

Albumin synthesis in preterm infants on the first day of life studied with [1-¹³C]leucine

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Bunt JE, Rietveld T, Schierbeek H, Darcos Wattimena JL, Zimmermann LJ, van Goudoever JB. Albumin synthesis in preterm infants on the first day of life studied with [1-¹³C]leucine. *Am J Physiol Gastrointest Liver Physiol* 292: G1157–G1161, 2007. First published January 18, 2007; doi:10.1152/ajpgi.00300.2006.—Albumin is the major binding protein in the human neonate. Low production of albumin will lower its transport and binding capacity. This is especially important in preterm infants, in whom albumin binds to potentially toxic products such as bilirubin and antibiotics. To study the metabolism of plasma albumin in preterm infants, we administered a 24-h constant infusion of [1-¹³C]leucine to 24 very low birth weight (VLBW) infants (28.4 ± 0.4 wk, 1,080 ± 75 g) on the first day of life. The caloric intake consisted of glucose only, and therefore amino acids for albumin synthesis were derived from proteolysis. The fractional synthesis rate (FSR) of plasma albumin was 13.9 ± 1.5%/day, and the absolute synthesis rate was 148 ± 17 mg·kg⁻¹·day⁻¹. Synthesis rates were significantly lower (*P* < 0.03) in infants showing intrauterine growth retardation. Albumin synthesis increased with increasing SD scores for gestation and weight (*P* < 0.05). The FSR of albumin tended to increase by 37% after administration of antenatal corticosteroids to improve postnatal lung function (*P* = 0.09). We conclude that liver synthetic capacity is well developed in VLBW infants and that prenatal corticosteroids tend to increase albumin synthesis. Decreased weight gain rates in utero have effects on protein synthesis postnatally.

synthesis of albumin; intrauterine growth retardation; gas chromatography-isotope ratio mass spectrometry

SERUM ALBUMIN IS THE SINGLE most abundant plasma protein and is synthesized exclusively in the liver. Albumin's numerous functions include maintenance of colloid osmotic pressure (accounting for ~80%), buffering, and transport of bilirubin, uremic toxins, porphyrins, fatty acids, metals, cortisol, thyroxine, endotoxins, medications, and endogenous nitric oxide. Furthermore, it is thought to be an important antioxidant and as such could play a role in neuronal survival during development (42). These functions are of vital importance to the critically ill preterm neonate. Albumin levels are often low in the postnatal period and variable between individuals. Very preterm infants show normal serum values of 20 g/l on day 1 (38) that rise by about 15% during the first 3 wk (8). Albumin infusions are indicated to treat oligouria and edema or hyperbilirubinemia in critically ill preterm infants whose plasma values are lower than a certain threshold (10–20 g/l) (24). Plasma albumin levels are not only influenced by plasma volume and transcap-

illary escape rate but also by the balance between synthesis and degradation. Therefore, it is important to study the regulation of albumin synthesis. Low synthesis rates in the critically ill preterm infant could result from immaturity of hepatic synthetic pathways, suboptimal nutritional status, and limited substrate availability. We studied plasma albumin synthesis in mechanically ventilated very low birth weight (VLBW) infants on the first day of life, when they were given only glucose for caloric intake. Among other variables, we related the synthesis rates to intrauterine growth and prenatal corticosteroids.

METHODS

Subjects and study design. Over a period of 3 yr, we studied infants with a gestational age of <32 wk admitted to the neonatal intensive care unit of the Erasmus Medical Center-Sophia Children's Hospital who required mechanical ventilation and an arterial catheter for clinical purposes. Exclusion criteria were congenital infection and maternal diabetes. Written parental informed consent was obtained, and the study was approved by the medical ethics committee of the Erasmus Medical Center.

Patients received a constant intravenous infusion of the stable isotope L-[1-¹³C]leucine (Campro Scientific, Veenendaal, The Netherlands) for 24 h at 1 mg·kg⁻¹·h⁻¹. Leucine is an essential amino acid, accounting for ~10% of the amino acids in albumin. The start of the study [time (*t*) = 0] was defined by the start of the isotope infusion, i.e., 5.3 ± 0.3 h after birth. Patients received glucose 10% [4.2 mg·kg⁻¹·min⁻¹ (25 kcal·kg⁻¹·day⁻¹) in adequate for gestational age (AGA) infants and 5.7 mg·kg⁻¹·min⁻¹ (33 kcal·kg⁻¹·day⁻¹) in small for gestational age (SGA) infants] with calcium gluconate and broad-spectrum antibiotics (penicillin and tobramycin) as routine neonatal care. The infants did not receive any amino acids or lipids. Albumin supplementation was withheld during the study. One milliliter of arterial blood was drawn just before the start of the isotope infusion and subsequently every 6 h for the determination of ¹³C enrichment of plasma α-ketoisocaproic acid (α-KICA) and albumin. Our clinical laboratory determined plasma albumin levels by routine measurements.

Analytical procedures. Blood was collected straight into lithium-heparin-containing vacutainers and directly centrifuged, and the plasma was stored at -70°C. Albumin was purified according to the method of Debro and Korner (15) and was hydrolyzed in 6 M HCl for 24 h. The amino acids were isolated by ion exchange and were converted to the *N*-ethoxyethylcarbonyl derivative by using ethylchloroformate. Plasma α-KICA was obtained from the supernatant after protein precipitation with TCA. α-KICA was derivatized to a butyldimethylsilylquinoxalinol derivative as previously described by us (49).

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Mass spectrometric analysis. The ^{13}C enrichment of α -KICA was determined by split injection (50:1) of 1 μl in a Carlo Erba GC 8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (Interscience, Breda, The Netherlands) and by measuring the intensity of the 259 and 260 fragments in electron-impact ionization mode. The ^{13}C enrichment of leucine in albumin was measured by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Aliquots of 0.5 μl of the suspension containing the amino acid derivatives were introduced in splitless mode on a Trace GC (Thermo Electron). A Sil-24 CB (Varian) capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.5 μm) was used for the chromatographic separation. The amino acids were online combusted and introduced as CO_2 into a Delta-XP IRMS (Thermo Finnigan, Bremen, Germany) (39). Samples were analyzed three times.

Calculations. Calculations were performed assuming a constant pool size of total amino acids and of albumin as well as constant rates of albumin synthesis and degradation over the study period (2, 53). To combine GC-MS and GC-C-IRMS measurements, the enrichment of leucine in plasma albumin was corrected for unlabeled carbon atoms due to $[1-^{13}\text{C}]$ leucine administration and unlabeled carbon atoms added during derivatization (five atoms); thus the correction factor was 11. Baseline enrichments of $[1-^{13}\text{C}]\alpha$ -KICA and $[1-^{13}\text{C}]$ leucine in plasma albumin were measured and subtracted from the respective enrichments during isotope infusion. The fractional synthesis rate (FSR) of albumin, i.e., the percentage of the total plasma pool of albumin synthesized per day, was calculated for each individual patient using the following equation.

$$\text{FSR}_{\text{albumin}} = \frac{(\text{IE}_{\text{leucine in albumin } t2-t1}) \times 100 \times 24}{(\text{IE}_{\alpha\text{-KICA at plateau}})} \quad (1)$$

where $\text{IE}_{\text{leucine in albumin } t2-t1}$ represents the slope of albumin-bound leucine enrichment over points $t2$ and $t1$ (in h) between $t = 6$ h and $t = 24$ h in that individual patient, and $\text{IE}_{\alpha\text{-KICA at plateau}}$ is the steady-state plasma enrichment of $[1-^{13}\text{C}]\alpha$ -KICA between $t = 6$ h and $t = 24$ h and represents the intracellular ^{13}C enrichment of leucine in that individual patient. The 100 is used to convert FSR to percentages, and 24 is used to normalize data per day. The absolute synthesis rate (ASR) was calculated as follows: $\text{ASR} = \text{FSR} \times 0.08 \times (1 - \text{hematocrit}) \times [\text{albumin}]_{\text{plasma}}$. The factor 0.08 corresponds to the assumed volume of blood per kilogram.

Data analysis. Data are presented as means \pm SE. Comparisons between groups were performed with a Student's *t*-test. Correlations were assessed by using Pearson's coefficient of correlation. A value of $P < 0.05$ was accepted as significant.

RESULTS

We studied 24 VLBW infants with a gestational age of 28.4 ± 0.4 wk and a birth weight of $1,080 \pm 75$ g. Thirteen infants (54%) had received corticosteroids prenatally to improve lung function after birth. All infants were mechanically ventilated for 11 ± 2.4 days and received surfactant when indicated. None had suffered from perinatal asphyxia.

The plasma albumin level was 25 ± 0.6 g/l and was stable during the study. The ^{13}C enrichment of plasma α -KICA reached steady state before $t = 6$ h in all infants (Fig. 1). The ^{13}C enrichment of leucine in plasma albumin increased linearly as shown in Fig. 2. The FSR was $13.9 \pm 1.5\%$ /day (range 2.0–25%/day). The ASR was 148 ± 17 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (range 19–286 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). The FSR and ASR data were scatter plotted in Microsoft Excel for Windows XP. The best curve-fitting trend line (i.e., the highest r^2 value) was used to define the relationship. Both FSR and ASR correlated with the SD score of birth weight vs. gestational age (Figs. 3 and 4) (47). The FSR ($8.3 \pm 3.1\%$ /day) and ASR (76 ± 22

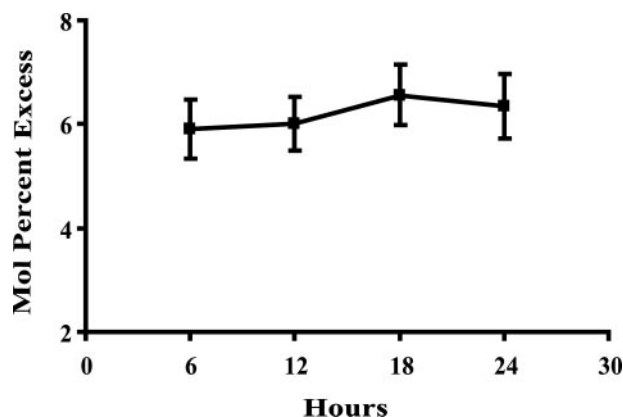


Fig. 1. Enrichment of plasma $[1-^{13}\text{C}]\alpha$ -ketoisocaproic acid ($[1-^{13}\text{C}]\alpha$ -KICA) during isotope infusion.

$\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) were significantly lower (both $P < 0.03$) in SGA infants (< -2 SD score, $n = 6$) compared with FSR ($15.7 \pm 1.5\%$ /day) and ASR (172 ± 18 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) of AGA infants ($-2 < \text{SD score} < +2$, $n = 17$) (Fig. 5). One infant was large for gestational age (SD score +2.7) and showed a higher albumin synthesis rate (FSR = 24.5%/day, ASR = 274 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). The patients that did not receive prenatal corticosteroids ($n = 10$) had a FSR of albumin of $12.5 \pm 2.06\%$ /day; the patients that did receive prenatal corticosteroids within 24 h before the study had a FSR of $16.5 \pm 1.59\%$ /day ($P = 0.3$ vs. no steroids). The patients that did receive prenatal corticosteroids within 48 h before the study ($n = 8$) had a 36.8% higher FSR compared with the patients that did not receive prenatal corticosteroids (FSR $17.1 \pm 1.31\%$ /day; $P = 0.09$ vs. no steroids).

DISCUSSION

We measured the synthesis of albumin in VLBW infants on the first day of life. The FSR of plasma albumin was $13.9 \pm 1.5\%$ /day, and the ASR was 148 ± 17 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Infants with intrauterine growth retardation (IUGR) had lower rates of synthesis, and antenatal corticosteroids tended to increase albumin synthesis ($P = 0.09$). We studied albumin synthesis shortly after birth, when plasma bilirubin levels are elevated. High levels of unbound bilirubin can lead to bilirubin encephalopathy (kernicterus), which may prompt albumin supplementation in selected cases.

The turnover of albumin has been studied extensively in adults with cirrhosis, nephrosis, infection, or trauma and in pregnant women. Little is known, however, about these processes and their regulation in infants and neonates. To our knowledge, only one study (52) of albumin synthesis in preterm infants has been published. In that study, preterm AGA infants on day 7 had a FSR of albumin of $\sim 12\%$ /day, which is comparable with our findings. Comparing our data with studies in adults, it could be suggested that albumin synthesis in preterm infants is higher than in adults, who were found to have a FSR of $\sim 7\%$ /day and an ASR of ~ 115 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (1, 3–5, 14, 19, 26, 36, 37). The high ASR in the present study in VLBW infants is also found in young animals. However, in most animal studies the feed that was used contained amino acids or protein, which probably increases albumin synthesis versus similar nonprotein caloric intake (11, 12, 21), although

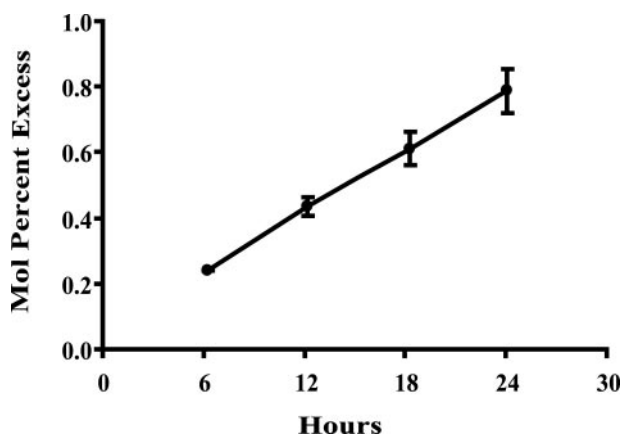


Fig. 2. Enrichment of $[1-^{13}\text{C}]$ leucine in plasma albumin during isotope infusion.

other findings on this issue disagree (46). The high albumin synthesis rates in premature infants and animals is probably attributable to a generalized intensification of whole body protein turnover, which is characteristic of such small infants (29, 30), although the reason for this high turnover is unclear.

It is known that dietary amino acid composition and energy intakes are important determinants of nitrogen retention and total body protein turnover (16). In our study, the infants received 25–35 nonprotein kcal·kg⁻¹·day⁻¹ (43, 44). There are data suggesting that increasing energy intake or increasing amino acid administration will, individually and in combination, stimulate protein accretion for growth (41). The amino acid composition in the diet plays a role in the availability of the different amino acids for protein synthesis. If one rate-limiting amino acid is not available, other amino acids will be present pro rata more abundantly and will be oxidized. The study of which amino acid is rate limiting is an interesting research field directed to improve feeding regimens for preterm infants. The concept of whole body protein turnover is necessarily artificial because it represents the integral of many different protein pools, each of which has a unique regulation and rate of metabolism (27, 28, 53). During protein administration, term infants (33) and adults (20) respond by decreasing proteolysis, whereas fetuses (25) and preterm infants (34, 45, 48, 51) increase protein synthesis, rather than suppress break-

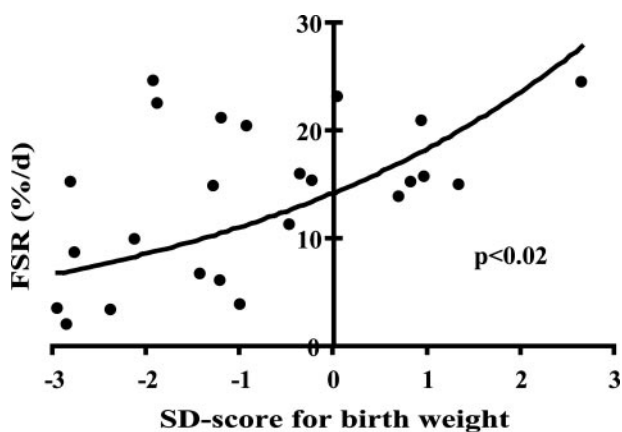


Fig. 3. Fractional synthesis rate (FSR) in relation to the SD score for birth weight. FSR: $y = 14.18e^{0.253x}$.

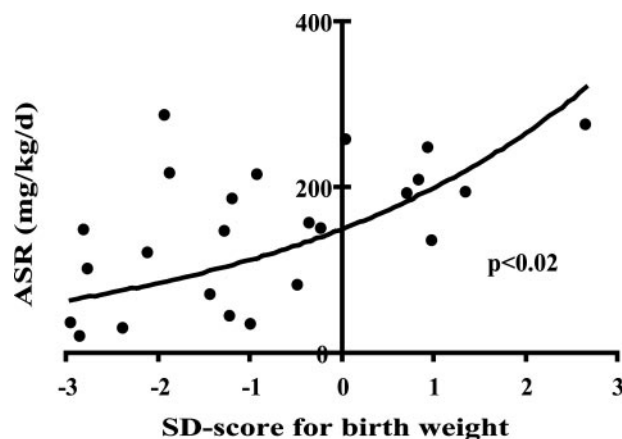


Fig. 4. Absolute synthesis rate (ASR) in relation to the SD score for birth weight. ASR: $y = 149.01e^{0.287x}$.

down. Only one study in premature infants found a concomitant decrease in proteolysis (10).

In our study, synthesis rates were correlated with SD scores of birth weight and gestational age and were significantly lower after IUGR. The reduced albumin synthesis after IUGR could reflect the low intrauterine albumin synthesis due to a lower availability of amino acids in utero. The data suggest that low intrauterine supply of nutrients may restrict growth and potentially reduce postnatal protein turnover, including albumin turnover, which has been shown in rats (28). It is unknown to what extent this reduced synthesis of albumin persists after birth and whether it is representative of the process of total body nitrogen accretion (growth), although there are suggestions that after IUGR, the infants have higher turnover rates when fed (32). The lower albumin synthesis rate in SGA infants is probably not due to reduced proteolysis as a result of higher intake of glucose (which is standard clinical treatment; see METHODS), because high intake of glucose was found not to reduce proteolysis (22). Although not done in the present study, administration of amino acids starting from birth has been shown to be safe and promote growth and is widely advocated (35, 43).

We did not find a correlation of albumin concentration and the FSR, which is usually present in adults (2, 53), probably

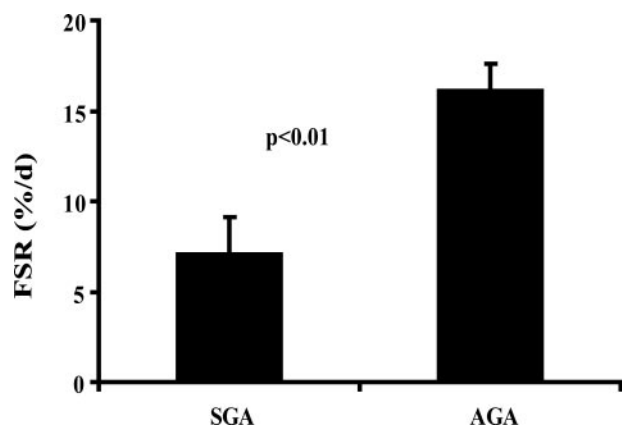


Fig. 5. FSR is significantly lower in small for gestational age (SGA, $n = 6$) compared with adequate for gestational age (AGA, $n = 17$) infants.

because the concentration of albumin varied within a narrow range (25 ± 0.6 g/l), and therefore the study did not have enough discriminative power. Additionally, normal albumin concentration at birth after 26 wk gestation is 19 g/l, increasing to 31 g/l at birth (8), and postnatal albumin concentration increases only slowly, at a rate of 5%/wk postnatal age (8). It could be speculated that in the infants with lower albumin levels, the albumin synthesis does not increase more because of lower set points for albumin concentrations (8).

The effect of illness (9) and stress (27) on albumin synthesis has been studied in adults, with conflicting results. Acute sepsis is often associated with low plasma albumin levels, probably due to increased capillary leak, in combination with increased albumin synthesis, which sometimes cannot fully compensate for loss of albumin from the circulation (17, 18, 23, 31, 40). McNurlan et al. (27) showed that a combination of stress hormones is a potent stimulator of albumin synthesis in healthy humans. In preterm human infants, postnatal steroids are known to increase protein breakdown without an effect on protein synthesis (7, 50). However, the effects of antenatally administered corticosteroids is less well studied; one study (13) did not show an effect on whole body amino acid metabolism. In our study the infants that received prenatal corticosteroids for lung maturation tended to have a 37% increase in albumin synthesis ($P = 0.09$), but this was not statistically significant for several possible reasons: 1) all infants were critically ill and mechanically ventilated and stressed, and therefore the study did not have enough discriminative power; 2) albumin synthesis was limited by the availability of amino acids or nonprotein energy; 3) the doses used to induce lung maturation were not sufficient to stimulate albumin synthesis; and 4) albumin synthesis is regulated differently in utero or in preterm infants compared with adults.

In the present study we isolated albumin by using ethanol (15). However, it has been shown recently (23) that the isolation of plasma albumin by using ethanol can result in contamination and overestimations of enrichment by as much as 10%, and a new isolation procedure would probably be superior for mass spectrometry purposes. Thus the rates of albumin synthesis may have been overestimated by us, but the comparison between patients and with earlier studies using the same isolation procedure remains valid.

The total body albumin pool is divided into an intravascular pool (~50% of total pool) and an extravascular pool (~50% of total pool). The turnover of intra- and extravascular albumin (transcapillary exchange) is unknown in infants and children. In adults it is estimated to be ~5%/h (6). This equilibration between the intra- and extravascular albumin pool may have caused us to label the extravascular albumin partially as well. Therefore, the ASR we calculated could be underestimated.

We conclude that direct postnatal albumin synthesis is high in preterm infants, even in the absence of exogenous amino acids. Prenatal corticosteroids tended to increase postnatal albumin synthesis. The synthetic capacity of the liver has probably developed well in these infants. Preterm infants with IUGR had significantly lower albumin synthesis rates. Further studies are needed to explore whether albumin synthesis, especially in these infants, could be increased.

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GRANTS

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