mass spectrometer, but with an additional derivatization step to determine the concentrations of serine and threonine.

Definitions Used for Clinical Diagnosis

Symptomatic patent ductus arteriosus was defined as ductus arteriosus necessitating pharmacological treatment and/or surgical ligation. Infant respiratory distress syndrome was classified according to the typical infant respiratory distress syndrome pattern at the first chest radiograph. Bronchopulmonary dysplasia was diagnosed at 36 weeks’ corrected gestational age as determined by the physiologic definition provided by Walsh et al., with an oxygen reduction test performed if indicated. Necrotizing enterocolitis was classified according to the staging criteria of Bell et al. Late-onset septicemia during the first 28 days was defined according to the criteria of Stoll et al. A blood culture positive for coagulase-negative staphylococci combined with elevated C-reactive

### Appendix

#### Urea Synthesis Rate

Good manufacturing practice–tested $[^{15}\text{N}_2]$urea (98% enriched and tested for sterility and pyrogenicity) was purchased from Cambridge Isotope Laboratories (Andover, Massachusetts). Vials of $[^{15}\text{N}_2]$urea for the priming dose and continuous infusion were manufactured by the hospital pharmacy according to good manufacturing practice guidelines. Tests were performed to reassure the correct identity and concentration and a sterile and pyrogen-free product. Immediately before infusion, the $[^{15}\text{N}_2]$urea was dissolved in 0.9% saline. On the second day of life, the infants received a primed (50 μmol/kg), continuous (5.0 μmol·kg$^{-1}$·h$^{-1}$) infusion with $[^{15}\text{N}_2]$urea for 8 hours via the use of a Perfusor fm infusion pump (B.Braun Medical B.V., Oss, The Netherlands). After 6, 7, and 8 hours of infusion, 0.5 mL of blood was sampled from the arterial catheter. The total amount of blood sampled did not exceed 5% of the patient’s estimated total blood volume of 75 mL/kg. Immediately after collection in ethylenediaminetetraacetic acid-containing tubes, the samples were placed on melting ice and centrifuged (10 minutes, 3500 g). The plasma was stored at $-80^\circ$C until analysis. During isotope infusion, no adjustments were made to the nutritional infusions.

Plasma urea enrichment was determined on the basis of enrichment calibration curves. To each plasma sample (20 μL), 500 μL of internal standard ($[^{15}\text{N}_2,^{18}\text{O}]$urea in ethanol, 0.0644 μmol/mL) was added and used as an internal standard to calculate the concentration. After thorough mixing and centrifugation (3 minutes, 2800 g), 20 μL of the supernatant was isolated. To the supernatant, 30 μL of water, 50 μL of 0.3 M malonaldehyde bis(dimethyl acetal), and 80 μL of 37% HCl (12N) were added and thoroughly mixed. After 1 hour at room temperature, the acid was evaporated with a speedvac (GeneVac miVac; GeneVac Ltd, Ipswich, United Kingdom). After the samples were completely dry, they were derivatized by adding 50 μL of acetonitrile and 50 μL of N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide, left at room temperature for 15 minutes, and analyzed in triplicate on a gas chromatograph mass spectrometer (MSD 5975C; Agilent Technologies, Amstelveen, The Netherlands) using a VF-17 ms, 30 m (0.25 mm ID capillary column (Varian Inc)). After this analysis was completed, the remaining samples were evaporated again under a stream of N$_2$ at room temperature, and 20 μL of pyridine and 50 μL of acetic anhydride was added to each sample. After 1 hour of derivatization at 60°C, the samples were evaporated under a stream of N$_2$ at room temperature and redissolved in 50 μL of ethylacetate. They were then reanalyzed in triplicate with the same gas chromatography mass spectrometer, but with an additional derivatization step to determine the concentrations of serine and threonine.

#### Definitions Used for Clinical Diagnosis

Symptomatic patent ductus arteriosus was defined as ductus arteriosus necessitating pharmacological treatment and/or surgical ligation. Infant respiratory distress syndrome was classified according to the typical infant respiratory distress syndrome pattern at the first chest radiograph. Bronchopulmonary dysplasia was diagnosed at 36 weeks’ corrected gestational age as determined by the physiologic definition provided by Walsh et al., with an oxygen reduction test performed if indicated. Necrotizing enterocolitis was classified according to the staging criteria of Bell et al. Late-onset septicemia during the first 28 days was defined according to the criteria of Stoll et al. A blood culture positive for coagulase-negative staphylococci combined with elevated C-reactive
protein (>10 mg/L) was also considered true sepsis. Intraventricular hemorrhage was graded according to Papile et al, and periventricular leukomalacia was defined according to De Vries et al. ROP was defined according to the International Classification for ROP.

### Table II. Total (parenteral + enteral) protein and nonprotein energy intake during the first week of life

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>AA + lipid group</th>
<th>High AA + lipid group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 4</td>
<td>Day 6</td>
</tr>
<tr>
<td>Total protein intake, g·kg⁻¹·d⁻¹</td>
<td>2.1 ± 0.6*</td>
<td>2.4 ± 0.9</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>Total nonprotein energy intake, kcal·kg⁻¹·d⁻¹</td>
<td>48.9 ± 11.0</td>
<td>71.4 ± 14.6</td>
<td>73.6 ± 16.8</td>
</tr>
</tbody>
</table>

*Data are presented as the mean ± SD.

*Significantly different from control group (ANOVA, \( P < .05 \)).

*Significantly different from AA + lipid group (ANOVA, \( P < .05 \)).

### References

Table V. Neonatal outcomes

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>AA + lipid group</th>
<th>High AA + lipid group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First 28 d</td>
<td>Discharge*</td>
<td>First 28 d</td>
</tr>
<tr>
<td>PDA, pharmacologically or surgically treated, n (%)</td>
<td>17 (35)*</td>
<td>21 (43)</td>
<td>21 (45)</td>
</tr>
<tr>
<td>IRDS, n (%)</td>
<td>36 (75)</td>
<td>38 (78)</td>
<td>33 (70)</td>
</tr>
<tr>
<td>Mechanical ventilation, days</td>
<td>9.1 ± 11.4</td>
<td>10.4 ± 12.3</td>
<td>10.4 ± 11.4</td>
</tr>
<tr>
<td>BPD, n (%)</td>
<td>16 (33)</td>
<td>11 (22)</td>
<td>16 (34)</td>
</tr>
<tr>
<td>NEC ≥ grade 2, n (%)</td>
<td>2 (4)</td>
<td>4 (8)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Late-onset sepsis, n (%)</td>
<td>8 (17)</td>
<td>17 (35)</td>
<td>16 (34)</td>
</tr>
<tr>
<td>Maximum total bilirubin, μmol/L</td>
<td>150.0 ± 32.2</td>
<td>141.3 ± 37.6</td>
<td>142.7 ± 31.9</td>
</tr>
<tr>
<td>Maximum direct bilirubin, μmol/L</td>
<td>10.0 ± 18.8</td>
<td>12.4 ± 32.8</td>
<td>8.0 ± 17.7</td>
</tr>
<tr>
<td>IVH ≥ grade 3, n (%)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>PVL, n (%)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ROP ≥ grade 3, n (%)</td>
<td>2 (4)</td>
<td>0</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Repeated failure on OAE hearing test, n (%)</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Duration of hospital stay, days</td>
<td>91.0 ± 39.9</td>
<td>86.5 ± 29.1</td>
<td>94.3 ± 31.3</td>
</tr>
<tr>
<td>Mortality, n (%)</td>
<td>5 (10)</td>
<td>10 (21)</td>
<td>7 (15)</td>
</tr>
</tbody>
</table>

*Data are presented as the mean ± SD; no significant differences between groups were observed (ANOVA).
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Figure 1. Consort flow diagram. N/A, not applicable.

Figure 2. A, Parenteral AA, B, lipid, and C, glucose intake during the first week of life (mean ± SD).
Figure 4. Urea rate of appearance on day 2 of life in a subset of infants. Boxes and whiskers indicate the medians, IQR, and 2.5th and 97.5th percentiles; tested with Kruskal-Wallis and Mann-Whitney U test.