

Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life^{1–3}

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ABSTRACT

Background: We recently showed that parenteral administration of amino acids to premature infants immediately after birth is safe and results in a positive nitrogen balance and increased whole-body protein synthesis. However, we did not determine organ-specific effects; albumin, produced by the liver, is an important protein, but its concentration is often low in premature neonates during the first few days after birth.

Objective: The objective of the study was to test the hypothesis that the fractional and absolute albumin synthesis rates would increase with the administration of amino acids after birth, even at low non-protein energy intake.

Design: Premature infants (<1500 g birth weight), who were on ventilation, received from birth onward either glucose only (control group, $n = 7$) or glucose and 2.4 g amino acid $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (intervention group, $n = 8$). On postnatal day 2, all infants received a primed continuous infusion of [$1\text{-}^{13}\text{C}$]leucine, and mass spectrometry techniques were used to determine the incorporation of the leucine into albumin. Results are expressed as medians and 25th and 75th percentiles.

Results: Albumin fractional synthesis rates in the intervention group were significantly higher than those in the control group [22.9% (17.6–28.0%)/d and 12.6% (11.0–19.4%)/d, respectively; $P = 0.029$]. Likewise, the albumin absolute synthesis rates in the intervention group were significantly higher than those in the control group [228 (187–289) $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ and 168 (118–203) $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, respectively; $P = 0.030$].

Conclusion: Amino acid administration increases albumin synthesis rates in premature neonates even at a low energy intake. *Am J Clin Nutr* 2007;86:1003–8.

KEY WORDS Albumin, stable isotopes, synthesis rates, amino acids, parenteral nutrition, metabolism, premature infants, intensive care units, neonates

INTRODUCTION

The plasma albumin concentration is a routinely measured variable in the neonatal intensive care unit (NICU) and is often found to be low in ill premature infants (1, 2). Albumin, produced by the liver, has several important roles in neonatal physiology (3, 4): it is the main preserver of the colloid osmotic pressure in plasma ($\approx 75\%$), it functions as an anticoagulant, and it is an important binding transporter of certain metabolites, eg, bilirubin, free fatty acids, and drugs. Moreover, albumin is an important antioxidant because it has specific binding sites for copper

ions and a free sulfhydryl group, which can scavenge harmful reactive oxygen species (5). The free sulfhydryl group can also bind nitric oxide (NO) to form a reservoir for this regulator of vascular tone (6). Furthermore, albumin synthesis probably provides for temporary storage of amino acids (AAs), which spares them from oxidation (7–9). Albumin consists of 585 AAs, and it is the most abundant plasma protein (2), although $\approx 60\%$ of the total albumin pool is in the interstitial space (10).

Albumin metabolism has been studied mainly in healthy adults and in adults during various stages of renal or liver disease. Most studies in neonates are limited to static properties such as concentrations. Measurement of albumin synthesis rates would no doubt provide more insight into the dynamics of albumin metabolism and its response to nutrition.

Several reports have described relations between a low albumin concentration and morbidity and mortality rates in premature neonates (11, 12). In the fasting state, albumin concentrations drop 2–3 g/L in the first 24 h after birth (2). Because there is discussion about the benefits and safety of exogenous albumin infusions in premature infants (13–15), stimulation of endogenous synthesis via adequate nutritional support may be an attractive alternative.

The latter strategy requires good knowledge of the protein metabolism of premature infants. Studies using stable isotopes have provided insights into anabolism and catabolism in general (16–18). For one, the administration of AAs directly after birth stimulates whole-body protein synthesis rather than depressing protein breakdown (16). The study of whole-body metabolism is limited, however, in that it provides information on the average of all metabolic processes in the body rather than on organ-specific changes.

It is not known whether the exogenous administration of AAs also stimulates organ-specific protein synthesis, eg, that of albumin, in premature infants. Albumin synthesis can be quantified

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by measuring the incorporation rate of a stable isotope-labeled AA into plasma albumin.

We report a study in preterm infants aimed at determining the effect of AA administration starting immediately after birth on subsequent albumin synthesis. We hypothesized that AAs added to glucose would increase albumin synthesis rates.

SUBJECTS AND METHODS

Subjects

Patients were eligible for the study if they were born in the Sophia Children's Hospital, had a birth weight of <1500 g, and required an arterial line for clinical purposes. Exclusion criteria were known congenital abnormalities, chromosome defects, and metabolic, endocrine, renal, or hepatic disorders. The cohort described here was also used in an earlier study on whole-body leucine metabolism (16). It comprises a subset of infants participating in a large trial on AA administration in the immediate postnatal phase (19); these infants were selected if they were born within a defined time span during that study and if they met current inclusion criteria. The study was designed as a randomized open trial and was performed in the NICU of the Erasmus MC–Sophia Children's Hospital (Rotterdam, Netherlands). The study was investigator initiated and received no funding from industry.

Written informed consent was obtained from the parents of each infant. The protocol was approved by the Erasmus Medical Center Medical Ethics Review Board.

Experimental design

Within 2 h after birth, infants were randomly assigned to receive during the first 2 postnatal days either glucose only (control group, $n = 7$) or glucose and 2.4 g protein \cdot kg⁻¹ \cdot d⁻¹ as AA (Primene 10%; Baxter, Clintec Benelux NV, Brussels, Belgium) (intervention group, $n = 8$).

The administration of glucose solution or the AA and glucose solution was accomplished by continuous infusion. Lipids or (minimal) enteral feedings (or both) were withheld during the study period. None of the infants received exogenous albumin infusions during the study. The hospital's pharmacy dissolved L-[1-¹³C]leucine (99% enriched; Cambridge Isotope Laboratories, Andover, MA) in normal saline and tested it for sterility and pyrogenicity. We infused it (prime: 15 μ mol/kg; continuous: 15 μ mol \cdot kg⁻¹ \cdot h⁻¹) with the use of an infusion pump (Perfusor fm; B Braun Medical BV, Oss, Netherlands).

Arterial blood samples (0.4 mL) were drawn before the isotope infusion (baseline) and after 4 and 5 h of infusion. The blood samples were immediately put on melting ice and centrifuged (2500 \times g, 10 min, 4 °C), after which the plasma was stored at -80 °C until it was analyzed.

Analytic methods

To isolate plasma albumin (20), 50- μ L plasma samples were deproteinized and washed with 10% trichloroacetic acid. Water and 1% trichloroacetic acid in 96% ethanol were added to the protein pellet, and the sample was centrifuged (2500 \times g, 10 min, 4 °C). The supernatant was mixed with 26.8% ammonium sulfate to precipitate albumin overnight. The pellet was then dissolved in 0.3 mol NaOH/L, which was precipitated with 2 mol perchloric acid/L. After washing, the new pellet was dissolved in 6 mol

HCl/L, which was hydrolyzed for 24 h, after which the acid was dried under nitrogen and dissolved in water. AAs were isolated with the use of a cation-exchange column and then derivatized with ethylchloroformate, and enrichment was measured on a gas chromatograph–combustion–isotope ratio mass spectrometer (Delta XP; Thermo Electron, Bremen, Germany) as previously described (21).

As albumin precursor, we used plasma [1-¹³C] α -ketoisocaproate (α -KIC, the keto acid of leucine) enrichment at a plateau that had already been measured, as described in our earlier report (16). Liver amino acyl-tRNA enrichment forms the true precursor, but its use requires tissue biopsies and technically demanding assays. Nevertheless, α -KIC enrichment adequately represents leucyl-tRNA enrichment and is valuable in this type of research (22, 23). Plasma albumin concentrations were routinely measured on a Hitachi 912 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Calculations

The fractional albumin synthesis rate (FSR) reflects the fraction of the intravascular albumin pool that is renewed per unit of time (%/d) and can be calculated by using the following equation:

$$\text{FSR} = \frac{[(E_{\text{leu-alb},t_2} - E_{\text{leu-alb},t_1})]}{E_{\text{KIC}}} \times \frac{24}{(t_2 - t_1)} \times 100\% \quad (1)$$

where $E_{\text{leu-alb}}$ is the enrichment in mole percent excess (MPE) of incorporated leucine in albumin at t_2 and t_1 (at 5 and 4 h after the start of infusion, respectively) and E_{KIC} is the mean enrichment in MPE of the precursor, ie, plasma α -KIC, at both time points.

The absolute albumin synthesis rate (ASR) represents the absolute amount of albumin that is produced per unit of time (mg \cdot kg⁻¹ \cdot d⁻¹), and it can be calculated by using the following equation:

$$\text{ASR} = \text{FSR} \times C_{\text{alb}} \times \text{vol}_{\text{bl}} \times (1 - \text{Ht}) \times \text{weight}^{-1} \quad (2)$$

where C_{alb} is the plasma albumin concentration in g/L, vol_{bl} is the infant's total blood volume in mL [assumed to be 75 mL/kg body wt (24, 25)], (1 - Ht) is the fraction of blood that is plasma, and weight is birth weight in kg.

We also calculated the contribution (%) of albumin ASR in relation to whole-body protein synthesis in percentage on the basis of previously measured leucine turnover data according to the following equation:

$$\text{Contribution} = [(\text{ASR} \times 0.104)/(\text{NOLD} \times 131.2 \times 24 \times 0.001)] \times 100\% \quad (3)$$

where NOLD is the nonoxidative leucine disposal representing whole-body protein synthesis (in μ mol \cdot kg⁻¹ \cdot h⁻¹), which was calculated in an earlier study by our group (16). In addition, 0.104 represents the fraction of leucine residues in albumin on a weight basis, 131.2 is the mole mass of leucine, and 24 and 0.001 convert to day and milligram, respectively.

Statistical analysis

Calculations were made with MICROSOFT OFFICE EXCEL software (version 2000; Microsoft Corp, Redmond, WA), and all statistical tests were conducted with the use of GRAPHPAD

TABLE 1
Clinical characteristics¹

	Control group	Intervention group
n (male/female)	7 (2/5)	8 (4/4)
Weight (kg)	0.960 (0.780–1.080) ²	0.940 (0.770–1.070)
Gestational age (wk)	26.7 (26.4–28.9)	26.9 (26.6–29.9)
SD score for weight ³	−0.53 (−0.75 to −0.11)	−1.14 (−2.22–0.19)
CRIB score ⁴	5 (2–8)	3 (1.3–5)
5-min Apgar score	9 (7–9)	9 (8–10)
Antenatal corticosteroids, no/yes (n)	(2/5)	(1/7)

¹ No statistical differences between groups were found (Mann-Whitney test).

² Median; 25th–75th percentile (all such values).

³ Reference 26.

⁴ Reference 27.

PRISM software (version 4.0; GraphPad, San Diego, CA). Differences between control and intervention groups were tested by using Mann-Whitney tests unless stated otherwise. Values are expressed as medians and 25th and 75th percentiles or as means \pm SDs, and significance was set at $P < 0.05$.

RESULTS

Fifteen premature infants were studied—7 in the control group and 8 in the intervention group. All infants were treated with mechanical ventilation. Birth weight, gestational age, SD score for weight (26), sex, disease scores [Apgar and Clinical Risk Index for Babies (27)], and antenatal steroid use to improve lung maturation did not differ significantly between the groups (Table 1). The birth weights of 1 infant in the control group and of 2 infants in the intervention group were <2 SD when related to gestational age. Blood gas variables, whole-blood glucose concentrations, and nonprotein energy intakes (only glucose) on the second day of life did not differ significantly between groups (Table 2). Because the intervention group received AAs, the blood urea nitrogen concentrations and nitrogen balance were higher in these infants than in the control group.

TABLE 2
Study variables on the second day of life¹

	Control group (n = 7)	Intervention group (n = 8)
Nonprotein energy intake (kcal \cdot kg ^{−1} \cdot d ^{−1})	33.3 (30.7–36.9)	31.2 (26.0–32.9)
AA intake (g \cdot kg ^{−1} \cdot d ^{−1})	0	2.3 (2.3–2.4) ²
Nitrogen balance (mg N \cdot kg ^{−1} \cdot d ^{−1})	−110 (−133 to −56)	156 (116–226) ²
Blood urea nitrogen (mmol/L)	6.2 (5.8–6.9)	9.7 (7.6–11.8) ²
Glucose concentration (mmol/L)	4.9 (3.1–6.2)	3.9 (3.0–4.8)
pH	7.31 (7.25–7.33)	7.31 (7.28–7.38)
Base excess (mmol/L)	−5 (−6 to −4)	−6 (−6 to −3)

¹ All values are median; 25th–75th percentile in parentheses. AA, amino acid.

² Significantly different from control group, $P < 0.05$ (Mann-Whitney test).

The mean leucine enrichments in albumin in the control group were 0.243 ± 0.12 and 0.289 ± 0.13 MPE after 4 and 5 h of infusion, respectively. In the intervention group, enrichments were 0.201 ± 0.050 and 0.249 ± 0.050 MPE, respectively. The mean α -KIC enrichments at plateau were 7.16 ± 0.56 MPE in the control group and 5.18 ± 0.46 MPE in the intervention group.

Albumin FSR was significantly higher in the intervention group than in the control group (Figure 1). The plasma albumin concentration was measured in 5 of 7 infants in the control group and in 6 of 8 infants in the intervention group; it was significantly higher in the intervention group (Figure 2). The calculated ASR was also higher in the intervention group than in the control group (Figure 3).

Because we had also obtained leucine turnover data (16), we were able to compare the albumin ASR with the whole-body protein synthesis rate. The median NOLD (a measure of protein synthesis) increased from 130 (122–172) to 185 (169–203) μ mol leucine \cdot kg^{−1} \cdot h^{−1} after administration of AAs ($P = 0.030$). The proportion of leucine used for albumin synthesis relative to whole-body NOLD was $\approx 4\%$ in both subject groups (Figure 4), which implies that AA administration stimulates albumin synthesis and whole-body protein synthesis at a similar rate.

DISCUSSION

Our data are consistent with the assumption that parenteral AA administration in premature neonates stimulates albumin synthesis. On the second day of life, albumin FSR and ASR and plasma albumin concentrations were significantly higher in premature infants who had received parenteral AAs from birth onward than in those who had not received parenteral AAs. The plasma albumin concentration is governed by 4 processes (or changes): changes in synthesis, degradation, intravascular space, and transcapillary escape. In the present study, we were not able to measure the latter 3 processes, but we speculate that greater synthesis is the primary cause for the higher plasma concentration noted.

Alternatively, a rise in albumin synthesis does not automatically coincide with a parallel rise in concentration. Albumin is a negative acute-phase protein, which means that its concentration will decrease during an inflammatory event. Such decreases have been described during cholecystitis (28), hemodialysis (29), and cancer (30) and in head trauma patients (31), despite a coinciding rise in albumin FSR. Cytokines might be responsible for this paradoxical increase (29, 32). The lowered concentrations probably result from concomitant increases in catabolic rate and transcapillary escape.

The albumin FSR in healthy adults is ≈ 6 – $8\%/d$ (8, 9, 32–36), and it seems unresponsive to intravenous nutrients (37). Meals, however, will increase albumin synthesis (7, 8, 38). A recent study showed that the protein portion of meals is the component responsible for this increase (9). Adults undergoing chronic hemodialysis also were found to be sensitive to nutrition, because albumin FSR improved after intradialytically administered nutrition (20). Overall, it seems that albumin synthesis in adults is more responsive to gastrointestinal nutritional uptake than to intravenous nutrition, as was also shown after surgery (39) and in rats (40). Our findings in human neonates and the findings of others in young piglets (41, 42) suggest that other metabolic mechanisms may be regulating albumin synthesis in younger

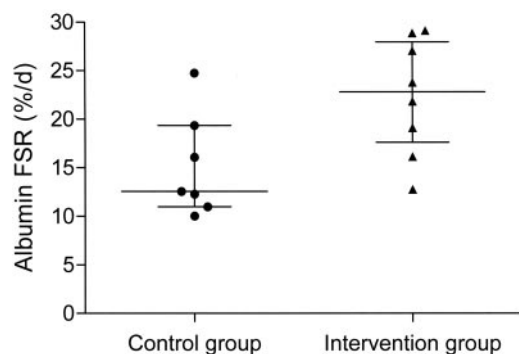


FIGURE 1. Medians and interquartile ranges of fractional albumin synthesis rates (FSR; in %/d) in the control ($n = 7$) and intervention ($n = 8$) groups. Albumin FSR was significantly higher in the intervention group than in the control group ($P = 0.029$, Mann-Whitney test).

persons, in whom albumin synthesis is also responsive to parenteral nutrition.

Consistent with the general finding that younger persons have higher metabolic rates than do adults, a higher albumin FSR, ranging from 15%/d to 20%/d, has been found in 12-mo-old infants (43, 44). Bunt et al (21) found values of 14%/d on the first postnatal day in fasted premature infants with a gestational age of 28 wk. Yudkoff et al (45) calculated a mean albumin FSR of 12%/d in parenterally fed, premature neonates with appropriate-size-for-gestational-age (which was 28 wk), after ≈ 1 wk of life. These figures correspond well with the synthesis rates we observed in the present study. Yet, unlike Bunt et al (21), we did not find clear correlations between the FSR and SD scores for weight related to gestational age. This may have been the result of reduced power in our study or of interference by our nutritional intervention.

We calculated that albumin constitutes $\approx 4\%$ of all proteins synthesized in the body. In addition, it was estimated that, of all proteins synthesized in the liver of healthy humans and rats, including those not excreted but produced for intrahepatic maintenance, 15% was albumin (34, 38). The combination of these figures shows that the liver would contribute $>25\%$ to whole-body protein synthesis. Normal hepatic functioning would, therefore, seem to be of vital importance.

Apart from all of the important roles of albumin mentioned earlier, the possibility of increasing the albumin FSR is interesting from a nutritional point of view. A higher albumin ASR

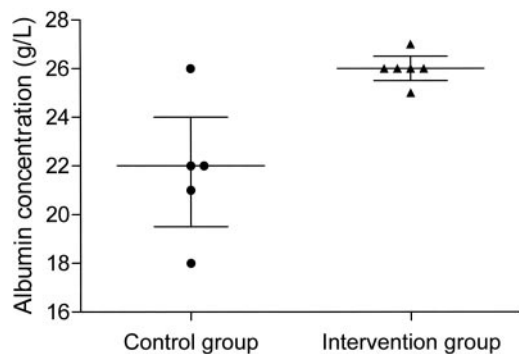


FIGURE 2. Medians and interquartile ranges of albumin plasma concentrations (in g/L) in the control ($n = 5$) and intervention ($n = 6$) groups. The plasma albumin concentration was significantly higher in the intervention group than in the control group ($P = 0.030$, Mann-Whitney test).

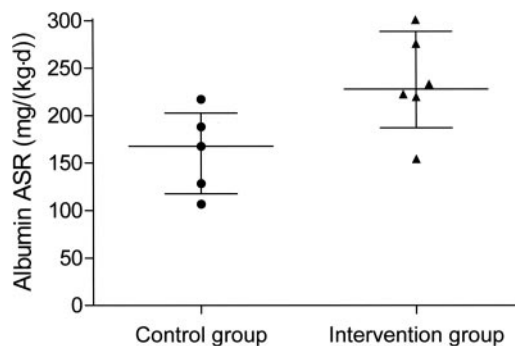


FIGURE 3. Medians and interquartile ranges of absolute albumin synthesis rates (ASR; in $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) in the control ($n = 5$) and intervention ($n = 6$) groups. The albumin ASR was significantly higher in the intervention group than in the control group ($P = 0.030$, Mann-Whitney test).

makes premature infants less vulnerable to catabolic insults through the temporary storage of AAs in albumin, which prevents excess AAs from being oxidized. Later, during low protein intake or increased protein demands, body protein stores can be spared—albeit at the cost of albumin breakdown—thereby releasing free AAs.

Especially in the first 24 h after premature birth, nonprotein energy intake is usually very low ($\approx 30\text{--}35 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and less than desirable. Recently, energy expenditure was measured in premature infants during the first few days of life, in which they had comparable energy intakes (46). Energy expenditure was estimated at $29\text{--}35 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, which, at an intake of $\approx 30 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, would leave no calories for net energy storage or growth. As a consequence, AA efficacy in terms of anabolism is usually moderate, in that a large fraction will be irreversibly oxidized (16).

Carbohydrate intake is limited because of potential hyperglycemia and fluid restrictions. Moreover, parenteral lipids are often withheld in the first 24 h after premature birth, because neonatologists fear pulmonary disease, hypertriglycerolemia, and high free fatty acid concentrations that could lead to competition with bilirubin for binding on albumin (47).

Albumin is the main transport vehicle for fatty acids to and from the tissues, according to metabolic demands. Notwithstanding the fact that lipids in blood are largely in the form of triacylglycerols, the turnover and utilization of fatty acids bound to

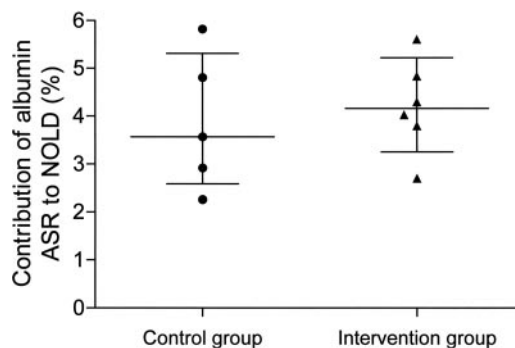


FIGURE 4. Medians and interquartile ranges of the contribution (in %) of absolute albumin synthesis rate (ASR) to whole-body protein synthesis [represented by nonoxidative leucine disposal (NOLD)] in the control ($n = 5$) and intervention ($n = 8$) groups. There was no significant difference between groups (Mann-Whitney test).

albumin are high, which makes fatty acids an important contributor to lipid metabolism (48). Providing AAs immediately after birth increases albumin synthesis and subsequent binding capacities, and those changes theoretically improve the infant's tolerance of intravenous lipids. Apart from the advantage of delivering essential fatty acids, the high energy content makes immediate commencement of lipids after birth beneficial by improving the energy balance, which, in turn, may stimulate protein synthesis even more.

A recent study with high-dose AAs and lipids initiated immediately after birth showed high anabolic use of AAs, probably because of a greater energy availability (49). We speculate that an increased albumin synthesis rate was at least partially responsible for the increased tolerance of lipids. More clinical trials are required to determine the efficacy and safety of parenteral administration to premature infants of lipids together with high-dose AAs that begins immediately after birth.

A potential limitation of this study is that the hydrolyzed protein pellet may not have contained pure albumin. Jacobs et al (50) reported earlier that, after simple ethanol extraction, $\approx 8\%$ of proteins were contaminants. By adding ammonium sulfate, we aimed to eliminate some of the contaminating proteins (20). However, even if purification of the protein pellet was still incomplete, nearly all of the proteins must have been albumin.

In conclusion, we have shown that introducing AAs immediately after birth to premature neonates stimulates not only whole-body protein synthesis but also albumin synthesis. This finding may have important implications in view of the vital roles of albumin, such as serving as an antioxidant and binding bilirubin and free fatty acids. Improving albumin synthesis may, therefore, have a major effect on later outcome.

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