

# Human fetal albumin synthesis rates during different periods of gestation<sup>1-3</sup>

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## ABSTRACT

**Background:** Despite nutritional intervention, albumin concentrations are often low in critically ill premature neonates.

**Objective:** Our aim was to quantify albumin synthesis rates during early life under physiologic circumstances. Human fetuses thereby reflect the developmentally related optimal condition.

**Design:** Pregnant women undergoing elective cesarean delivery received 3 different labeled amino acid infusions starting at different times before surgery. With the use of mass spectrometry techniques, this novel model enabled us to quantify fetal albumin synthesis from a single blood sample taken from the umbilical cord after cesarean delivery. The fractional synthesis rate reflects the fraction of the albumin pool that is daily renewed. The absolute synthesis rate is the absolute amount of albumin that is daily synthesized. Results are expressed as medians (25th-75th percentile).

**Results:** We studied 8 fetuses at 29.9 (28.4-35.4) weeks of gestation and 8 fetuses around term. Fractional synthesis rates in premature fetuses [17.5 (12.1-24.4) %/d] were higher ( $P = 0.02$ ) than in mature fetuses [10.4 (9.1-13.7) %/d]. Absolute synthesis rates were also higher ( $P = 0.02$ ) in premature than in mature fetuses: 280 (227-365) versus 205 (184-238)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ .

**Conclusions:** On a weight basis, albumin synthesis rates in premature fetuses were higher than in fetuses at term and were higher than the rates previously found in neonates after preterm birth. Considering that the premature fetal liver can synthesize albumin at a high rate, the observed hypoalbuminemia in premature infants therefore seems to suggest that current (nutritional) therapies fail to meet requirements necessary to sustain optimum albumin synthesis rates. *Am J Clin Nutr* 2008;88:997-1003.

## INTRODUCTION

Albumin concentrations are considered a marker of nutritional status, and albumin synthesis rates a measure of liver activity. Albumin is the major export protein produced by the liver and forms more than one-half of the total plasma protein mass. Albumin has been described as "the body's tramp steamer, shuttling cargo of various kinds between ports of call" (1). Its load includes bilirubin, cysteine, free fatty acids, calcium, and drugs. Besides, albumin preserves the colloid osmotic pressure and is an important antioxidant.

Recently, we determined albumin synthesis rates in premature infants immediately after birth who had received only glucose (2). These rates almost doubled in response to additional intravenous amino acid administration (3). Despite this increase,

plasma albumin concentrations were still very low. However, having knowledge of albumin synthesis rates during early life under physiologic circumstances, ie, pregnancy, would enable us to relate the intrauterine with the extrauterine synthesis rates. To this aim, we used a stable-isotope model allowing measurements on the human fetal albumin synthesis rates.

It has been long known that animal (4, 5) and human (6) fetuses are capable of endogenous albumin synthesis from early pregnancy on. Besides, all albumin in the fetus is from fetal origin because albumin does not cross the hemochorial placenta as shown in the rat (7), guinea pig (8), and the in vitro dually perfused human placenta (9). Also, after intravenous injection of radioiodinated albumin to pregnant women, only trace amounts were found in umbilical cord blood (10, 11). Furthermore, fetal plasma albumin concentrations at term are often higher than in maternal plasma (12, 13), which suggests no passive materno-fetal transport. In addition, normal concentrations of fetal plasma albumin were found during mild or severe maternal hypoalbuminemia (13, 14).

The only available kinetic information on albumin synthesis, however, is in the ovine fetus, where the albumin fractional synthesis rates (FSRs) were determined (15, 16). The albumin FSR reflects the fraction of the intravascular albumin pool that is renewed per unit of time. The FSR is usually calculated by infusing one stably labeled amino acid and obtaining multiple blood samples at consecutive time points. From the increase of tracer incorporation in albumin over time, one can calculate its synthesis rate. In humans, however, the insertion of catheters in

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<sup>2</sup> Supported by Sophia Children's Hospital Fund (SSWO) no. 459 (institutional grant, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands); Nutricia Research Foundation no. 2006-10 (independent charity, Nutricia, Wageningen, Netherlands).

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Received April 8, 2008.

Accepted for publication June 23, 2008.

the fetus or umbilical cord for repetitive blood sampling is impossible on ethical grounds. Obtaining blood from both the umbilical vein and artery is only possible at birth. We therefore modified the staggered infusion protocol proposed by Dudley et al (17) into a simplified model enabling us to measure the synthesis rate of albumin from a single blood sample taken at birth.

## SUBJECTS AND METHODS

### Setting and subjects

The study was performed at the Mother and Child Center of the Erasmus MC–Sophia Children's Hospital after approval by the Dutch (CCMO, The Hague) and the institutional medical ethical review board. Pregnant women scheduled to undergo elective cesarean delivery (repeat, breech, or multiple pregnancy) were eligible. We aimed to include fetuses who were close to term as well as fetuses who were still premature. Exclusion criteria were obesity (preconceptional body mass index, in kg/m<sup>2</sup>, >30), diabetes, or known fetal anomalies. Participants gave written consent after having been fully informed about the study.

### Experimental design

L-[1-<sup>13</sup>C,<sup>15</sup>N]Leucine, L-[1-<sup>13</sup>C]phenylalanine, and L-[U-<sup>13</sup>C<sub>5</sub>]valine were bought from Buchem BV, Apeldoorn, The Netherlands (local distributor of Cambridge Isotope Laboratories, Andover, MA, USA) (all 99% enriched and tested for sterility and pyrogenicity). Our hospital pharmacy dissolved the isotopes in 0.9% saline after which the solution was filtered (0.2 μm) and sterilized. Tests were performed to reassure the correct identity, concentration, and a sterile and pyrogen-free product.

Pregnant women received primed, continuous, stable-isotope infusions of L-[1-<sup>13</sup>C,<sup>15</sup>N]leucine (8 μmol · kg<sup>-1</sup> · h<sup>-1</sup>), L-[1-<sup>13</sup>C]phenylalanine (5 μmol · kg<sup>-1</sup> · h<sup>-1</sup>), and L-[U-<sup>13</sup>C<sub>5</sub>]valine (5 μmol · kg<sup>-1</sup> · h<sup>-1</sup>), starting at least 4, 3, and 2 h before planned surgery, respectively. The priming doses were half of the hourly doses. Tracers were given in a forearm vein with 3 separate Perfusor fm infusion pumps (B Braun Medical BV, Oss, Netherlands) until surgery was completed.

Maternal blood was sampled before the tracer infusions had begun (baseline) and from a contralateral maternal forearm vein immediately before anesthesia started. Fetal blood was sampled from both the vein and arteries of a doubly clamped segment of the umbilical cord immediately after delivery. After collection, blood samples were centrifuged (2000 × g) in heparin tubes and plasma was frozen at -80 °C until analyzed.

### Blood sample analyses

To isolate albumin from plasma, we used anti-human serum albumin affinity resin kits (Vivascience; Sartorius Group, Hannover, Germany). Fetal and adult albumin are indistinguishable (18). Enclosed spin columns were filled with 400 μL affinity resin and 25 μL of thawed plasma. According to the included protocol, the column was washed 3 times with a tris-buffer, and albumin was thereafter eluted from the affinity resin with 0.1 mol glycine/L (acidified to pH 2.5 with HCl). Eluted albumin was precipitated with 750 μL of 2 mol HClO<sub>4</sub>/L. A washing step was performed with 0.2 mol HClO<sub>4</sub>/L by resuspending and precipitating the pellet again. The protein pellet was then hydrolyzed in 140 μL of 6 mol HCl/L for 22 h at 110 °C. After hydrolyzation, the acid was evaporated by using a speedvac, after which the

dried amino acids were dissolved in H<sub>2</sub>O. Samples were derivatized using propylchloroformate (commercial kits: Phenomenex for hydrolysates, EZ:Faast, Bester BV, Amstelveen, Netherlands) and measured in triplicate on a gas chromatograph–combustion–isotope ratio mass spectrometer (GC-C-IRMS; Delta XP, Thermo Electron, Bremen, Germany) (2).

The enrichments of the true albumin precursors (intrahepatic amino-acyl tRNA) can obviously not be measured in the human fetus or mother. Because keto acids are intracellularly derived metabolites of amino acids, their enrichment has been advocated as a surrogate precursor (19, 20). However, keto acids are also transported transplacentally and it is thus not possible to discriminate whether the keto acids have undergone intracellular metabolism in the maternal, placental, or fetal compartment. Therefore, we chose to use plasma amino acid enrichments as the albumin precursors. As keto acid enrichment can only be lower than amino acid enrichment, the use of the latter results in a slight underestimation of synthesis rates.

Amino acids were extracted from plasma and derivatized by using the same Phenomenex kits that were also used for product (albumin) sample preparation. Enrichments of plasma leucine, phenylalanine, and valine were measured in triplicate with GC-C-IRMS as well.

Plasma albumin concentrations in maternal and umbilical plasma were measured on a Roche Hitachi 917 (Roche Diagnostics, Basel, Switzerland). Hematocrit was measured on an Advia 120 (Bayer Diagnostics, Leverkusen, Germany).

### Calculations

Baseline enrichment in the fetus could not be measured but was considered to be identical to that in the pregnant woman because the fetus consists of what the mother eats.

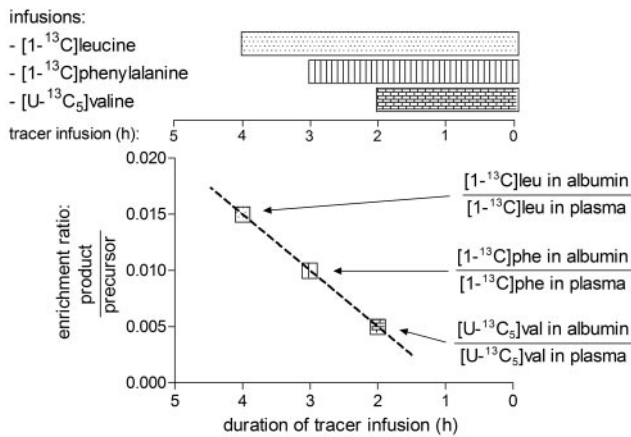
The fetal liver is perfused with blood directly from the umbilical vein (70%) and with blood that first passes the ductus venosus and then reenters the liver through the portal vein (20%) and hepatic arteries (10%) (21, 22). Blood from the portal vein and hepatic arteries has theoretically the same composition as in the umbilical arteries. Thus, the fetal liver is perfused with blood from both umbilical cord vessels. However, plasma amino acid enrichment in the umbilical arteries is slightly lower than that in the umbilical vein due to isotopic dilution by unlabeled amino acids released from fetal protein breakdown. Therefore, we calculated the precursor enrichment as the mean of umbilical venous and arterial plasma enrichment.

The enrichment of amino acids incorporated in fetal albumin was similar in blood from the umbilical vein and arteries, which indicates no maternofetal albumin transport. Nevertheless, we averaged the values. In each subject, the separate leucine, phenylalanine, and valine product/precursor enrichment ratios were plotted in a graph against the moment the corresponding infusion was started (**Figure 1**). Using computer software, we calculated the slope and the correlation coefficient of the linear trend line. The FSR was then derived by using the following equation:

$$\text{FSR}(\%/d) = \text{slope of trend line} \times -1 \times 24\text{h} \times 100\% \quad (1)$$

The absolute synthesis rate (ASR) represents the absolute amount of albumin that is produced per unit of time and can be calculated with the following equation:

$$\text{ASR}(\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = \text{FSR} \times C_{\text{alb}} \times \text{vol}_{\text{pl}} \times \text{weight}^{-1} \quad (2)$$



**FIGURE 1.** Study design. Pregnant women received 3 different stable-isotope-labeled amino acid infusions starting at different times before cesarean delivery. In maternal and umbilical cord blood, sampled at  $t = 0$ , we measured the product/precursor enrichment ratio of each of the 3 infused amino acids. These ratios were plotted in a graph against the moment the corresponding isotope infusion was started. Because labeled leucine had the longest infusion time, its incorporation into albumin will be highest. The slope of the trend line determines the albumin fractional synthesis rate.

where  $C_{\text{alb}}$  is the plasma albumin concentration in g/L,  $\text{vol}_{\text{pl}}$  is the plasma volume in mL, and weight is the maternal actual weight or infant's birth weight in kg. Maternal plasma volume was estimated from data by Whittaker et al (23) according to the following equation:

Plasma volume (mL)

$$= 36.1 \times \text{height (cm)} + 11.0 \times \text{weight (kg)} - 3029 \quad (3)$$

Fetal plasma volume (including placental and umbilical blood) was calculated by multiplying  $(1 - \text{hematocrit})$  with an estimated 105 mL blood/kg fetal body weight (24).

In our model, the use of one single amino acid with 3 different isotopomers (eg,  $[1-^{13}\text{C}]$ leucine,  $[\text{D}_7]$ leucine, and  $[^{18}\text{O}]$ leucine) could theoretically be preferred over infusing 3 different labeled amino acids as in our study. However, because the enrichment of incorporated amino acids in albumin is very low [ranging from 0.01 mol percent enrichment (MPE) for valine to 0.17 MPE for leucine in our study], enrichments can only be analyzed accurately by using GC-C-IRMS. Measuring hydrogen and oxygen on a GC-C-IRMS is technically very challenging. Besides, leucine with an oxygen label was at the time of the study prohibitively expensive. Unfortunately,  $[^{15}\text{N}]$ leucine could not be used because of label loss due to transamination. Owing to these technical difficulties and financial constraints, the single amino acid strategy could not be used. We thus chose to use carbon labels only, which necessitates using different amino acids when measuring with GC-C-IRMS. In addition, because of anticipated low enrichment in the last infused amino acid, valine was uniformly labeled to increase measurement accuracy.

## Statistics

Calculations were made with Microsoft Office EXCEL software (version 2007; Microsoft Corp, Redmond, WA), and statistical tests were done in GraphPad PRISM software (version 4.0; San Diego, CA). Because of our small groups, normality distribution of our data could not be determined or assumed.

**TABLE 1**  
Maternal characteristics ( $n = 11$ )

Characteristic	Value
Age (y)	35.0 (27.5–36.5) <sup>1</sup>
Preconceptional BMI (kg/m <sup>2</sup> )	22.8 (20.3–24.6)
Actual BMI (kg/m <sup>2</sup> )	29.4 (25.2–31.6)
Parity, 0:1:2:3 (n)	6:1:2:2

<sup>1</sup> Median; 25th–75th percentiles in parentheses (all such values).

Therefore nonparametric data analysis was performed. Consequently, values are expressed as medians (25th–75th percentile), and Mann-Whitney tests were used to detect statistical differences. The significance level was set at  $P < 0.05$ .

## RESULTS

We included 11 pregnant women, of whom 8 delivered at term, one at 31 wk gestation, one delivered a triplet at 35 wk (2 identical, one non-identical), and one delivered a quadruplet at 28 wk (all nonidentical). We thus studied 16 fetuses, classified into 2 groups: premature (<37 wk gestation) and mature. Maternal age, preconceptional and current body mass index, and parity are shown in **Table 1**. Descriptive characteristics of fetuses and neonates, which include birth weight, gestational age, birth weight  $z$  score (25), sex, umbilical pulsatility index, and Apgar score are shown in **Table 2**.

The enrichments of the 3 infused labeled amino acids both incorporated in albumin and free in plasma are shown in **Table 3** and **Table 4**, respectively. The trend lines through the leucine, phenylalanine, and valine product/precursor enrichment ratios in each studied subject are shown in **Figure 2**. The median linear regression coefficients ( $r^2$ ) of these trend lines were 0.995 (0.985–0.999) in pregnant women, 0.988 (0.981–0.993) in premature fetuses, and 0.996 (0.985–0.998) in mature fetuses. In **Figure 3**, the maternal, premature fetal, and mature fetal albumin FSRs are outlined. They were all significantly different from each other; pregnant women had the lowest FSRs, and premature fetuses had the highest.

Maternal albumin concentrations were 32.0 (29.5–34.5) g/L. Concentrations in premature fetuses were 28.8 (27.3–30.8) g/L and those in mature fetuses 33.5 (32.6–34.6) g/L, which was significantly different ( $P = 0.003$ ). Hematocrit in umbilical cord

**TABLE 2**  
Characteristics of the premature (<37 wk gestation) and mature groups of fetuses

	Premature ( $n = 8$ )	Mature ( $n = 8$ )
Gestational age (wk)	29.9 (28.4–35.4) <sup>1</sup>	38.5 (37.6–38.9)
Birth weight (kg)	1.3 (1.2–1.9)	3.3 (2.7–3.4)
Birth weight $z$ score (SD) <sup>2</sup>	-0.19 (-0.70–0.22)	-0.11 (-0.86–0.52)
Sex (M:F)	3:5	4:4
PI <sup>3</sup>	1.28 (1.18–1.36)	0.89 (0.78–0.96)
Apgar score at 5 min <sup>4</sup>	9 (9–10)	10 (10–10)

<sup>1</sup> Median; 25th–75th percentiles in parentheses (all such values).

<sup>2</sup> Birth weight corrected for gestational age (28).

<sup>3</sup> The umbilical pulsatility index (PI) is a Doppler ultrasound-derived index on the bloodstream velocity profile through the umbilical arteries and is a marker of fetal well-being. A normal PI decreases slightly over gestation.

<sup>4</sup> Apgar score is a postnatal scoring scale ranging from 0 to 10.

**TABLE 3**

Enrichments of the infused amino acids incorporated into albumin (product enrichments) in the maternal and fetal (mean of arterial and venous umbilical cord plasma) compartment<sup>1</sup>

	Pregnant women (n = 11)	Premature fetuses (n = 8)	Mature fetuses (n = 8)
[1- <sup>13</sup> C]Leucine	0.075 (0.061–0.083) <sup>1</sup>	0.105 (0.097–0.119)	0.096 (0.089–0.112)
[1- <sup>13</sup> C]Phenylalanine	0.063 (0.054–0.078)	0.103 (0.089–0.116)	0.095 (0.087–0.108)
[U- <sup>13</sup> C <sub>5</sub> ]Valine	0.015 (0.013–0.022)	0.019 (0.017–0.024)	0.025 (0.023–0.030)

<sup>1</sup> Enrichment is expressed in mole percent excess. All values are median; 25th–75th percentiles in parentheses.

blood (mean of venous and arterial blood) was 0.43 (0.40–0.50) in the premature fetuses and 0.46 (0.46–0.48) in the mature group. The albumin ASRs are shown in **Figure 4**. Similar to the fractional values, premature fetuses had the highest ASRs, followed by the mature fetuses and the pregnant women.

## DISCUSSION

This is the first study addressing albumin synthesis rates in human fetuses. These values are of great importance because they give guidance as what to strive for in aiming optimal nutrition for premature infants. The fetal measurements were possible as the result of a relatively novel multiple-stable-isotope infusion method. This enabled us to measure a protein's synthesis rate from a single blood sample. Given the high correlation coefficients, our method proves to be valid.

In this study, we compared albumin synthesis between pregnant women, fetuses at term, and fetuses that were still premature. In mothers of the latter group, however, elective cesarean sections are rarely performed because these are usually in the acute setting because of sudden (worsening of) fetal or maternal distress. Thus, there is usually no time for obtaining informed consent followed by a infusion protocol lasting 4 h for research purposes. Still, we were able to include 3 women who underwent a planned cesarean delivery before term and whose infants were assumed to be in relatively good condition. One woman had to give birth early because of maternal cervical carcinoma; the 2 other women delivered early because of anticipated complications due to triplet and quadruplet pregnancy. Whether the results in the premature group of fetuses were influenced by the effects of multiple pregnancy itself or by genetic relations remain unknown. However, a common genetic background does not imply having equal fetal metabolic nutrient availability. In normal pregnancy, the latter depends more on placental activity in each individual than on maternal nutrient availability. Thus, among multiplets, it is likely that the intrauterine metabolic environments are different, which was also reflected by different synthesis rates between siblings.

Because the maternal blood sample used for calculation of the albumin FSR was taken before spinal anesthesia was initiated

and surgery had started, the latter 2 procedures could not have influenced our results. It is unknown, however, to what extent maternal surgery influences fetal metabolism. Yet, surgery until the infant was born only lasted some 10 min, which is only a short period relative to the total infusion time. Thus, potential effects of maternal surgery would only minimally influence fetal synthesis rates.

The maternal plasma albumin concentrations in this study are low compared with those in nonpregnant women, but a 10-g/L drop in concentration starting early in pregnancy is common (23). However, rather than simple dilution because of a pregnancy-associated plasma volume expansion, actual alterations in albumin metabolism during pregnancy have been observed. During late gestation, albumin FSRs and ASRs as well as the total intravascular albumin pool were found to be higher than those in nonpregnant women (23, 26). Our measured maternal synthesis rates were similar to the rates in those studies. Increased synthesis could be necessary to compensate for the albumin loss caused by placental uptake and subsequent degradation, thereby releasing free amino acids available for transport to the fetus (27, 28).

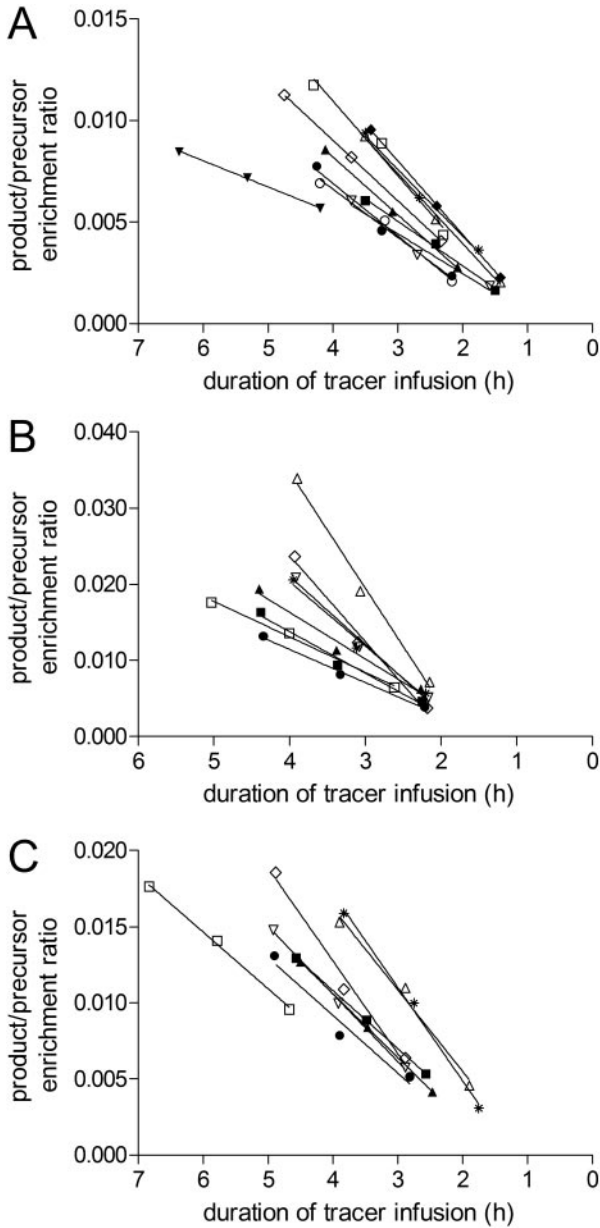
Two of the mature fetuses had birth weights that were only on the 5th percentile. These 2 small-for-gestational age infants had the lowest 2 albumin FSRs and ASRs. When nutrient availability is compromised, ultimately leading to reduced growth, oxygen and nutrient-rich blood entering the fetus through the umbilical vein is shunted away from the liver through the ductus venosus toward the upper body half (29). Bypassing the fetal liver ensures a more or less constant supply of essential substrates to the myocardium and brain. Underperfusion of the fetal liver, however, results in diminished liver growth. Small-for-gestational age infants are known to have smaller liver volumes, also when corrected for total body weight (30, 31). Interestingly, these 2 fetuses did not up-regulate albumin synthesis rates so as to compensate for their supposedly smaller liver size. In fact, the opposite was true because the albumin synthesis rates were the lowest. This could have important implications because impaired liver functioning might have lifelong effects on metabolism. Summarized as the “fetal origins of adult disease” or “Barker

**TABLE 4**

Enrichments of the infused amino acids in plasma (precursor enrichments) in the maternal and fetal (mean of arterial and venous umbilical cord plasma) compartment<sup>1</sup>

	Pregnant women (n = 11)	Premature fetuses (n = 8)	Mature fetuses (n = 8)
[1- <sup>13</sup> C]Leucine	8.89 (8.27–9.27)	5.40 (4.84–6.12)	6.55 (5.98–7.24)
[1- <sup>13</sup> C]Phenylalanine	12.7 (11.1–13.0)	8.55 (8.27–8.96)	10.1 (9.19–11.4)
[U- <sup>13</sup> C <sub>5</sub> ]Valine	6.73 (6.02–7.06)	3.75 (3.24–4.34)	4.97 (4.56–5.20)

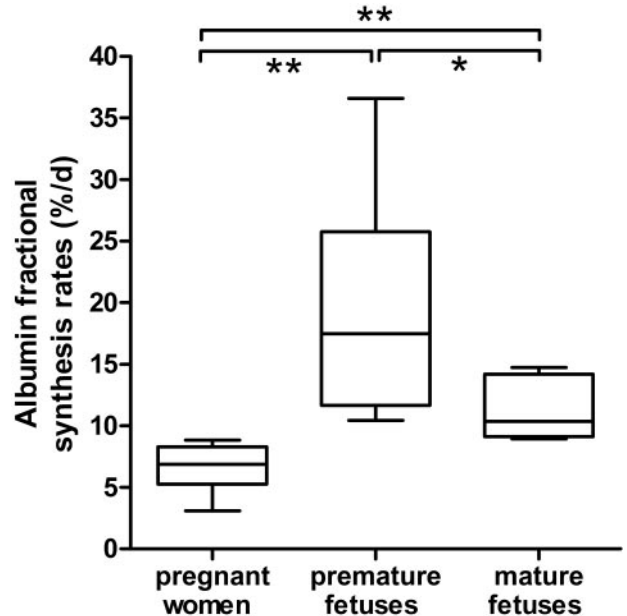
<sup>1</sup> Enrichment is expressed in mole percent excess (MPE). All values are median; 25th–75th percentiles in parentheses.



**FIGURE 2.** Individual trend lines through the 3 product/precursor trend lines in (A) pregnant women ( $n = 11$ ), (B) premature fetuses ( $n = 8$ ), and (C) mature fetuses ( $n = 8$ ). In each case, leucine had the longest infusion time, followed by phenylalanine and valine, respectively.

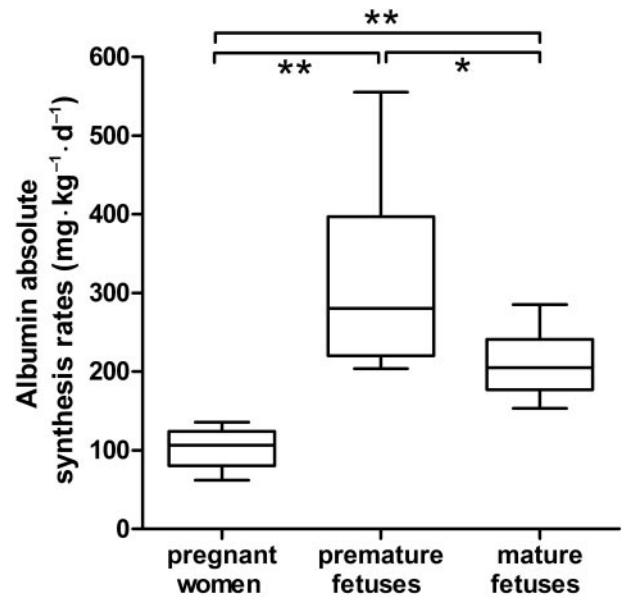
hypothesis,” compromised growth during early life of organs such as the liver, pancreas, spleen, kidneys, and adrenal glands predisposes an individual to cardiovascular disease, stroke, and type 2 diabetes (32, 33).

Considering the functions of albumin, which include acting as an antioxidant and transporting bilirubin and free fatty acids, one may wonder why normally grown fetuses, especially earlier in gestation, have such high synthesis rates. During intrauterine life, oxygen tension in blood is low, thereby generating only low amounts of radicals, which could damage albumin. The low oxygen tension is compensated for by the increased oxygen affinity of fetal hemoglobin. After birth, fetal hemoglobin is rapidly broken down, thereby releasing large amounts of bilirubin



**FIGURE 3.** Albumin fractional synthesis rates in pregnant women ( $n = 11$ ), premature fetuses (<37 wk gestation,  $n = 8$ ), and mature fetuses ( $n = 8$ ). Boxes and whiskers indicate the medians and interquartile and outer ranges. \*\*Significantly different (Mann-Whitney): \* $P < 0.05$ , \*\* $P < 0.001$ .

that should be transported off by albumin. Also, during the beginning of the third trimester, fatty acid concentrations are low and will be of no burden to albumin. The surge in albumin synthesis would therefore be expected just before term birth, as a preparation against an elevated radical exposure and for a higher transport load consisting of hemoglobin breakdown products and fatty acids, the latter found in high amounts in postnatal nutrition (breast milk). In addition, all mothers of the prematurely born infants had received corticosteroids in the 2 d before their



**FIGURE 4.** Albumin absolute synthesis rates in pregnant women ( $n = 11$ ), premature fetuses (<37 wk gestation,  $n = 8$ ), and mature fetuses ( $n = 8$ ). Boxes and whiskers indicate the medians, and interquartile and outer ranges. \*\*Significantly different (Mann-Whitney): \* $P < 0.05$ , \*\* $P < 0.001$ .

planned cesarean delivery. Antenatally given steroids accelerate fetal lung maturation in preparation for postnatal life. These stress hormones, however, can also elicit a catabolic response in the fetus. Albumin synthesis might therefore even have been down-regulated in the premature group at the time of the measurements.

The reason for a decreasing albumin synthesis rate during gestation could either be functional or depend on the general metabolic rate. During ovine pregnancy, fetal whole-body protein synthesis rates decrease significantly throughout gestation (34). Oxygen consumption by the ovine fetal liver has also been shown to decrease (35). In human preterm infants, whole-body protein metabolic rates are also higher when compared with infants born at term (36). However, human fetal liver volume as a percentage of body weight does not decrease as much throughout gestation as it does in fetal sheep (37).

The albumin ASR in intravenously fed premature babies (27 wk gestation) was 228 (187–289)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (3). The ASRs of premature fetuses measured in the current study are higher than the postnatal values from premature infants. Having low albumin concentrations and ASRs after birth is an unfortunate situation considering that sick premature infants experience more oxidative stress after high oxygen pressure ventilation and have to deal with increased bilirubin and drug transport. In our previous study, we showed that albumin synthesis in premature neonates is responsive to parenteral nutrition (3). Yet, the current recommended nutrient intakes for premature infants still do not appear to be sufficient to increase the albumin synthesis rates to levels observed in fetuses. This can be speculated because premature infants should theoretically be able to synthesize albumin in larger quantities as they also did while still in utero. Although the traditional method of measuring an FSR used in premature infants is different from our infusion model, the 2 should theoretically give comparable results.

In conclusion, we showed that mature fetuses produce twice as much albumin as do their mothers per kilogram bodyweight and premature fetuses 3 times as much. Premature fetuses have higher albumin synthesis rates than do parenterally fed premature neonates, indicating that postnatal synthesis capacity is reduced or that recommended nutrient intake is not sufficient.

Our method is not only applicable in fetal research, but also could be of benefit in all situations where multiple sampling is impossible or inconvenient to a subject. In organ protein metabolism studies (for example, liver, bowel, or muscle protein synthesis), the required number of tissue biopsies can be reduced to 1, instead of 2 or 3 with many currently used models (38, 39). In addition, our single sample method shortens sample preparation and analysis time and reduces risk on measurement artifacts.

Most of all, we thank the participating women. Furthermore, Willemijn Corpeleijn, Frans te Braake, Ad de Bruijn, and all staff from the obstetric and anesthesiology departments were a great helping hand in collecting all material and providing the facilities.

The authors' responsibilities were as follows: CHPvdA, JJD, EAPS, and JBvG participated in the design and implementation of the study, including recruitment of patients; AV prepared and tested all intravenous stable-isotope solutions; CHPvdA collected and prepared blood samples for analysis; HS and TR provided technical supervision of blood sample preparation; HS performed mass spectrometry analyses; CHPvdA, HS, and JBvG analyzed the data; CHPvdA wrote the manuscript draft; and all authors reviewed the manuscript and approved the final version. None of the authors had a personal or financial conflict of interest. Neither grant supplier had any involvement

whatsoever in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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