Aspects of protein metabolism in children in acute and chronic illness

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High-carbohydrate/low-protein–induced hyperinsulinemia does not improve protein balance in children after cardiac surgery

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ABSTRACT

**Background:** In pediatric cardiac surgery, fluid-restricted low-protein (LoProt) diets account for cumulative protein deficits with increased morbidity.

**Objective:** In this setting, we aimed to inhibit proteolysis by a high-carbohydrate (HiCarb)-intake–induced hyperinsulinemia and improve protein balance.

**Design:** The effect of a HiCarb/LoProt (glucose 10 mg/kg/min and protein 0.7 g/kg/d) versus a normal-carbohydrate (NormCarb)/LoProt (glucose 7.5 mg/kg/min and protein 0.3 g/kg/d) enteral diet on whole-body protein breakdown and balance was compared in a prospective, randomized, single-blinded trial in 24 children after cardiac surgery. On the second postoperative day, plasma insulin and amino acid concentrations, protein breakdown (endogenous rate of appearance of valine), protein synthesis (non-oxidative disposal of valine), protein balance, and the rate of appearance of urea were measured by using an isotopic infusion of [1-13C]valine and [15N2] urea.

**Results:** The HiCarb/LoProt diet led to a serum insulin concentration that was three times higher than the NormCarb/LoProt diet (596 pmol/L, 80–1833, and 198 pmol/L, 76–1292, respectively, \( p = 0.02 \)), without differences in plasma glucose concentrations. There were no differences in plasma amino acid concentrations, non-oxidative disposal of valine, and endogenous rate of appearance of valine between the groups, with a negative valine balance in the two groups (-0.65 mmol/kg/min, -1.91 to 0.01, and -0.58 mmol/kg/min, -2.32 to -0.07, respectively, \( p = 0.71 \)). The serum cortisol concentration in the HiCarb/LoProt group was lower compared with the NormCarb/LoProt group (204 nmol/L, 50–544, and 532 nmol/L, 108–930, respectively, \( p = 0.02 \)).

**Conclusion:** In children with fluid restriction after cardiac surgery, a HiCarb/LoProt diet compared with a NormCarb/LoProt diet stimulates insulin secretion but does not inhibit proteolysis further and therefore cannot be advocated for this purpose.
INTRODUCTION

Analyses of nutritional support strategies in patients after an acute admission to a pediatric intensive care unit have frequently shown an initial administration of basic carbohydrate infusions with only a slow introduction of macronutrients in the consecutive days, resulting in cumulative caloric and protein deficits (1–4). Especially young infants and children after cardiac surgery are at risk for acute protein malnutrition and delayed recovery owing to higher metabolic demands and strict fluid restriction (5, 6). In critically ill children, as in adults, a negative protein balance with a loss of lean body mass is associated with a longer stay in a pediatric intensive care unit, an important risk factor for mortality and long-term morbidity (1, 7–10).

Insulin has a clear inhibitory effect on proteolysis in healthy individuals (11–13) but also in intensive care patients (14, 15). In contrast, the protein synthesis rate is highly dependent on the availability of amino acids (AAs), with only an additive anabolic effect of insulin (16, 17). In modern adult critical care, a tight glycemic control by insulin infusion is standard care. However, in young critically ill children, the use of an exogenous insulin infusion is limited because of the risk of serious hypoglycemia with potential detrimental effects on brain function (18, 19). We hypothesized that in children after cardiac surgery with a low protein (LoProt) intake owing to fluid restriction, an enteral high-carbohydrate (HiCarb) diet-induced hyperinsulinemia could attenuate proteolysis and thus improve the net protein balance. Carbohydrates can be given at a higher concentration than protein, without increasing the total fluid load, to pediatric patients in the intensive care unit and could therefore be a clinically relevant manipulator of protein metabolism, because influencing the protein balance with the administration of protein demands a significant increase of fluid load.

We conducted a prospective, randomized, single-blinded trial in young children after cardiac surgery for the correction of congenital heart defects of two short-term, iso-energetic, enteral diets by continuous gastric drip. We compared the effect of a HiCarb/LoProt diet with that of a normal carbohydrate (NormCarb)/LoProt diet on serum concentrations of anabolic and catabolic hormones and on whole-body protein breakdown and balance using stable isotopic infusion technique.
MATERIALS AND METHODS

Subjects

Children with congenital heart defects in the preoperative phase of surgical repair were recruited from the cardiac surgery department of the Shanghai Children’s Medical Center, Shanghai Second Medical University (Shanghai, People’s Republic of China). During the research period, 24 consecutively admitted patients who fulfilled the inclusion and exclusion criteria were enrolled. The inclusion criteria were an age 3 to 48 mos and pending surgical repair with cardiopulmonary bypass (CPB). The exclusion criteria were infection (i.e., fever > 39 °C for > 4 hrs with positive culture), mechanical ventilation or inotropic medication at the start of the isotopic infusions, an intolerance for enteral tube feeding, and the postoperative use of medication with modulating effects on protein metabolism (e.g., insulin and steroids).

All procedures were explained to the subjects’ parents in Chinese, and written informed consent in Chinese was obtained from all subjects. The medical ethics committees of the Academic Medical Center, Amsterdam, and the Shanghai Children’s Medical Center approved the study protocol.

Study design

At enrollment, a medical history was obtained and a physical examination was performed. Preoperatively, the CPB circuit was primed with standardized, body weight–related amounts of packed cells, albumin, and methylprednisolone, among other components. In the operation room, all patients received a multilumen 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. During anesthesia and surgery, unexpected events, vascular clamping time, and total CPB time were noted. After surgery, the patients were extubated in the operating room or ventilated postoperatively for a maximum of 4 hrs in the cardiac intensive care unit according to the clinical judgment of the attending anesthetist and pediatric intensivist. During the entire postoperative intensive care period, the appropriate medical treatment was instigated by the medical staff of the cardiac intensive care unit. Notes were made of the amounts of prescribed sedative and analgesic medications.

After approximately 4 hrs in the cardiac intensive care unit, at time 0 ($t = 0$ h), the liquid study diet was administered in increasing amounts through a nasogastric tube using a feeding pump
High carbohydrate/low protein intake in acute illness

(Applix Smart; Fresenius Kabi AG, Bad Homburg, Germany), such that the required feeding rate was reached at \( t = 8 \) hrs (Figure 2.1). The diets consisted of a mixture of a carbohydrate powder (Polycal; Nutricia, Zoetermeer, the Netherlands), a whey protein/carbohydrate powder (Whey Protein for Kids; Natural Elements, Los Angeles, CA, USA), and a fat emulsion (Solagen; Nutricia) dissolved in water. Using continuous drip feeding, the carbohydrate/protein intake was set at glucose 10 mg/kg/min and protein 0.7 g/kg/d in the intervention (HiCarb/LoProt) group and glucose 7.5 mg/kg/min and protein 0.3 g/kg/d in the control (NormCarb/LoProt) group. The remaining non-protein calories were supplied by the aforementioned fat emulsion as predicted by age-related Schofield equation (20). Because all patients had fluid restrictions after cardiac surgery, the volume of water in which the macronutrients was dissolved was limited to 60 mL/kg/d for the first day and to 100 mL/kg/d for the subsequent days.

At \( t = 25 \) hrs, continuous infusion of \( \text{NaH}^{13}\text{CO}_3 \) was started at a rate of 0.16 \( \mu \text{mol/kg/min} \), after a prime of 12 \( \mu \text{mol/kg} \). At \( t = 27 \) hrs, after the baseline blood and breath samples were taken for the determination of background isotopic enrichments, continuous infusions of \([1-^{13}\text{C}]\text{-valine at a rate of } 0.153 \mu\text{mol/kg/min (prime 9.1 \mu mol/kg)} \) and \([^{15}\text{N}_2]\text{-urea at a rate of } 0.28 \mu\text{mol/kg/min (prime 157 \mu mol/kg)} \) were started (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). The isotopes were dissolved in normal saline and infused by infusion pumps (IVAC P3000; Alaris Medical Systems Inc, Dublin, OH, USA; and SIGO TCI-II; Beijing Silugo High Technology Development Co. Ltd., Beijing, People’s Republic of China) after sterilization by a passage through a 0.20-\( \mu \)m Millipore filter (Minisart; Sartorius AG, Göttingen, Germany). All infusions were tested for pyrogenic properties.

Figure 2.1  Experimental design showing the timing of surgery (open arrow) and the sampling of blood (open crosses) and breath (small arrows). The ventilated hood procedure during 30 min (X) and the duration of primed infusions of isotopes (straight lines) are displayed. \( T \), time.
At $t = 42$ hrs, the production of carbon dioxide was measured for 30 min using a ventilated hood system (Ultima CCM; Medical Graphics Corporation, St. Paul, MN, USA). During this period, at 10-min intervals, three concurrent breath and blood samples were collected. The breath samples for the enrichment of $^{13}$CO$_2$ were aspirated through a nasopharyngeal tube with a 20-mL syringe and collected in screw-capped glass vials (Exetainer 12-mL $^{13}$C Breath Test Vial; Labco Ltd., High Wycombe, UK). In the same period, three blood samples were collected for the measurement of the isotopic enrichment of valine, $a$-ketoisovalerate (KIV), and urea at 10-min intervals. At the end of the 30-min sampling period, blood samples were taken for the measurement of plasma concentrations of insulin, glucagon, cortisol, cortisol-binding globulin, serum urea nitrogen, and catecholamines. For the latter, blood was collected in 5-mL plain glass Vacutainer tubes containing 100 mL of a solution of ethylene glycol-bis-(2-aminoethyl) tetraacetic acid (0.24 mol/L) and glutathione (0.20 mol/L) in NaOH (0.25 mol/L) adjusted to pH 6.5. All blood samples were centrifuged immediately at 1860 x $g$ for 10 min at room temperature (90-2 Centrifuge; Shanghai Surgical Instruments Factory, Shanghai, People’s Republic of China). After separation, the plasma was stored at -20 °C until analysis. Between $t = 42$ and 42.5 hrs, the serum glucose concentrations were measured bedside twice (Synthesis 45 Critical Care Laboratory; Instrumentation Laboratory, Lexington, MA, USA).

At $t = 42.5$ hrs, the study was ended, and all children resumed to the standard hospital age-related diets.

**Assays**

Valine and urea were isolated in a cation exchange column (AG50W-X8; Bio-Rad Laboratories, Inc., Hercules, CA, USA) after the precipitation of the plasma proteins with trichloroacetic acid. The proteins were eluted with ammonia and evaporated to dryness. Valine was derivatized to the N(O,S)-methoxy-carbonylmethyl derivative as described by Hušek (21). After extraction with chloroform, an aliquot was injected into a gas chromatography/combustion/isotope ratio mass spectrometric (IRMS) system that was validated for the measurement of isotope enrichment up to 10% (HP 6890 series gas chromatographic system; Hewlett-Packard, Palo Alto, CA, USA; and Finnigan Deltaplus IRMS; Finnigan-MAT, Bremen, Germany). Separation was achieved in a CP-SIL 19CB capillary column (25 m x 0.32 mm x 0.2 μm; Varian, Middelburg, the Netherlands). After combustion of the gas chromatographic effluent to carbon dioxide, the $^{13}$C enrichment of valine was measured using the IRMS system. Data acquisition and delta calculations were performed using ThermoFinnigan ISODAT NT 0.144 (Finnigan-MAT). Urea was measured as the bis-trimethylsilyl derivative described by Matthews et al (22). Isotopic enrichment was measured on a gas chromatography/MS system (HP 6890 series gas
chromatographic system and 5973 Mass Selective Detector; Hewlett-Packard) equipped with a J&W Scientific DB 17 capillary column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies, Palo Alto, CA, USA).

Selected ion monitoring (i.e., electron impact ionization), data acquisition, and quantitative calculations were performed using Chemstation D.01.02.16 (Agilent Technologies). The bis-trimethylsilyl derivative was monitored at a mass-to-charge ratio (m/z) of 189 for urea and an m/z of 191 for [15N2]-urea. The ratio of urea tracer to that traced was calculated as described by Patterson et al (23). The [1-13C]KIV enrichment was determined in the O-t-butyl-dimethyl-silyl-quinoxalinol derivative according to the method of Kulik et al (24). The same gas chromatographic/MS system was used for the urea analysis. The KIV derivative was monitored at an m/z of 245 for KIV and an m/z of 246 for [1-13C]-KIV. The KIV tracer/traced ratio was calculated as described by Patterson et al (23). Plasma AA concentrations were measured by ultrahigh-performance liquid chromatographic MS/MS using multiple-reaction monitoring and stable isotopically labeled internal standards.

Plasma insulin concentrations were ascertained by using a chemiluminescent immunometric assay on a Immulite analyzer (DPC, Los Angeles, CA, USA), with an intra-assay coefficient of variation (CV) lower than 6%, an interassay CV lower than 6%, and a detection limit of 15 pmol/L. Glucagon was measured by radioimmunoassay (Linco Research, St. Charles, MO, USA), with an intra-assay CV lower than 10%, an interassay CV of lower than 7%, and a detection limit of 15 ng/L. Cortisol was measured by a chemiluminescent immunoassay in an Immulite analyzer, with an intra-assay CV lower than 8%, an interassay CV lower than 7%, and a detection limit of 50 nmol/L. Plasma concentrations of catecholamines were determined with an in-house highperformance liquid chromatographic method. Essentially, norepinephrine and epinephrine were selectively isolated by liquid–liquid extraction and derivatized with the fluorescent 1,2-diphenylethylenediamine (25, 26). The fluorescent derivatives were separated by reverse-phase liquid chromatography and detected by scanning fluorescence detection (Waters X Terra RD18 5 μm, 3.9 x 150 mm, Waters 474 Scanning Fluorescence Detector excitation 340 nm, emission 480 nm; Waters, Milford, MA, USA), with intra-assay CVs lower than 2% and lower than 9% and interassay CVs lower than 10% and lower than 18% for norepinephrine and epinephrine, respectively. The detection limit for the two catecholamines was 0.05 nmol/L. Free fatty acids were measured using an enzymatic method (NEFAC; Wako Chemicals GmbH, Neuss, Germany) with an intra-assay CV of 2% to 4%, an interassay CV of 3% to 6%, and a detection limit of 0.02 mmol/L.

In the breath samples, the enrichment of 13CO2 was measured (Breath-MATplus; Finnigan-MAT).
Calculations and statistical analysis

In this model of single AA tracer kinetics, the non-oxidative disposal of valine (NOD\textsubscript{val}) represents protein synthesis (S), and the endogenous rate of the appearance of valine (Endo-R\textsubscript{aval}) represents the protein breakdown (B) proportionally (27). The rate of appearance of valine in plasma (R\textsubscript{aval}) and valine oxidation (Oxid\textsubscript{val}) were calculated according to standard equations (28–31).

\[ S = \text{NOD}_{\text{val}} \ (\mu\text{mol/kg/min}) = R_{\text{aval}} - \text{valine oxidation} \]  \[ \text{[1]} \]

\[ B = \text{Endo-R}_{\text{aval}} \ (\mu\text{mol/kg/min}) = R_{\text{aval}} - \text{valine (diet)} \]  \[ \text{[2]} \]

Protein balance (g/kg/d) = (S – B) x 1440 / (450 \mu\text{mol/g protein})  \[ \text{[3]} \]

where 1440 is the number of minutes in 24 hrs, and 450 mmol/g protein represents the estimated contribution of valine to whole-body protein (32). From the kinetics of the urea isotope, we calculated the urea production (27).

In small samples, data are not normally distributed. Therefore, data are presented as median and range. The Mann-Whitney \textit{U} test was used to investigate differences between groups. Curve estimation was used to construct a curve that had the best fit to the insulin and endogenous R\textsubscript{aval} data. To investigate the association between cortisol plasma concentrations and the number of times a child had received pain medication or sedation (range 0–3), the Kruskal-Wallis test was performed. All analyses were performed by using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). \( P < 0.05 \) was considered statistically significant.

RESULTS

Patients

Baseline characteristics of the NormCarb/LoProt and HiCarb/LoProt groups are listed in Table 2.1. There were slightly more male than female patients in each group (total 15 of 24). In either group, one patient dropped out of the study because no isotope studies could be performed: in the NormCarb/LoProt group, one girl developed chylothorax after correction of her ventricular septal defect; in the HiCarb/LoProt group, one boy needed additional surgical tricuspid valvuloplasty after correction of his aberrant pulmonary venous return and subsequent failure to wean him from the ventilator. In the NormCarb/LoProt group, one additional patient had missing values only for protein kinetics because of a protocol violation for isotopic valine and urea infusion. In this latter subject, the data for hormonal response were accepted according to the intention-to-treat principle.
Between groups, there were no differences in caloric intake, intraoperative clamping time, postoperative body temperature, and number of sedative boluses (Table 2.2). The actual carbohydrate intake as aimed for in the protocol was reached (Table 2.2). Intakes of carbohydrate and fat differed by study design, the latter to achieve isoenergetic diets. Because the study feeding formulas were dissolved in 60 mL/kg of water, the maximal caloric density was 1.6 kcal/mL, which is comparable to commercially available formulas. Owing to the use of

Table 2.1  Baseline characteristics of groups

<table>
<thead>
<tr>
<th></th>
<th>NormCarb/LoProt (n = 12)</th>
<th>HiCarb/LoProt (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls</td>
<td>7/5</td>
<td>8/4</td>
</tr>
<tr>
<td>Age (mos)</td>
<td>8.8 (5.0 to 38.8)</td>
<td>12.6 (3.0 to 27.0)</td>
</tr>
<tr>
<td>Body weight (SDS)</td>
<td>-1.3 (-2.3 to 0.0)</td>
<td>-1.3 (-2.3 to 2.5)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>0.0 (-1.8 to 0.7)</td>
<td>-0.7 (-2.3 to 2.5)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSD</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>ASD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AVSD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOF</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>APVR</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are presented as number of subjects or median (range). NormCarb/LoProt, carbohydrate 7.5 mg/kg/min and protein 0.3 g/kg/d; HiCarb/LoProt, carbohydrate 10 mg/kg/min and protein 0.7 g/kg/d; SDS, standard deviation score; APVR, aberrant pulmonary venous return; ASD, atrial septal defect; AVSD, atrioventricular septal defect; TOF, tetralogy of Fallot; VSD, ventricular septal defect. *SDSs according to Chinese growth charts (Beijing, 1995).

Table 2.2  Actual dietary intake, intraoperative aortic clamping time, and postoperative body temperature

<table>
<thead>
<tr>
<th></th>
<th>NormCarb/LoProt (n = 12)</th>
<th>HiCarb/LoProt (n = 12)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric intake (kcal/kg/d)</td>
<td>94.2 (82.3 to 103.9)</td>
<td>95.6 (86.9 to 106.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>Non-protein calories (kcal/kg/d)</td>
<td>93.0 (81.1 to 102.6)</td>
<td>92.6 (84.0 to 103.3)</td>
<td>0.95</td>
</tr>
<tr>
<td>Carbohydrate intake (mg/kg/min)</td>
<td>7.6 (6.9 to 8.5)</td>
<td>10.2 (9.6 to 10.9)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Protein intake (g/kg/d)</td>
<td>0.3 (0.3 to 0.3)</td>
<td>0.7 (0.7 to 0.8)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fat intake (g/kg/d)</td>
<td>5.4 (4.1 to 6.0)</td>
<td>3.8 (3.1 to 4.7)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Aortic clamping time (min)</td>
<td>39 (16 to 72)</td>
<td>34 (10 to 75)</td>
<td>0.69</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>37.8 (37.2 to 38.5)</td>
<td>37.7 (37.0 to 38.6)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Values are presented as median (range). HiCarb/LoProt, carbohydrate 10 mg/kg/min and protein 0.7 g/kg/d; NormCarb/LoProt, carbohydrate 7.5 mg/kg/min and protein 0.3 g/kg/d. *Mann-Whitney U test.
a carbohydrate/protein mixture, there was a low, but statistically different, protein intake in the two groups (0.3 and 0.7 g/kg/d in the NormCarb/LoProt and HiCarb/LoProt groups, respectively; this equals approximately 12% and 30% of the age-related recommended protein intake).

**Protein metabolism**

Between the NormCarb/LoProt and HiCarb/LoProt groups, there were no statistically significant differences in total valine flux, oxidation, and nonoxidative disposal (Table 2.3). Also, the endogenous Ra_{val} and the (negative) valine balance did not show significant differences between groups. The data for nitrogen kinetics showed no differences in urea production or the consequent serum urea nitrogen concentration between the NormCarb/LoProt and HiCarb/LoProt groups (Table 2.3). There were no differences in plasma concentrations of standard essential and non-essential AAs between groups (Table 2.4).

**Glucose and hormones**

There was a significantly higher serum concentration of insulin in the HiCarb/LoProt group compared with the NormCarb/LoProt group (596 pmol/L, 80–1833, and 198 pmol/L, 76–1292, respectively, p = 0.02; Table 2.5). Despite the significantly different carbohydrate intakes between the two groups, there was no difference in plasma glucose concentrations (6.2 mmol/L, 4.2–8.6, in the NormCarb/LoProt group; 6.4 mmol/L, 5.2–12.8, in the HiCarb/LoProt

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**Table 2.3** Absolute and relative contribution to total valine flux of oxidation, NOD, exogenous (diet) and endogenous Ra (total flux corrected for exogenous supply) of valine, Ra of urea, total body protein balance, and serum urea nitrogen

<table>
<thead>
<tr>
<th></th>
<th>NormCarb/LoProt (% flux) (n = 10)</th>
<th>HiCarb/LoProt (% flux) (n = 11)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total valine flux (μmol/kg/min)</td>
<td>1.91 (1.38 to 2.78) [100]</td>
<td>1.94 (1.67 to 2.37) [100]</td>
<td>1.00</td>
</tr>
<tr>
<td>Valine oxidation (μmol/kg/min)</td>
<td>0.68 (0.17 to 2.41) [36.3]</td>
<td>0.89 (0.24 to 2.17) [44.1]</td>
<td>0.28</td>
</tr>
<tr>
<td>NOD of valine (μmol/kg/min)</td>
<td>1.16 (0.37 to 1.85) [63.7]</td>
<td>1.23 (-0.23 to 1.73) [55.9]</td>
<td>0.51</td>
</tr>
<tr>
<td>Endogenous valine Ra (μmol/kg/min)</td>
<td>1.81 (1.27 to 2.69) [94.9]</td>
<td>1.68 (1.43 to 2.11) [86.8]</td>
<td>0.31</td>
</tr>
<tr>
<td>Urea Ra (μmol/kg/min)</td>
<td>2.38 (1.29 to 6.59)</td>
<td>2.50 (1.62 to 4.08)</td>
<td>0.80</td>
</tr>
<tr>
<td>Protein balance (g/kg/d)</td>
<td>-2.13 (-7.43 to -0.22)</td>
<td>-2.32 (-6.12 to 0.03)</td>
<td>0.71</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>2.8 (1.4 to 7.0)</td>
<td>2.0 (1.5 to 4.2)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Values are presented as median (range), absolute [and relative] contribution. HiCarb/LoProt, carbohydrate 10 mg/kg/min and protein 0.7 g/kg/d; NormCarb/LoProt, carbohydrate 7.5 mg/kgmin and protein 0.3 g/kgd; BUN, serum urea nitrogen; NOD, non-oxidative disposal; Ra, rate of appearance. *Mann-Whitney U test.
Table 2.4  Plasma concentrations of standard essential and non-essential amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>NormCarb/LoProt (n = 11)</th>
<th>HiCarb/LoProt (n = 11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine (mmol/L)</td>
<td>60 (45 to 85)</td>
<td>63 (50 to 90)</td>
<td>0.62</td>
</tr>
<tr>
<td>Tyrosine (mmol/L)</td>
<td>44 (30 to 58)</td>
<td>43 (27 to 59)</td>
<td>0.60</td>
</tr>
<tr>
<td>Tryptophan (mmol/L)</td>
<td>23 (13 to 41)</td>
<td>22 (14 to 32)</td>
<td>0.87</td>
</tr>
<tr>
<td>Alanine (mmol/L)</td>
<td>134 (94 to 264)</td>
<td>186 (82 to 253)</td>
<td>0.25</td>
</tr>
<tr>
<td>Methionine (mmol/L)</td>
<td>10 (9 to 12)</td>
<td>10 (9 to 12)</td>
<td>0.77</td>
</tr>
<tr>
<td>Glycine (mmol/L)</td>
<td>117 (62 to 167)</td>
<td>103 (61 to 148)</td>
<td>0.97</td>
</tr>
<tr>
<td>Valine (mmol/L)</td>
<td>107 (66 to 209)</td>
<td>109 (71 to 141)</td>
<td>0.90</td>
</tr>
<tr>
<td>Leucine (mmol/L)</td>
<td>49 (27 to 91)</td>
<td>50 (30 to 69)</td>
<td>0.82</td>
</tr>
<tr>
<td>Isoleucine (mmol/L)</td>
<td>27 (22 to 40)</td>
<td>35 (25 to 62)</td>
<td>0.09</td>
</tr>
<tr>
<td>Glutamine (mmol/L)</td>
<td>47 (13 to 175)</td>
<td>37 (19 to 111)</td>
<td>0.79</td>
</tr>
<tr>
<td>Asparagine (mmol/L)</td>
<td>7 (2 to 18)</td>
<td>6 (1 to 9)</td>
<td>0.37</td>
</tr>
<tr>
<td>Citrulline (mmol/L)</td>
<td>12 (8 to 24)</td>
<td>13 (10 to 18)</td>
<td>0.32</td>
</tr>
<tr>
<td>Ornithine (mmol/L)</td>
<td>17 (13 to 46)</td>
<td>21 (8 to 34)</td>
<td>0.67</td>
</tr>
<tr>
<td>Lysine (mmol/L)</td>
<td>72 (49 to 111)</td>
<td>71 (40 to 104)</td>
<td>0.95</td>
</tr>
<tr>
<td>Arginine (mmol/L)</td>
<td>36 (21 to 50)</td>
<td>40 (18 to 52)</td>
<td>0.51</td>
</tr>
<tr>
<td>Serine (mmol/L)</td>
<td>93 (65 to 131)</td>
<td>89 (59 to 127)</td>
<td>0.67</td>
</tr>
<tr>
<td>Proline (mmol/L)</td>
<td>91 (56 to 132)</td>
<td>93 (54 to 127)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Values are presented as median (range). HiCarb/LoProt, carbohydrate 10 mg/kg/min and protein 0.7 g/kg/d; NormCarb/LoProt, carbohydrate 7.5 mg/kg/min and protein 0.3 g/kg/d. *Mann-Whitney U test.

Table 2.5  Plasma concentrations of glucose and regulatory hormones of protein metabolism

<table>
<thead>
<tr>
<th>Hormone</th>
<th>NormCarb/LoProt (n = 11)</th>
<th>HiCarb/LoProt (n = 11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/L)</td>
<td>198 (76 to 1292)</td>
<td>596 (80 to 1833)</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.2 (4.2 to 8.6)</td>
<td>6.4 (5.2 to 12.8)</td>
<td>0.35</td>
</tr>
<tr>
<td>Glucagon (ng/L)</td>
<td>104 (66 to 168)</td>
<td>91 (60 to 226)</td>
<td>0.16</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>532 (108 to 930)</td>
<td>204 (50 to 544)</td>
<td>0.02</td>
</tr>
<tr>
<td>CBG (mg/L)</td>
<td>37 (23 to 47)</td>
<td>40 (25 to 60)</td>
<td>0.75</td>
</tr>
<tr>
<td>Epinephrine (nmol/L)</td>
<td>0.32 (0.05 to 1.25)</td>
<td>0.28 (0.05 to 0.74)</td>
<td>0.67</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)</td>
<td>1.60 (0.07 to 3.95)</td>
<td>0.76 (0.18 to 1.67)</td>
<td>0.13</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.30 (0.16 to 0.69)</td>
<td>0.29 (0.17 to 1.39)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Values are presented as median (range). HiCarb/LoProt, carbohydrate 10 mg/kg/min and protein 0.7 g/kg/d; NormCarb/LoProt, carbohydrate 7.5 mg/kg/min and protein 0.3 g/kg/d. CBG, cortisol-binding globulin; FFA, free fatty acids. *Mann-Whitney U test.
group; $p = 0.35$). There was no effect of serum insulin concentration on the endogenous $R_{\text{Va}}$ (protein breakdown) in either group (Figure 2.2A).

In the HiCarb/LoProt group, there was a lower concentration of the catabolic hormone cortisol compared with the NormCarb/LoProt group (204 ng/L, 50–544, and 532 ng/L, 108–930, respectively, $p = 0.02$). Plasma concentrations of other catabolic hormones (glucagon, norepinephrine, and epinephrine) and CBG did not differ significantly between groups. Within groups, serum concentrations of cortisol and norepinephrine and epinephrine showed an inverse relation to the insulin concentration (Figure 2.2B–D).

Figure 2.2  Scatterplots of individual serum insulin concentrations in relation to (A) endogenous $R_{\text{Va}}$ (protein breakdown) and serum concentrations of (B) cortisol, (C) norepinephrine, and (D) epinephrine for the high-carbohydrate (10 mg/kg/min)/low-protein (0.7 g/kg/d) group (open circles) and the normal-carbohydrate (7.5 mg/kg/min)/low-protein (0.3 g/kg/d) group (closed circles). Ra, rate of appearance.
Association between cortisol and number of sedative boluses

The Kruskal-Wallis test showed no significant association between cortisol plasma concentration and the number of sedative boluses ($p = 0.55$, data not shown).

**DISCUSSION**

In the present study, in young children with congenital heart defects after cardiac surgery with CPB, the HiCarb/LoProt diet induced an insulin concentration that was three times higher than in the NormCarb/LoProt diet without an additional inhibitory effect on proteolysis. This resulted in an equally negative protein balance and plasma AA profile in the HiCarb/LoProt group compared with the NormCarb/LoProt group. Also, the rate of appearance of urea was the same in the two groups.

The relation between serum insulin concentration and protein metabolism is complex. In healthy humans, it is well known that hyperinsulinemia decreases proteolysis with no stimulation of protein synthesis, whereas the infusion of large amounts of AAs increases protein synthesis without the suppression of endogenous proteolysis (33). In patients with insulin resistance, the suppressive effect on proteolysis has been reported to occur at higher plasma insulin concentrations (34–36). In anticipation of insulin resistance in the present surgical patient population, we designed the carbohydrate intake in the two groups to produce high insulin responses. The observed median serum insulin concentrations of ~200 and ~600 pmol/L in the NormCarb/LoProt and HiCarb/LoProt groups, respectively, were approximately three and nine times higher than has been reported previously in a similar patient group with a low carbohydrate intake (37). However, despite the observed high serum insulin concentrations, in the present study, there was no additional suppression of proteolysis in the higher range of serum insulin concentrations within or between groups (Figure 2.2). We therefore speculate that, unexpectedly, proteolysis was already maximally suppressed by insulin concentrations in response to the normal carbohydrate intake, and that a higher carbohydrate intake with the consequent higher insulin concentrations did not have an additional effect. Also, in our study design with low-protein diets, hypoaminoacidemia might have blunted the responsiveness of insulin’s suppression of the protein breakdown in the two groups, because the proteolytic suppressive effect of insulin is enhanced by the sufficient availability of AAs (17, 38). Recently, it has been shown that in critically ill, septic children, a high AA intake increases protein synthesis, whereas increasing insulin levels do not have an additional positive effect on the protein balance (39). This observation suggests that the degree of insulin resistance on protein metabolism in these septic children was not...
overcome by the chosen insulin concentration and supports our conclusion that high insulin levels alone cannot improve the whole-body protein balance. Future research should be aimed on the effects of lower insulin concentrations on proteolysis in response to lower carbohydrate intakes, with or without the sufficient availability of AAs.

The lack of difference in the non-oxidative disposal of valine, as an estimate of protein synthesis, between groups was expected, because protein synthesis is stimulated mainly by the AA supply, rather than plasma insulin concentration. We acknowledge that the small but statistically significantly different protein intake between groups (0.3 and 0.7 g/kg/d in the NormCarb/LoProt and HiCarb/LoProt groups, respectively, \(p < 0.01\)), as a direct result of the use of a carbohydrate/protein mixture, is a limitation in the study design. However, in the chosen study design, the protein supply was very low in the two groups (10% and 30% of the recommended dietary allowance in the NormCarb/LoProt and HiCarb/LoProt groups, respectively). As listed in Table 2.4, plasma AA concentrations showed no differences between groups. Moreover, the higher protein intake in the HiCarb/LoProt group did not lead to an increased inhibition of proteolysis or an increased protein synthesis and therefore does not undermine the main conclusion of our study.

The rates of whole-body protein synthesis and breakdown in our study are comparable to other isotopic studies in critically ill infants with sepsis or ventilatory support for viral bronchiolitis and neonates on extracorporeal life support (40–42). Two studies (40, 41) that had provided patients with protein intakes of 1.2 to 2.4 g/kg/d and 40 to 90 kcal/kg/d reported negative whole-body protein balances. In their study with higher than recommended intakes of protein and calories (3.1 ± 0.3 g/kg/d and 119 ± 25 kcal/kg/d, respectively), de Betue et al reported only positive protein balances (42). In none of the studies was serum insulin concentrations measured. These observations support our conclusion that a mere provision of calories without a sufficient (i.e., higher than currently recommended) intake of proteins does not improve the protein balance in critically ill children.

We observed a lower serum concentration of cortisol in the HiCarb/LoProt group compared with the NormCarb/LoProt group. This difference is unexplained because clinical factors that stimulate cortisol secretion were equally distributed between the two groups. In all patients, the CPB circuit was primed with methylprednisolone in a body weight-dependent fashion and could not have affected the stress response in a selective group. The median and range of serum concentrations of cortisol that we found are comparable to those published in another cohort of pediatric patients after cardiac surgery (37). Although a hypercortisolemic condition increases proteolysis (43), it does not affect our observation of a lack of effect of
higher insulin concentrations on proteolysis, because cortisol was significantly lower in the HiCarb/LoProt group.

The catabolic hormones epinephrine and norepinephrine showed an inverse relation to serum insulin concentrations within groups. Although insulin is known to have anti-inflammatory properties (44, 45), lower serum concentrations of stress hormones concomitant with higher insulin concentrations do not necessarily represent a decreased metabolic stress response, because other neuroendocrine hormonal changes (e.g., thyroid and growth hormone) and proinflammatory mediators (e.g., cytokines and tumor necrosis factor) also play a very important role in this response. We did not measure these factors owing to the restrictions in blood sample volumes in these small children.

CONCLUSIONS

In children after cardiac surgery, a high-carbohydrate/low protein diet compared with a normal-carbohydrate/low protein diet stimulates insulin secretion but does not influence proteolysis or the whole-body protein balance. In the usual clinical setting of a low-protein intake after cardiac surgery, increasing the carbohydrate intake to mitigate the protein loss is not a meaningful strategy.

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REFERENCES


