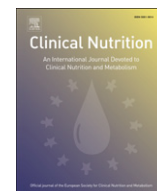


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Clinical Nutrition

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Original Article

Albumin synthesis rates in post-surgical infants and septic adolescents; influence of amino acids, energy, and insulin

Sascha C. Verbruggen^{a,b,c}, Henk Schierbeek^a, Jorge Coss-Bu^{b,c}, Koen F.M. Joosten^a, Leticia Castillo^d, Johannes B. van Goudoever^{a,e,f,*}

^a Department of Pediatrics, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands

^b Children's Nutrition Research Center, USDA, Houston, TX, USA

^c Critical Care Section, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX, USA

^d Division of Critical Care Medicine, Children's Medical Center Dallas, University of Texas Southwestern, Dallas, TX, USA

^e Department of Pediatrics, VU University Medical Center, Amsterdam, The Netherlands

^f Department of Pediatrics, Emma Children's Hospital, Amsterdam Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Article history:

Received 19 September 2010

Accepted 3 February 2011

Keywords:

PICU

Parenteral nutrition

Hepatic protein synthesis

Anabolism

SUMMARY

Background & aims: To investigate the effects of glucose, parenteral amino acids, and intravenous insulin on albumin synthesis rates in critically ill children.

Methods: Two studies were performed in 8 post-surgical infants (age 9.8 ± 1.9 months; weight 9.5 ± 1.1 kg) and 9 septic adolescents (age 15 ± 1 yr; BMI 23 ± 4 kg m⁻²), respectively. All received a primed, constant, tracer infusion with [1-¹³C]Leucine. The infants in study 1 were randomized to receive low (2.5 mg kg⁻¹ min⁻¹) and standard (5.0 mg kg⁻¹ min⁻¹) glucose intake in a cross-over setting of two periods of 4 h each. The adolescents in study 2 were randomized to receive total parenteral nutrition with standard (1.5 g kg⁻¹ day⁻¹) and high (3.0 g kg⁻¹ day⁻¹) amino acid intake in a two day cross-over setting. On both study days, during the last 3 h of the tracer study, they received insulin infused at 80 mU m⁻² min⁻¹.

Results: The post-surgical infants and the septic adolescents were mildly hypoalbuminemic (~ 2.5 g dL⁻¹) with high synthesis rates, which were not affected by different intakes of glucose, amino acids, or insulin infusion.

Conclusions: Albumin synthesis rates in hypoalbuminemic critically ill children are high but were not upregulated through nutrient supply, and in septic adolescents are unaffected by insulin.

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1. Introduction

Albumin is the most abundant protein in human plasma with a normal plasma concentration of around 4.0 g dL⁻¹, while about 60% of the total albumin pool is located in the interstitial space.¹ Albumin holds several important functions, both in health as well as in critically ill patients. It is the main preserver of colloid oncotic pressure ($\sim 75\%$), it functions as an anticoagulant and anti-oxidant and it is an important binding transporter of metabolites and drugs.^{1,2}

Abbreviations: PICU, pediatric intensive care unit; LG, low glucose intake; SG, standard glucose intake; SAA, standard amino acid intake; HAA, high amino acid intake; TPN, total parenteral nutrition; APE, atom percent excess; KIC, α -ketoisocaproate; Ra, rate of appearance; Rd, rate of disappearance.

* Corresponding author. Kamer 9D11, VU Medical Center, De Boelelaan 1117, Postbus 7057, 1007 MB, Amsterdam, The Netherlands.

E-mail address: S.verbruggen@erasmusmc.nl (S.C. Verbruggen).

Critically ill patients are often hypoalbuminemic, primarily due to dilution and redistribution secondary to an altered vascular permeability.¹ In critically ill patients hypoalbuminemia has been documented as a marker for disease severity, nutritional status, prolonged ventilator support and prolonged length of stay.^{3,4} In critically ill adults and children, low albumin plasma levels (<3.3 g dL⁻¹) are inversely related to morbidity and mortality, where in adults each 1.0 g dL⁻¹ drop in serum albumin raised the odds of morbidity by 87% and mortality by 137%.^{3–5} However, plasma concentrations are static measurements. Dynamic measurements by means of albumin synthesis rates actually show a consistent increase in critically ill adults.^{6,7}

Despite the clear association between hypoalbuminemia and poor outcome, there is still a debate on the benefits and safety of intravenous albumin administration, partially due to the increased risk of escape in the extravascular space and inflammation.^{2,5} Therefore, stimulation of endogenous albumin synthesis seems an appealing alternative.

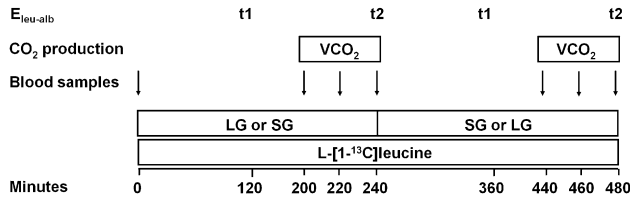


Fig. 1. Experimental design of study 1 in 8 post-surgical infants admitted to the pediatric intensive care unit receiving only parenteral glucose as nutrition, LG = low glucose intake ($2.5 \text{ mg kg}^{-1} \text{ min}^{-1}$), SG = standard glucose ($5.0 \text{ mg kg}^{-1} \text{ min}^{-1}$), $E_{\text{Leuc-alb}}$ = Enrichment of $[1-^{13}\text{C}]$ Leucine incorporated into albumin, VCO_2 = Carbon dioxide production.

Albumin synthesis can be stimulated by an increase in energy (glucose and fat) but is particularly responsive to amino acid intake.^{8–10} Hyperinsulinemia has shown to increase albumin synthesis as well, with an additive effect of increased amino acids in healthy adults.¹⁰ This latter intervention is of particular interest as insulin is more frequently used to treat hyperglycemia in critically ill adults and children.¹¹ Moreover, the increase in synthesis rates in response to these interventions is immediate and fast. Albumin synthesis rates increased by 40% within 2 h following intravenous endotoxin in healthy volunteers.¹² Furthermore, nutritional supplementation^{13,14} and hyperinsulinemia¹⁵ increased albumin synthesis rates, within 4, 6, and 3 h respectively.

The effect of various nutritional interventions on albumin synthesis rates have not been investigated in critically ill children, other than in premature infants.¹⁶ Increasing amino acid availability resulted in higher albumin synthesis rates, although not as high as reached in utero.¹⁶ Given the limited knowledge of albumin synthesis rates and the impact of nutrition in the critically ill pediatric population, we set out to ascertain these. We hypothesized that in critically ill children albumin synthesis rates are increased and responsive to nutrients and hyperinsulinemia. Therefore, our first objective was to quantify albumin synthesis rates in critically ill infants and adolescents. Our second objective was to determine the

impact of nutrients on albumin synthesis rates in these children, with special emphasis on parenteral glucose and amino acids. Our third objective was to determine whether additional hyperinsulinemia in combination with parenteral glucose and amino acid intake would increase albumin synthesis rates through a synergistic fashion. The here described studies were part of two larger studies aiming to investigate the effect of reduced glucose intake on glucose homeostasis and protein catabolism (Study 1) and the effect and interactions of increased amino acid intake and hyperinsulinemia on substrate metabolism and insulin resistance (Study 2).

2. Methods

2.1. Patients

Study 1 was a one day study with infants (0.5–1.0 y) admitted after surgical repair of non-syndromal craniosynostosis to the pediatric intensive care unit (PICU) at Erasmus MC – Sophia Children’s Hospital in Rotterdam, The Netherlands. Study 2 was a two day study in adolescents (13–18 y) who were admitted with a diagnosis of severe sepsis or Systemic Inflammatory Response Syndrome (SIRS), as defined by the criteria of the First International Pediatric Sepsis Forum¹⁷ to the PICU at Texas Children’s Hospital, Houston, Texas. All patients had drawing and infusing catheters in place for clinical purposes. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded. The study protocols were approved, respectively by the Institutional Review Board of Erasmus Medical Center, Rotterdam, The Netherlands and by the Institutional Review Board of Baylor College of Medicine, Houston, Texas. Studies were carried out after written informed consent from the parents.

2.2. Study design and data collection

The experimental design of study 1 is shown in Fig. 1 and consisted of an 8 h glucose infusion in a randomized, cross-over design,

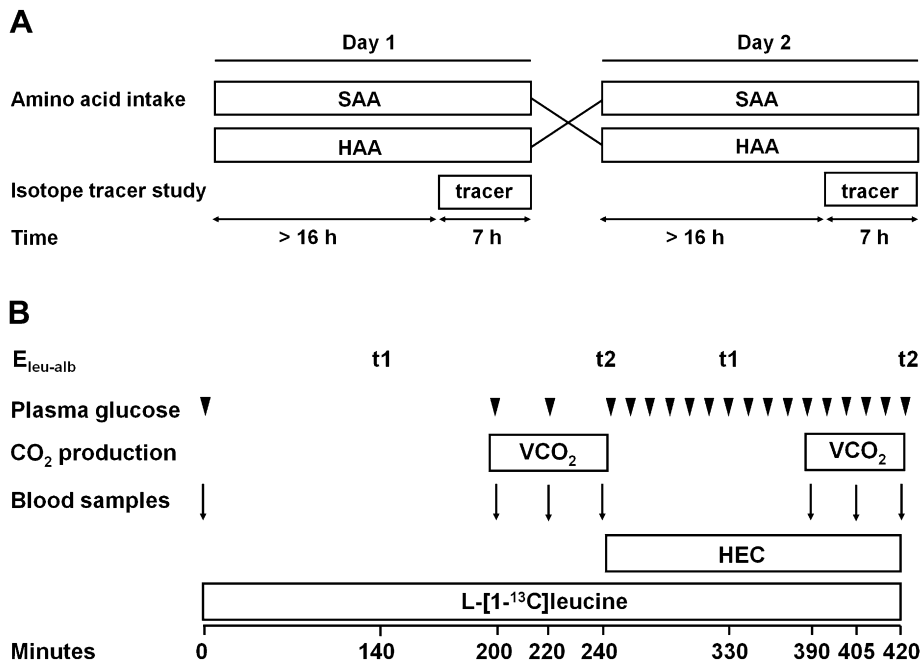


Fig. 2. Experimental design of study 2 in 9 adolescents admitted to the pediatric intensive care unit receiving full parenteral nutrition with two different amino acid intakes at baseline and during hyperinsulinemia, Panel A Two day study protocol with two different amino acid intakes in a randomized cross-over fashion, Panel B Isotope tracer study on both days during baseline and during hyperinsulinemia, SAA = Standard amino acid intake ($1.5 \text{ g kg}^{-1} \text{ day}^{-1}$), HAA = High amino acid intake ($3.0 \text{ g kg}^{-1} \text{ day}^{-1}$), $E_{\text{Leuc-alb}}$ = Enrichment of $[1-^{13}\text{C}]$ Leucine incorporated into albumin, VCO_2 = Carbon dioxide production.

4 h with low (LG; 2.5 mg kg⁻¹ min⁻¹) and 4 h with standard (SG; 5.0 mg kg⁻¹ min⁻¹) glucose intake rates. Patients were randomized for the order of glucose intake through a computer generated envelop. Laboratory personnel, the clinical team and investigators were blinded for glucose intake until after analyses were finished. Prior to the study, infants did not receive (par)enteral nutrition other than intravenous glucose infusion in the range of 4–6 mg kg⁻¹ min⁻¹ as per standard care before start of the study. Eight hours after admission to the PICU and after obtaining baseline blood samples, the intravenous glucose intake as per standard care (4.0–6.0 mg kg⁻¹ min⁻¹) was stopped and the study glucose intake started (t = 0), after which the patients received a primed, continuous, 8 h intravenous tracer infusion with L-[1-¹³C]leucine at 8 μmol kg⁻¹ and 8 μmol kg⁻¹ h⁻¹ respectively. Four hours after start of the study (t = 240) the study glucose was switched as per cross-over design (Fig. 1).

Study 2 consisted of 2 study days in a randomized cross-over fashion, i.e. one with standard (SAA; 1.5 g kg⁻¹.day⁻¹) and one with high (HAA; 3.0 g kg⁻¹.day⁻¹) amino acid intake via total parenteral nutrition (TPN) (Fig. 2a). Patients received full parenteral nutrition (Aminosyn (Hospira Inc. Lake Forest, IL) or Clinisol (Baxter, Deerfield, IL)) for at least 24 h before the start of the study. Both days consisted of two experimental periods, a basal and a period with insulin infusion (see subsequent description at the end of the paragraph) (Fig. 2b). Amino acid intake was randomized by pharmacy through a computer generated envelop. Insulin infusion always followed a baseline period because of the expected wash-out effect of insulin on protein metabolism. Laboratory personnel was blinded for both the amino acid intake and the insulin allocation, while investigators were blinded for amino acid intake, but could not be blinded for insulin allocation due to the risk of hypoglycemia. Energy intake provided as parenteral glucose and lipids were prescribed by the clinical team according to standard care. The total energy intake supplied remained unchanged during both study days. After an adaptation period of at least 12 h of the randomized TPN, the patients received a primed, continuous, 7 h infusion with L-[1-¹³C]leucine at 6 μmol kg⁻¹ and 6 μmol kg⁻¹ h⁻¹ respectively, of which the last 3 h in combination with a hyperinsulinemic euglycemic clamp as previously described¹⁸ (Fig. 2b). Briefly, a 3 h infusion of insulin (Actrapid, Novo Nordisk Inc., Princeton, NJ), dissolved in sterile isotonic NaCl was started at 80 mU m⁻² min⁻¹ in order to achieve both normoglycemia between 90 and 110 mg dL⁻¹ and a plasma insulin concentration greater than 100 μU mL⁻¹. During insulin infusion, small blood samples were obtained from the indwelling arterial catheter every 5–10 min to monitor whole blood glucose concentration, at the bedside with the aid of a Y.S.I. 2300 STAT Plus analyzer (YSI Life Sciences, Yellow Springs, OH). To maintain the plasma glucose between 90 and 110 mg dL⁻¹ (5.0–6.1 mM) for the duration of the study, a 30% glucose solution was infused with a Harvard syringe pump (PHD 22/2000, Harvard apparatus, Holliston, MA).¹⁸

3. Materials

L-[1-¹³C]leucine (99 atom%) was purchased from Cambridge Isotope Laboratories (Andover, MA) and tested for sterility and pyrogenicity after they were compounded at the investigational pharmacy at Texas Children's Hospital or Erasmus MC – Sophia Children's Hospital.

3.1. Measurements and sample analysis

Basal metabolic rate was predicted using the Schofield equation.¹⁹ All were assessed for severity of disease by the Pediatric Logistic Organ Dysfunction (PELOD) score²⁰ and the Pediatric Risk of Mortality III (PRISM III) score.²¹

Blood samples were obtained from the arterial line at standard frequent intervals (Fig. 1) and immediately centrifuged and frozen at –80 °C until samples were analyzed. To isolate albumin from plasma, we used anti-human serum albumin affinity resin kits (Vivascience; Sartorius Group, Hannover, Germany). Enclosed spin columns were filled with 400 μL affinity resin and 25 μL of thawed plasma. 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 HClO₄/L. A washing step was performed with 0.2 mol HClO₄/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 H₂O. Samples were derivatized using propylchloroformate (commercial kits: Phenomenex for hydrolyzates, EZ:Faast, Bester BV, Amstelveen, the Netherlands) and measured in triplicate on a gas chromatograph–combustion–isotope ratio mass spectrometer (GC–C-IRMS; Delta XP, Thermo Electron, Bremen, Germany).²² As albumin precursor, we used plasma [1-¹³C]α-ketoglutarate (α-KIC; the keto-acid of leucine, a measure of intracellular leucine enrichment) enrichment at a plateau. 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.^{23,24} Plasma isotope enrichment of [1-¹³C]α-KIC were, after derivatization to butyldimethylsilylquinoxalinol derivatives, determined by gas chromatography mass spectrometry (GC–MS) as previously described.²⁵ Plasma albumin concentrations were routinely measured on a Hitachi 912 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Carbon dioxide production (VCO₂) was obtained with a respiratory profile monitor (Deltatract™ I MBM-200, Datex Division Instrumentarium Corp. Finland/CO₂SMO Plus, Novamatrix Medical System, Wallingford, CT, USA) during the last 30 min of each study period. To determine the enrichment of ¹³CO₂ (E¹³CO₂) in whole blood, 1 mL of perchloric acid 10% was added to 1.0 mL of whole blood in a vacutainer to release the CO₂. The ¹³CO₂ in gas was subsequently determined on gas combustion isotope ratio mass spectrometry (GC–IRMS).^{26,27}

Plasma glucose levels >110 mg dL⁻¹ (~>6.1 mmol L⁻¹) were considered hyperglycemic and plasma albumin levels <3.5 g dL⁻¹ were considered hypoalbuminemic.

3.2. Calculations

Whole body kinetics of leucine were calculated by conventional isotope dilution technique using a stochastic model during steady state plateau.²⁸ The rate of appearance (Ra) of unlabeled leucine can be derived from the plasma isotope enrichment calculated by:

$$Ra_{leu} = i \times \left(E_{inf} / E_{pl} - 1 \right) \quad (1)$$

where *i* is the infusion rate of [1-¹³C]leucine, *E*_{inf} is the tracer enrichment of the infusate and *E*_{pl} the tracer enrichment in plasma, respectively. At steady state plateau rate of appearance (Ra) is equal to rate of disappearance (Rd).

The fractional albumin synthesis rate (FSR) reflects the fraction of the intravascular albumin pool that is renewed per unit of time (%·d⁻¹) and can be calculated by using the following equation¹⁶:

$$FSR = (E_{leu-alb,t2} - E_{leu-alb,t1}) / E_{\alpha-kic} \times (24 \times 60) / (t2 - t1) \times 100\% \quad (2)$$

where $E_{\text{leu-alb}}$ is the enrichment in mole percent excess (MPE.) of incorporated leucine in albumin at t_1 and t_2 (Figs. 1 and 2), and $E_{\alpha\text{-kic}}$ is the mean enrichment in MPE of the precursor, ie, plasma α -KIC, at these time points in minutes.

The absolute albumin synthesis rate (ASR) represents the absolute amount of albumin that is produced per day ($\text{mg kg}^{-1} \text{d}^{-1}$), and it can be calculated by using the following equation¹⁶:

$$\text{ASR} = \text{FSR} \times C_{\text{alb}} \times \text{vol}_{\text{bl}} \times (1 - \text{Ht}) \times \text{weight}^{-1} \quad (3)$$

where C_{alb} is the plasma albumin concentration in g L^{-1} , vol_{bl} is the child's total blood volume in mL (assumed to be 75 and 70 mL/kg body wt for the infants and adolescents respectively), Ht stands for hematocrit and $(1 - \text{Ht})$ is the fraction of blood that is plasma.

We also calculated the contribution (%) of albumin ASR in relation to whole body protein synthesis in percentage on the basis of measured leucine turnover data. To do so we needed to determine leucine oxidation and the non-oxidative leucine disposal (NOLD) representing leucine utilized into whole body protein synthesis (in $\mu\text{mol kg}^{-1} \text{h}^{-1}$).

Leucine oxidation rates were calculated as follows;

$$\text{Leucine Oxidation} = \text{VCO}_2 \times \left(E^{13}\text{CO}_2 / 69.18 \right) / [^{13}\text{C}]\alpha - \text{KIC} \quad (4)$$

where 69.18 is the $^{13}\text{CO}_2$ refraction correction factor for critically ill children.²⁹ VCO_2 is measured in milliliters per minute and converted to millimoles per hour by multiplying by 60 min and dividing by 22.4, which is the number of 1 in 1 mol of an ideal gas at standard temperature and pressure to convert to milliliters per minute.

NOLD is the leucine oxidation subtracted from the leucine rate of disappearance;

$$\text{NOLD} = \text{Ra}_{\text{leu}} - \text{Leucine Oxidation} \quad (5)$$

This allows us to calculate the contribution of albumin synthesis in ratio to the whole body protein synthesis according to the following equation¹⁶:

$$\text{Contribution} = \left[(\text{ASR} \times 0.104) / (\text{NOLD} \times 131.2 \times 24 \times 0.001) \right] \times 100\% \quad (6)$$

where 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.

3.3. Statistical analysis

A prospective power analysis on our previous data on albumin synthesis rates¹⁶ revealed that 8 patients with complete data, would detect a difference of 20% (80% power, type I error of 5%) in synthesis rates. Data are presented as the mean \pm standard deviation unless non-parametric in which case they are presented as median and 95% CI. Due to the variation between the two studies, not only in nutritional and metabolic support, but moreover in age and diagnosis, statistical comparison would not be justified. Differences within study groups were tested by use of the Bonferroni multiple comparison for selected pairs after repeated measurements ANOVA, after which a paired student's *t* test was used. For non-parametric data the Wilcoxon matched pairs or Mann Whitney was used. Statistical significance was considered at

$p < .05$. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, La Jolla, CA., USA).

4. Results

4.1. Patients

The demographic characteristics of all patients are described in Table 1.

4.1.1. Study 1

Study 1 included 8 post-surgical infants (age 0.8 ± 0.2 months; 9.5 ± 1.1 kg). All patients were hemodynamically stable without vasoactive drugs and all patients were breathing spontaneous with an inspiratory fraction of oxygen less than 0.6.

4.1.2. Study 2

Study 2 included nine septic adolescents (age 15 ± 1 yr; BMI $20 \pm 4 \text{ kg m}^{-2}$, Tanner-score 4.0 ± 0.8). All patients were mechanically ventilated and hemodynamically stable. One patient received epinephrine as vasopressor therapy and 4 patients received glucocorticoids as additional therapy (Table 1). One patient randomized to start first with the standard amino acid intake group (SAA) died the next day after the first study (SAA) was completed, therefore the high amino acid intake (HAA) part of the study could not be conducted. In a second patient a technical error occurred during the insulin infusion protocol on the study day with SAA intake. Therefore, complete two day study data were available on 7 patients. Data available from the two incomplete studies were included according to intention-to-treat principle.

4.2. Nutritional interventions

The nutritional characteristics of all patients are described in Table 2.

4.2.1. Study 1

The infants received glucose intake in two different amounts and did not receive any amino acids. As anticipated, the caloric intake was below the caloric requirements according to the Schofield equations,¹⁹ although significantly higher during SG. During LG the infants were normoglycemic, and during SG glucose levels were higher and hyperglycemic, while the insulin plasma levels did not significantly differ.

Table 1

Patient demographics of 8 infants and 9 adolescents admitted to the PICU^a

	Study 1	Study 2		
Age (years)	0.8 ± 0.2	15.0 ± 1.2		
Gender (male:female)	6:2	3:6		
Tanner-score		4.0 ± 0.8		
Body mass index (kg m^{-2})		20 ± 4		
Weight (kg)	9.5 ± 1.1	49.1 ± 13.1		
Length (cm)	74.3 ± 3.0	154.3 ± 11.6		
			SAA ^b	HAA ^c
PICU LOS ^d (days)	1	5.9 ± 3.3	6.3 ± 3.6	
C-Reactive protein (mg dL^{-1})	2.4 ± 1.3	16.5 ± 9.4	15.1 ± 12.0	
PELOD ^e	10 ± 9	9 ± 11	6 ± 7	
PRISM III ^f	7 ± 4	10 ± 4	8 ± 4	
Catecholamines (n)	0	1	0	
Glucocorticoids (n)	0	4	3	

^a All values are mean \pm SD.

^b SAA = standard amino acid intake ($1.5 \text{ g kg}^{-1} \text{d}^{-1}$).

^c HAA = high amino acid intake ($3.0 \text{ g kg}^{-1} \text{d}^{-1}$).

^d PICU LOS = Length of stay in the PICU at start of the study.

^e PELOD = Pediatric Logistic Organ Dysfunction.²⁰

^f PRISM III = Pediatric Risk of Mortality III.²¹

Table 2
Nutritional parameters of 8 infants and 9 adolescents admitted to PICU^a

	Study 1		Study 2			
	LG ^b	SG ^c	SAA ^d		HAA ^e	
			Base	Insulin	Base	Insulin
Schofield ^f (kcal.kg ⁻¹ day ⁻¹)		53.7 ± 5.3		29.3 ± 6.0		29.3 ± 6.0
Caloric intake (kcal kg ⁻¹ day ⁻¹)	12.7 ± 0.2	25.3 ± 0.5 ^g	32.7 ± 10.0	41.8 ± 8.4	37.8 ± 9.9	43.0 ± 14.3
Caloric intake (% of Schofield)	24 ± 1	47 ± 2 ^g	115 ± 39	147 ± 44	136 ± 31	156 ± 55
Glucose intake (mg kg ⁻¹ min ⁻¹)	2.6 ± 0.1	5.2 ± 0.1 ^g	4.5 ± 1.7	6.2 ± 1.9	4.6 ± 1.5	5.6 ± 2.2
Protein intake (g kg ⁻¹ day ⁻¹)	0	0	1.5 ± 0.2	1.5 ± 0.2	2.8 ± 0.4 ⁱ	2.8 ± 0.4 ⁱ
Glucose calories (kcal kg ⁻¹ .day ⁻¹)	12.7 ± 0.2	25.3 ± 0.5 ^g	21.8 ± 8.1	30.9 ± 8.9	22.4 ± 7.6	27.6 ± 10.8
Amino acid calories (kcal kg ⁻¹ day ⁻¹)	0	0	5.8 ± 0.8	5.8 ± 0.8	11.2 ± 1.4 ⁱ	11.2 ± 1.4 ⁱ
Lipid calories (kcal kg ⁻¹ day ⁻¹)	0	0	5.7 ± 3.9	5.7 ± 3.9	4.7 ± 3.5	4.7 ± 3.5
Glucose plasma level (mg dL ⁻¹)	105 ± 10	133 ± 30 ^g	168 ± 58	98 ± 6 ^h	172 ± 62	101 ± 15 ^h
Insulin plasma levels (μU mL ⁻¹)	54 (19–159)	73 (24–186)	32 (17–122)	144 (94–2385) ^h	51 (29–153)	168 (93–2239) ^h

^a All values are mean ± SD, except insulin plasma levels which are depicted in median (range).

^b LG = low glucose (2.5 mg kg⁻¹ min⁻¹).

^c SG = Standard glucose (5.0 mg kg⁻¹ min⁻¹).

^d SAA = standard amino acid intake (1.5 g kg⁻¹ d⁻¹).

^e HAA = high amino acid intake (3.0 g kg⁻¹ d⁻¹).

^f Resting Energy Expenditure according to Schofield equations.¹⁹

^g LG vs. SG, *p* < .05.

^h Base vs. Insulin *p* < .05.

ⁱ SAA vs. HAA, *p* < .05.

4.2.2. Study 2

The adolescents received full parenteral nutrition in two different amounts of amino acids (Table 2). The caloric intake was adequate or above requirements according to the Schofield equations¹⁹ (Table 2), but did not significantly differ between protocols. During baseline the adolescents were hyperglycemic, while during insulin infusion they were normoglycemic and had significantly higher insulin concentrations (Table 2).

4.3. Albumin synthesis rates

All patients were hypoalbuminemic (Table 3).

4.3.1. Study 1

Albumin synthesis rates, NOLD, and the contribution of albumin synthesized in relation to the whole body protein synthesis were not affected with different glucose intakes (Fig. 3, Table 3).

4.3.2. Study 2

Neither an increased amino acid intake nor the supra-physiological insulin concentrations affected albumin synthesis

rates. NOLD was not significantly affected by the different amino acid intakes, but was lower (*p* < .05) during insulin infusion in the adolescents (Table 3). The contribution of albumin synthesized in relation to the whole body protein synthesis was comparable between protocols and not affected by either intervention (Fig. 3, Table 3).

We did not observe an effect of timing or order of protocol sequence on the outcome measures.

5. Discussion

Our study provides insight in albumin synthesis rates in critically ill children of two different age groups. The data obtained in our study are consistent with previous observations in critically ill adults that, although in a hypoalbuminemic condition, albumin synthesis rates were high during inflammation and metabolic stress. Nutritional interventions with various levels of glucose and amino acid intake, and insulin infusion did not further stimulate albumin synthesis rates, nor did they increase the contribution of albumin to whole body protein synthesized.

Table 3
Albumin synthesis of 8 infants and 9 adolescents admitted to the PICU^a

	Study 1		Study 2			
	LG ^b	SG ^c	SAA ^d		HAA ^e	
			Base	Insulin	Base	Insulin
Albumin concentration (g dL ⁻¹)		2.6 ± 0.4		2.6 ± 0.6		2.5 ± 0.3
FSR ^f (%.d ⁻¹)	16.3 ± 3.2	16.0 ± 2.2	23.7 ± 13.8	27.5 ± 19.0	22.6 ± 8.3	23.8 ± 11.0
ASR ^g (mg kg ⁻¹ day ⁻¹)	243 ± 45	243 ± 47	299 ± 172	330 ± 211	276 ± 99	296 ± 148
Non-oxidative leucine disposal (μmol kg ⁻¹ h ⁻¹)	122 ± 14	124 ± 20	153 ± 36	128 ± 38 ^h	173 ± 31 ⁱ	149 ± 38 ^{h i}
Contribution to total protein synthesis (%)	6.7 ± 1.9	6.7 ± 1.9	6.9 ± 4.4	8.4 ± 4.4	5.3 ± 1.7	6.5 ± 2.5

^a All values are mean ± SD.

^b LG = low glucose (2.5 mg kg⁻¹ min⁻¹).

^c SG = Standard glucose (5.0 mg kg⁻¹ min⁻¹).

^d SAA = standard amino acid intake (1.5 g kg⁻¹ d⁻¹).

^e HAA = high amino acid intake (3.0 g kg⁻¹ d⁻¹).

^f FSR = fractional synthesis rate.

^g ASR = absolute synthesis rate.

^h Base vs. Insulin; *p* < .05.

ⁱ SAA vs. HAA; *p* < .05.

^j SAA vs. HAA, *p* = .05.

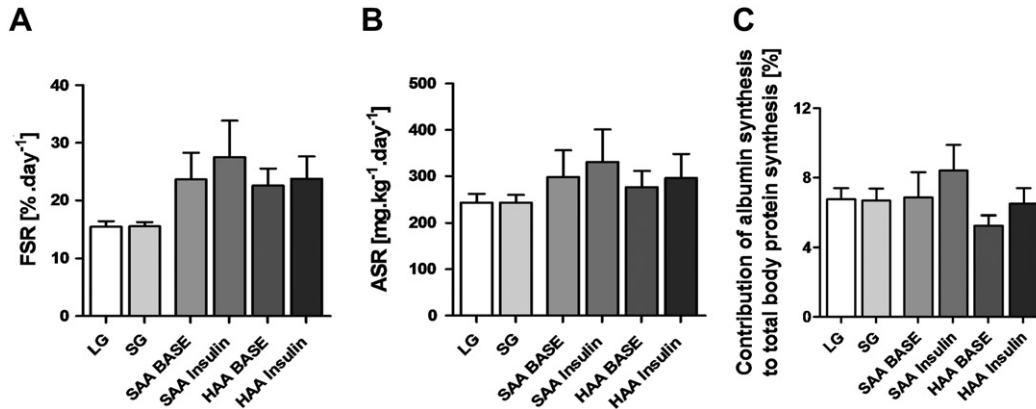


Fig. 3. Albumin synthesis in 8 infants and 9 adolescents receiving various nutritional support and insulin, Panel A Albumin fractional synthesis rate (%·day⁻¹), Panel B Albumin absolute synthesis rate (mg kg⁻¹ day⁻¹), Panel C Contribution of albumin synthesis as ratio to total whole body protein synthesis per day LG = low glucose intake (2.5 mg kg⁻¹ min⁻¹), SG = standard glucose (5.0 mg kg⁻¹ min⁻¹), SAA = Standard amino acid intake (1.5 g kg⁻¹ day⁻¹), HAA = High amino acid intake (3.0 g kg⁻¹ day⁻¹), Base = during baseline, Insulin = during insulin infusion (80 mU m⁻² min⁻¹).

It has been shown previously that fractional synthesis rates are higher in neonates (12.5–23%)^{16,22} and infants (15–20%)^{30,31} than healthy adults (6–15%)^{7–10,14,32,33} (Table 4). However, the populations studied in these studies, as well as the experimental conditions, methods and interventions studied differ markedly (Table 4) and one should be cautious to compare these historical data. However, in our study high synthesis rates were more obvious in the septic adolescents. The synthesis rates measured in our infants were consistent with those measured in age-related peers.^{30,31} The synthesis rates in the adolescents were even slightly higher than those in (critically) ill adults (11–18%).^{7,34–37} These relative high synthesis rates might be explained by their condition, as an inflammatory and catabolic insult, which usually follows after trauma, surgery or infection, is a strong stimulant for albumin synthesis in patients.¹² These findings suggest that the adolescents were in a higher inflammatory and catabolic state, compared to the infants, explaining their relative high synthesis rates for their age. The latter is supported with a higher leucine flux in the adolescents (*data not shown*), an indicator for amino acid turnover as a derivative measure of catabolism. Furthermore, the C-reactive protein, a hepatic acute phase protein, was substantially higher in the septic adolescents.

In contrast to our hypothesis and data in healthy adults and neonates^{9,14,16} increasing both parenteral energy (glucose) as well as amino acid intake did not affect synthesis rates. Although an unexpected finding at first, it is nevertheless consistent with published data in healthy³⁸ as well as post-surgical³⁷ adults receiving intravenous nutrients. Albumin synthesis rates are less responsive to parenteral than enteral nutrients.^{39,40} Parenteral nutrition bypasses the splanchnic uptake and amino acids are presented to the liver through the portal venous circulation in lower concentrations.⁴¹ Studies in piglets suggest that after enteral feeding, portal rather than arterial amino acids are preferentially used for hepatic protein synthesis.⁴¹ Therefore, the parenteral route of nutrient supply in our children might be responsible for the lack of effect of our nutritional interventions, stressing the need for early enteral nutrition in critically ill children even more.

The lack of nutritional effect could also have been caused by the medical or surgical condition our patients were in. In health, the major regulator of (muscle and hepatic) protein synthesis is increased amino acid availability, since amino acids themselves modulate cellular processes leading to protein synthesis via enhanced translation initiation as well as through promoting translation elongation.^{42,43} Under conditions of inflammation and cytokine release, muscle protein synthesis is inhibited and less

responsive to amino acid supply, while hepatic synthesis of immune/inflammatory proteins is greatly increased.^{44–46} An increased supply of amino acids moderately attenuates the inhibitory response of inflammation on muscle protein synthesis.⁴⁷ In contrast, a pathway other than nutrient signaling is responsible for the increased hepatic protein synthesis during an inflammatory insult.⁴⁸ Our study now shows that albumin synthesis, already amplified in our patients, was also not further affected with changes in nutrient supply.

Of further interest, administration of insulin, a strong anabolic hormone, previously reported to stimulate albumin synthesis^{15,49} did not have any effect. Insulin decreased whole body protein synthesis, indicated by the NOLD, even when a high amino acid intake was provided. Lang and coworkers⁵⁰ reported that insulin failed to stimulate protein synthesis in an animal model of sepsis via a defect in insulin signaling to a step in translation initiation. Inflammation induces insulin resistance to protein synthesis, which might explain why hyperinsulinemia in our septic adolescents did not increase albumin synthesis rates. Because our study is the first to describe the synthesis rates in critically ill infants and adolescents, it is difficult to characterize our results. It is therefore possible that the synthesis rates measured in our patients are not further increased by either intervention because they are already at their maximum.

There are some limitations to our study which need to be taken into account, some of them inherent to studying critically ill children. As mentioned in the statistical description, statistical comparison between the two protocols was not justified due to the wide variations in between the two groups. Furthermore, absolute albumin synthesis rates are calculated with albumin plasma levels. However, as we mentioned in the introduction, hypoalbuminemia is primarily caused by dilution and redistribution secondary to an altered vascular permeability. Therefore, we potentially underestimated the absolute synthesis rates as our study was not able to correct for dilution and redistribution. However, the data within the groups are consistent and justify our conclusions that synthesis rates were high, and unresponsive to change in parenteral nutrients as well as hyperinsulinemia. We further acknowledge that we only performed short term nutritional changes in our study. However, as has been shown in previous studies, albumin synthesis rates can react acute and fast to these interventions.^{12–15} Finally, we acknowledge that 4 septic adolescents received glucocorticoids as adjuvant therapy. Glucocorticoids impair protein anabolism, also in the pediatric population⁵¹ and are capable of obstructing the anabolic effects of insulin.⁵² However, the catabolic properties are

Table 4
Overview of populations and experimental conditions in historical data describing albumin synthesis rates in comparison with present study.

	Age	Diagnosis	n =	Route of nutrition	Caloric intake (kcal kg ⁻¹ d ⁻¹) ^a	Protein intake (g kg ⁻¹ d ⁻¹) ^a	Albumin plasma level (g.dL ⁻¹)	Albumin FSR (%.d ⁻¹)	Albumin ASR (mg.kg ⁻¹ d ⁻¹)
Present study	Infants (0.8 ± 0.2 yrs)	Post-craniofacial surgery	8	Parenteral	12.5–25.9	0	24.8 (20–31)	15.3 ± 1.7	224 ± 42
Present study	Adolescents (15.0 ± 1.2 yrs)	Sepsis/SIRS	9	Parenteral	17.7–52	1.2/3.2	25.5 (22–38)	25.6 ± 16.2	301 ± 158
Van den Akker et al. ¹⁶	Premature infants	VLBW	15	Parenteral	31.2 (26.0–32.9)	2.4	26 (25–27)	22.9 (17.6–28.0)	228 (187–289)
Bunt et al. ²²	Premature infants	VLBW	24	Parenteral	25–33	0	25 ± 0.6	13.9 ± 1.5	148 ± 17
Jahoor et al. ³⁰	Children (7–17 months)	Healthy/HIV infected	4/6	Enteral	168 ± 12/ 101 ± 12	3.4 ± 0.3/ 2.3 ± 0.5	34 ± 2.4/ 26 ± 2.9	16 ± 3.3/17 ± 2.0	309 ± 48/247 ± 15
Morlese et al. ³¹	Children (~1 yr)	Recovery from marasmus/kwashiorkor	14	Enteral	30–50	3	~34–40	15.6 ± 1.2/~17	277 ± 30/368 ± 36
De Feo et al. ¹⁴	Adults (18–36 yrs)	Healthy	9	–/Enteral	0/40	0/?	–	12 ± 2/23 ± 3	–
Caso et al. ⁸	Adults (21–35/>60 yrs)	Healthy	8/8	Enteral	1.5 daily REE	15:30:55% ^b	38.8 ± 0.7/40.8 ± 1.0	10.1 ± 0.5/9.2 ± 0.4	182 ± 6/144 ± 7
Thalacker-Mercer et al. ⁹	Adult (21–43/63–79 yrs)	Healthy	18/18	Enteral	1.75 daily REE	0.5–1.0	–	14.7 ± 5.6	–
Ballmer et al. ³³	Adults (33.4 ± 6.5 yrs)	Healthy	7	–	0	0	47 ± 4	7.2 ± 1.3	157 ± 39
Mansoor et al. ⁷	Adults	Healthy/Head injury	5/6	Enteral/Parenteral	34 ± 2/39 ± 2	1.4 ± 0.1/1.5 ± 0.1	33.7 ± 1.2/25.2 ± 1.2	7.3 ± 0.4/11.4 ± 1.0	91 ± 5/ 133 ± 14
Barle et al. ³²	Adults	Laparoscopic cholecystectomy	9	–	0	0	39.9 ± 1.7	5.9 ± 1.2	109 ± 21
Essen et al. ³⁵	Adults	Critically ill	15	Parenteral	20–30	0.6–1.1	27 ± 1	12.8 ± 1.2	184 ± 19
Barle et al. ³⁶	Adults	Critically ill	11	Parenteral and Enteral	20–30	0.6–1.3	28.2 ± 6.6	16.3 ± 4.1	233 ± 67
Barle et al. ³⁷	Adults	Post-surgery	7	Parenteral	17.5	0.6	–	15.2 ± 4.7	–

Values are depicted as mean ± SD, or as median (range). FSR = Fractional synthesis rate, ASR = Absolute synthesis rate, REE = Resting Energy Expenditure. ~ Data from Morlese et al. were partially adapted from figures.

^a Nutritional data were either copied from the manuscript or, when possible, calculated from the data provided.

^b Percentage of energy content of meal derived from protein, fat and carbohydrates respectively.

exerted primarily through increased proteolysis, more than suppression of protein synthesis.⁵¹ We did not find a difference in synthesis rates or effect of insulin therapy in the 4 adolescents who received glucocorticoids, although our study was not powered to discover such an effect.

6. Conclusion

Although mildly hypoalbuminemic, albumin synthesis rates were high in post-surgical infants and in septic adolescents. Synthesis rates did not respond to short term changes in parenteral intakes of glucose, amino acids, and insulin. The data from our study, the first in the critically ill pediatric population, confirm previous observations that the low plasma albumin levels are not due to decreased synthesis rates. Furthermore, during critical illness albumin synthesis rates were not influenced by nutrients or insulin and might be regulated through pathways other than nutritional signaling.

Financial disclosure

This study was supported by the Sophia Foundation of Scientific Research (SSWO). The sponsor had no influence on protocol design, data acquisition, the writing or the decision to publish the data.

Statement of authors' contributions

S.V. Study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript. H.S. Technical and material support; analysis and interpretation of data, critical revision of the manuscript. J.C. Study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript. K.J. Obtained funding; analysis and interpretation of data; statistical analysis; critical revision of the manuscript. L.C. Study concept and design; obtained funding; analysis and interpretation of data; statistical analysis; critical revision of the manuscript. J.v.G. Study concept and design; obtained funding; technical and material support; analysis and interpretation of data; critical revision of the manuscript; study supervision.

Conflict of interest

None of the authors had a conflict of interest regarding the study, nor do none of the authors have anything to disclose.

Acknowledgments

Our gratitude goes out to the patients and their families at Texas children's hospital and Erasmus MC Sophia Children's hospital for their selfless contribution to this study. We thank the nursing staff, Gardi Minderman-Voortman and Kristien Dorst for their contribution.

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