Aspects of protein metabolism in children in acute and chronic illness
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Short-term protein intake and stimulation of protein synthesis in children after cardiac surgery

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Submitted
ABSTRACT

Background: Infants undergoing cardiac surgery are at risk of a negative protein balance, due to increased endogenous proteolysis in response to surgery and the cardiopulmonary bypass circuit, and limited intake due to fluid-restriction.

Objective: We aimed to improve whole-body protein balance, via stimulation of protein synthesis, with a short-term high-protein (HP) diet in infants following cardiac surgery.

Design: In a prospective, double-blinded, randomized trial we compared the effects of a HP (5 g/kg/d) versus normal protein (NP, 2 g/kg/d) enteral diet on protein kinetics in young children < 24 mos, on day 2 following surgical repair of their congenital heart disease. Non-oxidative disposal (synthesis) and endogenous rate of appearance of valine (breakdown), net valine balance, and the fractional synthesis rate of albumin were measured using an isotopic infusion of [1-13C]valine. We measured plasma concentrations of blood urea nitrogen, albumin, insulin, cortisol, and inflammatory mediators directly after surgery, and on the second postoperative day.

Results: We observed no statistically significant differences in valine kinetics (synthesis of valine 2.73 [range: 0.94 to 3.36] versus 2.26 [1.85 to 2.73] μmol/kg/min, \( p = 0.28 \); breakdown 2.06 [1.12 to 3.64] versus 1.90 [1.63 to 2.48] μmol/kg/min, \( p = 0.50 \); and net balance 0.54 [-0.73 to 1.75] versus 0.24 [-0.20 to 0.63] μmol/kg/min, \( p = 0.57 \) in the HP diet, compared to the NP diet, respectively). The fractional synthesis rate of albumin did not show differences between groups. In the HP group, there was significant higher valine oxidation together with higher blood urea nitrogen concentrations, as indicators of excess AA intake in the HP group, compared to the NP group.

Conclusion: Whole-body protein balance cannot be improved by a high-protein (5 g/kg/d) diet, compared to normal protein (2 g/kg/d) intake, in young children following cardiac surgery.
INTRODUCTION

Critically ill children on a Pediatric Intensive Care Unit (PICU) usually receive less than recommended protein intake, with 50% of cumulative deficits developing in the first 48 hrs of admission (1). As a result, lean body mass (LBM) deteriorates during the admission period, superimposed on the fact that approximately a quarter of patients is already undernourished on admission (1–3). Especially infants and young children after cardiac surgery, due to higher metabolic demands and fluid restriction, may be at risk of undernutrition (4). Poor pre-operative nutritional status with further decrease of LBM during admission increases morbidity, infection rate, and length of stay (LOS) in the ICU (5, 6).

In pediatric surgical patients, increased endogenous proteolysis with negative net whole-body protein balance, is the result of an endocrine and inflammatory response to surgery (7). Additionally, in cardiac surgical procedures, the use of a cardiopulmonary bypass circuit (CPB) induces complement activation, endotoxin release, leukocyte activation, and the release of many pro-inflammatory mediators, adding to the stress response (8–10).

The importance of early enteral nutrition (EN) in the PICU population was underlined by a recent multi-center trial including > 5,000 critically ill children from 12 North-American PICUs with LOS of ≥ 96 hrs, showing a strong and statistically significant association between early EN and lower mortality (odds ratio, 0.51; 95% confidence interval: 0.34 to 0.76, \( p = 0.001 \)) (11). However, data on optimal protein intake in the early phase following pediatric cardiac surgery are lacking. In adult cardiothoracic patients, recommended protein intake is 1.5–2.0 g/kg/d (12). In critically ill children, recommended protein intake for children 0–2 yrs is 2–3 g/kg/d (13). Higher protein intake (up to 3 g/kg/d) can further improve protein balance in children with acute illness (14). In a study in children with chronic illness (cystic fibrosis), we demonstrated that protein synthesis could be stimulated and net balance improved, at a protein intake of 5 g/kg/d, compared to 1.5 and 3 g/kg/d (15).

We hypothesized that whole-body protein synthesis rate and net balance, and albumin synthesis rate, but not endogenous proteolysis, can be improved with protein intake of 5 compared to 2 g/kg/d. We did not expect a difference in postoperative endocrine and inflammatory stress response between groups. In this prospective, randomized, controlled study in infants after cardiac surgical repair of a low-complex congenital heart defect (CHD), we investigated the short-term (< 48 hrs) effects on whole-body valine kinetics and albumin synthesis rate, of high protein (HP; 5 g/kg/d) dietary intake, compared to normal protein diet (NP; 2 g/kg/d). Also, we studied the effect of the HP diet on the postoperative endocrine (i.e., insulin, cortisol) and inflammatory (i.e., C-reactive protein, cytokines) response.
METHODS

Subjects

Children with CHD in the pre-operative phase of surgical repair were recruited from the pediatric cardiology departments of the Wilhelmina Children’s Hospital/University Medical Center Utrecht (UMCUtrecht), the Netherlands, Radboud University Medical Center, Nijmegen, the Netherlands and Maximá Medical Center, Veldhoven, the Netherlands. Inclusion criteria were age 3–48 months, and pending low-complex surgical repair of ventricular septal defect (VSD) and/or atrial septal defect (ASD) with CPB. Exclusion criteria were: trisomy of chromosome 21, infection (i.e. fever > 38.5 °C for > 4 hrs with positive blood culture < 48 hrs), mechanical ventilation or inotropic medication during the period of isotopic infusions, intolerance to enteral tube feeding, and post-operative use of medication with modulating effects on protein metabolism (insulin, steroids). We allowed the use of routine sedation medication (low-dose propofol 10%) during transport of the mechanically ventilated patient from the operation room (OR) to the PICU until extubation. Additionally, postoperative pain medication (morphine, paracetamol) and diuretics were allowed. The results of patients who dropped out of the study were analyzed following the intention to treat principle.

All procedures were explained to the subjects’ parents, and written informed consent was obtained. The Central Committee on Research Involving Human Subjects, The Hague, Netherlands, and the Medical Ethics Committees of the UMCUtrecht approved the study protocol.

Study design

At enrollment, a medical history was obtained and physical examination performed. Preoperatively, the cardiopulmonary bypass circuit was primed with standardized, body weight-related amounts of packed cells and albumin, but not steroids. In the OR, all patients received a multiluminal central venous catheter with the tip in the superior or inferior caval vein, a catheter in a peripheral artery for blood sampling and invasive monitoring of the blood pressure, and a nasogastric feeding tube, according to standard cardiac surgical procedures. Additionally, for study purpose, a nasopharyngeal tube was inserted for aspiration of breath samples. General anesthesia was induced using sevoflurane and fentanyl, sporadically combined with midazolam. During anesthesia and surgery, unexpected events, vascular clamping time and total CPB time were noted. After surgery, the patients were transferred to the PICU for postoperative care and ventilated for 4–6 hrs under mild sedation using propofol (standard dose: 1–2 mg/kg/h)
and morphine (standard dose: 10 μg/kg/h). After cessation of sedation they were extubated at the attending anesthetist’s and pediatric intensivist’s clinical judgment. During the entire postoperative intensive care period, medical treatment was instigated by the medical staff of the PICU. Notes were made of the amounts of prescribed sedative and analgesic medication.

After approximately 4 hrs in the PICU, at time zero ($t = 0$ h), the liquid study diet was administered in increasing amounts via a nasogastric tube with the use of a feeding pump (Kangaroo 324, Kendall Healthcare products, Mansfield, MA) such that the required feeding rate was reached before $t = 12$ hrs (Figure 3.1). The diets consisted of a mixture of whey protein/carbohydrate powder (Hydrolyzed Whey Protein Powder/Maltodextrin Mix), carbohydrate powder (Fantomalt), and fat emulsion (Calogen; all products of Nutricia, Zoetermeer, Netherlands) dissolved in water. The caloric need was estimated by age-related Schofield equation (16). Via continuous drip-feeding the protein intake was set at 5 g/kg/d in the intervention group (high protein, HP) and 2 mg/kg/d in the control group (normal protein, NP). Patients in both groups received a glucose intake of 6 mg/kg/min, with the remaining non-protein calories supplied by fat. Since all patients were fluid restricted after cardiac surgery, the volume of water in which the macronutrients were dissolved was limited at 60 mL/kg/d.

Also, at $t = 0$ h, both a blood and breath sample were taken for measurement of background isotopic enrichment of $\alpha$-ketoisovalerate (KIV) and $^{13}$CO$_2$, respectively. The end-tidal breath samples for enrichment of $^{13}$CO$_2$ were slowly aspirated through a nasopharyngeal tube with a syringe, and collected in 10 mL sterile, air evacuated, glass tubes with silicon-coated stopper.

![Experimental design](image)

**Figure 3.1** Experimental design. Timing of surgery (open arrow), and sampling of blood (cross) and breath (small arrows, with each arrow representing a duplicate sample). Duration of primed infusions of isotopes is represented by straight lines.
Plasma concentrations of insulin, cortisol, cortisol binding globulin (CBG), and blood urea nitrogen (BUN) were measured, together with inflammatory markers; C-reactive protein (CRP), interleukins IL-1β-6-8-10, and TNF-α.

At $t = 31$ hrs, $\text{NaH}^{13}\text{CO}_3$ (Cambridge Isotope Laboratories Inc, Andover, MA) was continuously infused at a rate of $0.14 \mu\text{mol/kg/min}$, after a prime bolus of $12 \mu\text{mol/kg}$, to determine CO2 production rate. At $t = 33.5–34$ hrs, after one blood and three duplicate breath samples were taken for determination of background isotopic enrichments, infusion of [1-$^{13}$]C-valine (Cambridge Isotopes) was administered at a rate of $0.184 \mu\text{mol/kg/min}$ (prime $9.1 \mu\text{mol/kg}$). The isotopes were dissolved in normal saline and infused by volumetric infusion pumps (Infusomat Space Infusion Pump; B.Braun Melsungen AG, Melsungen, Germany), after passage through a 0.20-$\mu$m Millipore filter (Minisart; Sartorius AG, Göttingen, Germany). All infusates were tested for pyrogenicity, purity and concentration.

From $t = 45.5$ to 46.5 hrs, after reaching presumed isotopic equilibrium, 5 concurrent breath samples were collected in duplicate at 15-min intervals. In the same period, at 30-min intervals, three blood samples were collected for measurement of isotopic enrichment of KIV and $^{13}$C-valine incorporation in albumin. In this 30-min sampling period an extra blood sample was taken for second measurement of plasma concentrations of insulin, cortisol, CBG, BUN, and inflammatory markers. Also, at $t = 46$ hrs, as part of the routine clinical assessment of discomfort, the FLACC (face, legs, activity, cry, consolability) score was noted (18, 19).

All blood samples were immediately centrifuged at 4000 rpm for 10 min at room temperature (Labofuge 300; Heraeus Instruments GmbH, Hanau, Germany). After separation, plasma was stored at -20 °C until analysis. At $t = 46$ hrs, serum glucose concentration was measured bedside (i-STAT r1 analyzer MN300 series; Abbott Laboratories, Chicago, IL). At $t = 46.5$ hrs, the study was ended, and all children resumed to the standard hospital age-related diets.

**Assays**

Plasma insulin concentrations were ascertained by using a chemiluminescent immunometry assay on an Immulite analyzer (DPC, Los Angeles, CA) with an intra-assay CV of < 6%, an inter-assay CV of < 6%, and a detection limit 15 pmol/L. Cortisol was measured by chemiluminescent immunoassay on an Immulite analyzer with an intra-assay CV of < 8%, an inter-assay CV of < 7%, and a detection limit of 50 nmol/L. CBG was measured by radio-immuno assay (BioSource Europe S.A., Nivelles, Belgium) with intra-assay CV of 4–5%, an inter-assay CV of 9–12%, and a detection limit of 10 mg/L.
Plasma albumin and BUN concentrations were measured by spectrophotometry (both Roche Diagnostics, Basel, Switzerland).

CRP values were measured by immunoturbidimetry (Roche Diagnostics; lower limit of detection [LOD]: 0.3 mg/L). Cytokine levels were determined by multiplex fluorescent bead assay (eBioscience, Vienna, Austria) for IL-1β (LOD in pg/mL: 0.20), IL-6 (LOD: 2.27), IL-8 (LOD: 0.79), IL-10 (LOD: 0.47) and TNF-α (LOD: 6.97) on a Bioplex 200 (BioRad). All samples were analyzed in parallel and for values below detection level we took half the LOD values for statistical analyses.

**Isotopic analysis**

A detailed description of KIV-measurement was described in recent articles of our group (20, 21). 13C isotopic enrichment in the breath samples was analyzed by an infrared isotope analysis technique (Helifan, Analytic Fischer Instruments, Leipzig, Germany). The 13C enrichment was expressed as the atom percentage excess (APE) above baseline.

**Albumin isolation and enrichment measurements**

To isolate pure 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. According to the included protocol, the column was washed three 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 re-suspending and precipitating the pellet again. The protein pellet was then hydrolyzed in 140 μL 6 mol HCl/L for 22 hours at 110 °C. Amino acids (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 (GC/C/IRMS; Delta XP; Thermo Electron, Bremen, Germany) as previously described (22–24).

**Calculations**

The rate of appearance of valine in plasma (Ra_{val}), VCO₂ carbon dioxide production (in millimoles per h), and valine oxidation (Oxid_{val}) were calculated according to standard equations (25–28):
\[ Ra_{val} = I_{val} \times (E_{inf}/E_{val} - 1) \]  
\[ VCO_2 = I_{bicarb} \times 0.81 / E_1^{13}CO_2 \text{ bicarbonate plateau} \]  
\[ Oxid_{val} = VCO_2 \times (E_1^{13}CO_2 \text{ valine plateau} - E_1^{13}CO_2 \text{ bicarbonate plateau}) / E_{KIV}^{13C} \]  

where \( I_{val} \) is the infusion rate of labeled valine, \( E_{inf} \) tracer enrichment of the labeled valine solution (= 99%), \( E_{val} \) valine enrichment in plasma during plateau phase, \( VCO_2 \) carbon dioxide production (in millimoles per hour), \( I_{bicarb} \) is the infusion rate of labeled bicarbonate, \( E_1^{13}CO_2 \) enrichment of carbon dioxide in the breath samples, and \( E_{KIV}^{13C} \) enrichment in plasma of KIV.

In this model of single amino acid tracer kinetics non-oxidative disposal of valine (\( NOD_{val} \)) represents protein synthesis (\( S \)), and endogenous rate of appearance of valine (\( \text{Endo-Ra}_{val} \)) represents protein breakdown (\( B \)) proportionally (29).

\[ S = NOD_{val} (\mu\text{mol/kg/min}) = Ra_{val} - Oxid_{val} \]  
\[ B = \text{Endo-Ra}_{val} (\mu\text{mol/kg/min}) = Ra_{val} - \text{val(diet)} \]  
\[ \text{Protein balance (g/kg/d)} = ([4] - [5]) \times 1440 / 450 \]

where 1440 is the amount of minutes in 24 hrs, and 450 represents the estimated contribution of valine to whole body protein in \( \mu\text{mol/g protein} \) (30).

Using plasma KIV enrichment at plateau level, as albumin precursor, we also calculated albumin fractional synthesis rate (\( FSR_{alb} \)) as described by Verbruggen et al (24). The \( FSR_{alb} \) 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:

\[ FSR_{alb} = (E_{val-alb} / E_{KIV}) / \Delta t \times 100\% \]  

where \( E_{val-alb} \) is the enrichment in mole percent excess (MPE) of incorporated valine in albumin, and \( E_{a-KIV} \) is the mean enrichment in MPE of the precursor, \( i.e., \) plasma KIV. \( \Delta t \) is the time between start of the valine infusion and time of blood sample. This calculation slightly underestimates actual \( FSR_{alb} \), since KIV plasma concentrations approximately need 0.5–1 h to reach plateau after the start of infusion. However, since infusion time of valine was 11.5–12.5 hrs, this calculation error can be neglected.
Statistical analysis and study power

In small sample sizes data are not normally distributed. Therefore, data are presented as median and range. The Mann-Whitney $U$ test was used to investigate differences between groups. For comparison of the changes of hormone levels and inflammatory mediator concentrations in time, we used the Wilcoxon signed rank test. All analyses were performed by using SPSS for WINDOWS statistical software (version 20.0; SPSS Inc, Chicago, IL). P-values less than 0.05 were considered statistically significant.

From a previous study in young children after cardiac surgery, with net whole-body valine balance of $-0.65 \, \mu\text{mol/kg/d}$, we estimated that we needed 12 patients per randomization group to detect sufficient difference (with $\alpha = 2.5\%$ and $\beta = 82\%$) in whole-body valine balance to reverse the negative balance ($> 0 \, \mu\text{mol/kg/d}$) (31).

RESULTS

Patients

We were able to enroll 28 patients in the study who fulfilled the inclusion criteria in the period September 2012–January 2014 (Figure 3.2). From 47 eligible patients prior to surgery, we were not able to obtain consent from 18 individual’s caretakers for reasons of continuation of breast feeding, stressful situation around surgery, and reluctance for randomization of nutrition. As listed in Table 3.1, there were no differences in baseline characteristics of the NP and HP groups. In our study model, there were no differences in aortic clamping time, time on CPB circuit, and priming of the CPB circuit between groups (Table 3.2). In the postoperative phase, according to randomization, the actual protein and carbohydrate intakes were reached as targeted, with no difference in total caloric intake between groups. Intakes of non-protein calories (i.e., calories derived from fat and carbohydrates) and fat differed by study design, the latter to achieve isoenergetic diets (Table 3.3). The contribution to fat intake of the propofol infusion (propofol dissolved in a 10% fat emulsion at rate 0.1–0.2 mL/kg/h) in the immediate postoperative phase until extubation within 6 hrs after surgery, was $< 0.1 \, \text{g/kg/d}$ fat.

There were 3 dropouts in the NP group and 5 in the HP group, respectively (Figure 3.2): the study protocol was violated 1 time in each group (premature removal of sample line and accidental removal of nasogastric feeding tube, respectively), and in 3 patients the study was stopped due to postoperative complications (sternum dislocation, cardiac tamponade, and junctional ectopic tachycardia). In the HP group, 2 patients had gastric residues and/or
diarrhea, which in 1 patient was caused by viral gastroenteritis. One patient in the NP was withdrawn from the study by the parents due to personal circumstances.

At \( t = 45.5 \) to 46.5 hrs, data regarding valine kinetics, endocrine and inflammatory response mediators could be obtained and analyzed in 9 patients in the NP group and 11 patients in the HP group, respectively. In either group, from one patient oxidation data are missing, due to sampling error of breath samples.

**Protein metabolism**

There was, by study design with higher dietary protein intake and consequent higher valine intake in the HP group compared to the NP group, a significant difference in total valine flux (turnover) between groups. We observed no statistically significant differences in non-oxidative disposal of valine, representing protein synthesis, endogenous rate of appearance
Table 3.1  Baseline characteristics of groups

<table>
<thead>
<tr>
<th></th>
<th>NP  (n = 12)</th>
<th>HP  (n = 16)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls</td>
<td>5/7</td>
<td>7/9</td>
<td></td>
</tr>
<tr>
<td>Age (mos)</td>
<td>12 (3 to 17)</td>
<td>7 (3 to 20)</td>
<td>0.13</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>7.6 (4.4 to 12.0)</td>
<td>7.0 (3.6 to 13.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>Body weight (SDS)</td>
<td>-1.8 (-4.4 to 0.8)</td>
<td>-1.1 (-4.1 to 1.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-1.3 (-3.2 to 2.1)</td>
<td>-0.9 (-1.9 to 1.6)</td>
<td>0.63</td>
</tr>
<tr>
<td>Weight for height (SDS)</td>
<td>-1.2 (-2.8 to 0.1)</td>
<td>-1.2 (-2.9 to 0.6)</td>
<td>0.87</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSD</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ASD I</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>VSD + ASD II</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>pAVSD</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Preop O₂ saturation (%)</td>
<td>99 (94 to 100)</td>
<td>98 (96 to 100)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Values are presented as number of subjects or median (range). NP, normal protein (2 g/kg/d) diet; HP, high protein (5 g/kg/d) diet; SDS, standard deviation score; VSD, ventricular septal defect; ASD I, atrial septal defect type 1; ASD II, atrial septal defect type 2; pAVSD, partial atrioventricular septal defect; Preop, preoperative. *Mann-Whitney U test.

Table 3.2  Intraoperative aortic clamping time, priming and time on cardiopulmonary bypass circuit

<table>
<thead>
<tr>
<th></th>
<th>NP  (n = 12)</th>
<th>HP  (n = 16)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic clamping time (min)</td>
<td>36.5 (19 to 76)</td>
<td>31.5 (18 to 79)</td>
<td>0.84</td>
</tr>
<tr>
<td>Time on CPB (min)</td>
<td>60.5 (36 to 97)</td>
<td>54.0 (37 to 138)</td>
<td>0.54</td>
</tr>
<tr>
<td>Priming CPB circuit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes (mL/kg)</td>
<td>140 (100 to 260)</td>
<td>125 (80 to 197)</td>
<td>0.16</td>
</tr>
<tr>
<td>Plasma (mL/kg)</td>
<td>0 (0 to 25)</td>
<td>0 (0 to 15)</td>
<td>0.66</td>
</tr>
<tr>
<td>Albumin 20% (mL/kg)</td>
<td>50 (50 to 100)</td>
<td>50 (50 to 150)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are presented as median (range). NP, normal protein (2 g/kg/d) diet; HP, high protein (5 g/kg/d) diet; CPB, cardiopulmonary bypass. *Mann-Whitney U test.

of valine, representing protein breakdown, and net whole-body protein balance between groups (Table 3.4). Additionally, the fractional synthesis rate of albumin and plasma albumin concentrations did not show differences between groups. In the HP group, there was statistically higher valine oxidation with significant higher BUN concentrations, compared to the NP group. There was no correlation between weight-for-height score on admission and protein balance (data not shown).
Endocrine response

Between the NP and HP group, there were no statistically significant differences in plasma concentrations of glucose at $t = 46$ hrs, and plasma concentrations of insulin, cortisol and CBG at $t = 0$ h and $t = 46$ hrs (Table 3.5). Between $t = 0$ h and $t = 46$ hrs, we observed a significant increase in plasma insulin concentration in both groups. At $t = 46$ hrs, carbohydrate intake...
was 5.9 and 5.7 mg/kg/min in the NP and HP group, respectively, compared to absence of carbohydrate intake at $t = 0$ h.

Between time points $t = 0$ h and $t = 46$ hrs, cortisol concentration increased from 175 (range: 49–833) to 417 (79–1548) nmol/L in the NP group ($p = 0.02$), and from 211 (83–411) to 345 (37–1162) nmol/L in the HP group, respectively ($p = 0.08$). In the same time-interval plasma concentrations of CBG increased in the NP group from 34 (13 to 44) to 39 (30 to 48) mg/L ($p = 0.01$), and in the HP group from 29 (18 to 51) to 36 (26 to 54) mg/L ($p < 0.01$), respectively. There were no statistically significant differences between cortisol-to-CBG ratios between groups and time points (data not shown).

**Inflammatory response**

At $t = 0$ h, we found relatively high concentrations of the anti-inflammatory cytokine IL-10 (177.4 with range 25.0–465.8 pg/mL in the NP group, compared to 120.2 with range 5.0–581.5 pg/mL in the HP group; $p = 0.58$) (Figure 3.3). In both groups, IL-10 had almost completely

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**Table 3.5  Clinical and endocrine response after surgery**

<table>
<thead>
<tr>
<th></th>
<th>NP ($n = 9$)</th>
<th>HP ($n = 11$)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLACC score at $t = 46$ hrs</td>
<td>0 (0 to 7)</td>
<td>0 (0 to 3)</td>
<td>0.55</td>
</tr>
<tr>
<td>Body temp (°C)</td>
<td>37.3 (36.8 to 37.8)</td>
<td>37.5 (37.0 to 38.1)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Endocrine measures at $t = 0$ h</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>19 (8 to 98)</td>
<td>8 (8 to 113)</td>
<td>0.66</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>175 (49 to 833)</td>
<td>211 (83 to 411)</td>
<td>0.87</td>
</tr>
<tr>
<td>CBG (mg/L)</td>
<td>34 (13 to 44)</td>
<td>29 (18 to 51)</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>Endocrine measures at $t = 46$ hrs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 (4.2 to 9.0)</td>
<td>4.9 (4.2 to 5.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>165 (35 to 226)*</td>
<td>158 (55 to 492)*</td>
<td>0.82</td>
</tr>
<tr>
<td>Glucose/insulin-ratio</td>
<td>0.04 (0.01 to 0.06)</td>
<td>0.03 (0.02 to 0.12)</td>
<td>0.50</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>417 (79 to 1548)†</td>
<td>345 (37 to 1162)†</td>
<td>0.46</td>
</tr>
<tr>
<td>CBG (mg/L)</td>
<td>39 (30 to 48)†</td>
<td>36 (26 to 54)†</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are presented as median (range). NP, normal protein (2 g/kg/d) diet; HP, high protein (5 g/kg/d) diet; FLACC, face, legs, activity, cry, consolability score; temp, temperature; CBG, cortisol binding globulin. *Mann-Whitney U test; †Wilcoxon signed rank test.
Figure 3.3  Box-whisker plots of CRP, IL-1β, IL-6, IL-8, IL-10, and TNF-α plasma concentrations at \( t = 0 \) h and \( t = 46 \) hrs for NP group (light blue bars) and HP group (dark blue bars). Values are presented as median (range). NP, normal protein (2 g/kg/d) diet; HP, high protein (5 g/kg/d) diet; CRP, C-reactive protein; IL, interleukin; TNF-α, tumor necrosis factor-α. *\( p < 0.05 \) within groups between time points (Wilcoxon signed rank test).
disappeared at $t = 46$ hrs ($p = 0.01$ and $p < 0.01$ in the NP and HP group, respectively, between time points).

We observed low plasma concentrations of the proinflammatory cytokines IL-1β, IL-6, and IL-8 at $t = 0$ h and $t = 46$ hrs, without statistical differences between groups. At $t = 46$ hrs compared to $t = 0$ h, the plasma concentrations of the proinflammatory cytokines were even lower in both groups, but only for IL-8 this difference was statistically significant [13.1 (range: 2.4–94.5) and 6.0 (2.9–17.7) pg/mL in the NP group, $p = 0.04$; compared to 17.7 (5.5–61.5) and 2.0 (0.4–29.0) pg/mL in the HP group, $p = 0.05$]. At both time points, TNF-α concentrations were below the limit of detection in both groups. Between $t = 0$ h and $t = 46$ hrs, CRP was the only inflammatory biomarker that showed an increase in plasma concentration in time [from 0.5 (0.2–1.9) to 34.0 (20.5–55.8) mg/L in the NP group ($p < 0.01$), and from 0.4 (0.2–3.2) to 20.8 (13.8–41.2) mg/L in the HP group ($p < 0.01$), respectively]. Between groups, at different time points, there were no statistically significant differences in concentrations of interleukins and CRP.

**DISCUSSION**

It is not clear what the optimal protein intake should be in infants and young children after cardiac surgery. In the present study, in infants after cardiac surgical repair of their low-complex CHD with use of CPB, we observed no statistically significant differences in whole-body protein synthesis (WBPS), -breakdown (WBPB), and net protein balance after feeding with a high-protein diet (HP, 5 g/kg/d), compared to the control group with an isocaloric normal-protein diet (NP, 2 g/kg/d). Additionally, we did not find improvement of fractional synthesis rate of albumin (FSR$_{\text{alb}}$) and plasma albumin concentrations in the HP group, compared to the NP group.

Our findings suggest that in this population protein synthesis is already maximally stimulated by a protein intake of 2 g/kg/d, and that excess dietary protein is converted to carbon dioxide and urea, as suggested by the significantly higher valine oxidation rate and plasma urea levels in the HP group. Therefore, the upper limit of optimal protein intake in infants after cardiac surgery is below 5 g/kg/d. We conclude that at present an intake of 2 g/kg/d of protein in these patients is recommended, and not 5 g/kg/d.

A systematic review of protein balance studies in mixed PICUs shows that in critically ill children a positive protein balance can relatively easily be achieved by moderate intake of calories 57 kCal/kg/d and protein 1.5 g/kg/d (14). This is in agreement with our own observations in
the NP group. In a small study in neonates following general surgery, Duffy et al observed an improvement of the protein balance due to decreased endogenous protein breakdown at higher protein intake of $3.9 \pm 0.5 \text{ g/kg/d}$, compared to normal protein intake of $2.3 \pm 0.4 \text{ g/kg/d}$ (32). However, in that study, the high protein intake group also received a higher amount of total calories compared to the normal protein group ($91$ and $75 \text{ kCal/kg/d}$, respectively), and therefore the observed decrease in proteolysis and effect on net protein balance may not be attributable solely to higher protein intake. In a systematic review in low birth weight infants, Premji et al identified 5 studies that showed improved nitrogen accretion rates with high intakes of $4–6 \text{ g/kg/d}$ balanced protein, compared to age-related reference protein intake of $3 \text{ g/kg/d}$ (33). However, the same studies also found adverse metabolic effects such as azotemia and metabolic acidosis (33). Also in our study, in the HP group compared to the NP group, we observed significantly higher valine oxidation rate and plasma urea levels. There were no differences of $\text{FSR}_{\text{alb}}$ between the 2 groups. The observed values in our study were lower than those in a study in infants following general surgery ($16 \pm 2.2 \% / \text{d}$) (34). In critically ill children, lower albumin synthesis rates may be observed as the result of increased synthesis rates of acute phase proteins such as CRP and fibrinogen, at the expense of decreased synthesis of selected proteins (e.g. albumin, transferrin, prealbumin, retinol-binding protein, and fibronectin) (35). Moreover, in our study in children following cardiac surgery, the CPB circuit was primed with albumin (median [range]: $1.5 [1.0–4.2] \text{ g/kg}$) as part of standard procedure, resulting in relatively high plasma albumin concentrations in the NP and HP groups, which might further have suppressed albumin synthesis in our patients.

Insulin inhibits proteolysis (36). In response to carbohydrate intake after start of the study diets, we observed a statistically significant increase of plasma insulin concentration at $t = 46$ hrs, compared to $t = 0 \text{ h}$, in both groups. The median values were comparable to those that we have found in an earlier study in pediatric cardiac surgical patients, but three times higher than reported in a similar setting with lower carbohydrate intake (31, 37). We speculate that the insulin concentrations in response to the relative high carbohydrate intake of $\sim6.0 \text{ mg/kg/min}$ in our studies maximally suppress proteolysis.

As secondary outcome of our study, we did not find a difference in postoperative endocrine and inflammatory stress response to cardiac surgery with use of CPB, between the 2 groups. We observed a mild and short-lived (< 46 hrs) postoperative production of the pro-inflammatory mediators IL-6 and IL-8, together with persistent low levels of IL-1β over time. In pediatric cardiac surgical patients, the plasma IL-6 concentration is positively correlated with increased postoperative morbidity (38). Simultaneous with the pro-inflammatory response,
there was a moderate increase in the plasma concentration of anti-inflammatory cytokine IL-10 in both groups in the immediate postoperative phase, with a sharp decrease in plasma IL-10 also in both groups at $t = 46$ hrs. In both groups, at both time points, TNF-$\alpha$ plasma concentrations were below the limit of detection. At $t = 46$ hrs, we observed an increase of plasma concentrations of CRP and cortisol in both groups, compared to $t = 0$ h.

These observations are in agreement with other studies in infants following cardiac surgery, that also report only limited stress response on day 1, with prompt resolution within 48 hrs (8, 39–41). Identical mild and short-lived stress responses can be observed in infants following general surgery (42–46). Interestingly, in an ex vivo experimental setting, it was demonstrated that plasma drawn from neonates during CPB-assisted cardiac surgery can inhibit LPS-induced TNF-$\alpha$ but not IL-6 synthesis by monocytes (47). In our study, we were able to demonstrate IL-6, IL-8 and IL-10 production, but not TNF-$\alpha$. Clinically, the mild stress response that we observed was translated to a favorable clinical course, with all of our patients weaned from the ventilator within 4–6 hrs after surgery, and transfer from the PICU to the general ward on the next day. Typically, in adult cardiac surgical patients, a more extensive inflammatory response can be seen after surgery with concomitant greater metabolic impact and prolonged clinical course (48, 49). As an explanation, neonates have an immature immune system, and react to injury with a decreased hyper-inflammatory response (50, 51). Newborns and infants react to cardiac surgery and exposition to a CPB circuit with pro- and anti-inflammatory response, without tendency to extremes of either side (9). With aging, gradually a low grade, chronic pro-inflammatory status arises, which is a concept that previously has been referred to as ‘inflammaging’ (52).

In our study, we have infused the bicarbonate and valine isotopes for 15 and 12 hrs, respectively (Figure 3.1). As a consequence of this long infusion period, we might have over-primed the distribution pools, resulting in premature release of the isotopic tracer from the tracee back into the blood compartment. This results in overestimation of total valine flux, and, consequently, overestimation of endogenous $R_{av}$ and underestimation of NOD$_{val}$. We estimated that this might have affected results in both groups proportionally.

We have also considered the possibility that our study was underpowered, since net valine balance is more than 2-fold higher in the HP group, compared to the NP group, but without statistical significance (0.24 [range: -0.20 to 0.63] $\mu$mol/kg/min and 0.54 [-0.73 to 1.75] $\mu$mol/kg/min in NP and HP group, respectively; $p = 0.57$). However, we consider this to be less likely, as the statistically significant increase of valine oxidation and BUN are the most plausible explanation of the metabolic fate of surplus protein intake in the HP diet.
In conclusion: in pediatric, low-to-moderate-complexity cardiac surgery, a short-term high-protein diet (HP, 5 g/kg/d), compared to an isocaloric normal-protein diet (NP, 2 g/kg/d), does not improve whole-body protein balance, possibly due to a mild postoperative stress response. Therefore, in young children following cardiac surgery a higher than standard protein intake cannot be recommended.

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High protein intake in acute illness


