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How much protein and energy are needed to equilibrate nitrogen and energy balances in ventilated critically ill children? Manuscript number: YCLNU-D-14-00642R1.

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ABSTRACT

Background & Aims: Protein and energy requirements in critically ill children are currently based on insufficient data. Moreover, longitudinal measurements of both total urinary nitrogen (TUN) and resting energy expenditure (REE) are lacking. The aim of this study was to investigate how much protein and energy are needed to equilibrate nitrogen and energy balances in ventilated critically ill children on the basis of daily measurements of TUN, REE and protein and energy intakes. Comparisons were made with the guidelines of the American Society for Parenteral and Enteral Nutrition and the Dietary Reference Intakes.

Methods: Children with an expected duration of mechanical ventilation ≥ 72 hours were prospectively recruited. TUN was measured by chemiluminescence, and REE was measured by indirect calorimetry. Generalised linear models for longitudinal data were used to study the relation between protein intake and nitrogen balance and to calculate the minimum intake of protein needed to achieve nitrogen equilibrium. A similar approach was used for energy. Results were compared to the recommended values.

Results: Based on 402 measurements performed in 74 children (median age: 21 months), the mean TUN was high at 0.20 (95% CI: 0.20, 0.22) g/kg/d and the REE was 55 (95% CI: 54, 57) kcal/kg/d. Nitrogen and energy balances were achieved with 1.5 (95% CI: 1.4, 1.6) g/kg/d of protein and 58 (95% CI: 53, 63) kcal/kg/d for the entire group, but there were differences among children of different ages. Children required more protein and less energy than the Dietary Reference Intakes.

Conclusions: In critically ill children, TUN was elevated and REE was reduced during the entire period of mechanical ventilation. Minimum intakes of 1.5 g/kg/d of protein and 58 kcal/kg/d can equilibrate nitrogen and energy balances in children up to 4 years old. Older children require more protein.

Keywords: Critical care; child; infant; nitrogen; energy expenditure; indirect calorimetry.

ABBREVIATIONS¹

¹ A.S.P.E.N.: American Society for Parenteral and Enteral Nutrition

CRP: C-Reactive Protein

DRI: Dietary Reference Intakes

REE: Resting Energy Expenditure

PICU: Paediatric Intensive Care Unit

PRISM: Pediatric Risk of Mortality

TUN: Total Urinary Nitrogen

WHO: World Health Organization

1. Introduction

In critically ill children, optimal nutritional support, especially adequate amounts of energy and protein are associated with decreased mortality and morbidity.¹ It can be difficult to estimate energy and protein requirements in healthy children because these requirements vary with age, growth velocity, modifications in body composition and physical activity.² The task is even more complex in patients in paediatric intensive care units (PICUs) where nutritional needs are influenced by various factors such as the patient's disease, treatments, fever and reduced physical activity.

Current nutritional recommendations for critically ill children are based on little evidence, as highlighted by the Cochrane Collaboration.³ The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) provides recommendations on protein intake while acknowledging that these recommendations are based on insufficient data.⁴ A recent systematic review on protein balance concluded that longitudinal studies including measurements of both protein and energy balances are needed in critically ill children.⁵

The paucity of studies can be explained by the difficulty in measuring nitrogen and energy balances as these measurements require technical and financial resources. According to the World Health Organization (WHO)⁶, the major method currently available to determine protein needs is to measure nitrogen balance, which requires a precise quantification of both nitrogen intake and nitrogen losses. Total urinary nitrogen (TUN) must be measured⁶ and not estimated from the urinary urea content, which is highly variable in critically ill patients.⁷ Similarly, determining the energy balance demands the precise measurement of resting energy expenditure (REE) by indirect calorimetry.⁴

Although researchers should measure TUN and REE in critically ill children, clinicians are often constrained to estimating protein and energy needs because of limited resources. In a European survey conducted in 111 PICUs, measurements of REE were only performed in 19 units. As an alternative, energy needs were estimated from weights and ages, according to methods developed for healthy children.⁸

The primary aim of this study was to assess how much protein and energy are needed to equilibrate nitrogen and energy balances in ventilated critically ill children on the basis of daily measurements of TUN and REE. The secondary aim was to compare these results with the recommended values of the A.S.P.E.N. guidelines⁴ and the Dietary Reference Intakes (DRIs)².

2. Material and methods

2.1. Subjects

Children (from birth to age 16 years) who were admitted from January 2008 to April 2010 to the PICU of the University Hospital of Lausanne, Switzerland, with an expected duration of mechanical ventilation of at least 72 hours were considered eligible for this study. Exclusion criteria were as follows: a fraction of inspired oxygen (FiO_2) > 60%, an air leak around the endotracheal tube > 10%, chylothorax, chronic or acute renal disease, severe loss of inflammatory fluid through a pleural or peritoneal drains, exudative enteropathy and therapeutic hypothermia. Out of 448 admissions, 78 children were eligible and three parents refused participation in the study. One patient was excluded after loss of his urine collection. Patients were prospectively studied until extubation or for a maximum of 15 days. The study was approved by the ethics committee of the University Hospital of Lausanne. Written informed consent was obtained from the parents of each child before measurements were made.

Demographic data, diagnoses and Pediatric Risk of Mortality (PRISM) II scores⁹ were obtained at admission. The C-reactive protein (CRP) level was recorded each day as a marker of inflammation. Body temperature was obtained during indirect calorimetry measurements. Nutritional status was classified by using the z-scores of either the WHO¹⁰ for children younger than 5 years old or those of the National Center for Health Statistics (NCHS, 2000) for older children. A deviation of more than -2 standard deviations for weight-for-height was considered acute malnutrition, and a deviation of more than -2 standard deviations for height-for-age was considered chronic malnutrition. Weight and height were measured at PICU admission and discharge. For patients admitted as emergency cases, parents provided the last weight value, and height was measured at the patient's bedside.

The research staff did not intervene in the daily management of nutrition support. Energy and protein goals were determined by using the Schofield equation with weight and height¹¹ and the A.S.P.E.N recommendations⁴. Our protocol uses a progressive increase of nutritional intakes to achieve these goals by at least day 5.

2.2. Measurements of TUN and REE

In all patients, urine was collected through urinary catheters from midday to midday and frozen at -80°C until the day of measurement. TUN was measured by the chemiluminescence technique with an Antek 7000 analyser (Houston, TX, USA).¹²

REE was measured by indirect calorimetry as soon as possible after PICU admission. Measurements were performed daily until extubation, at the same time each day. The calorimeter (Deltatrac II, Datex-Ohmeda, Helsinki, Finland) was calibrated monthly by the alcohol-burn technique with a burning kit specifically designed for paediatric use, a precise syringe (Model TLL 1010, Hamilton, Switzerland) and a syringe pump (Model 789100, KD Scientific, USA). Before each measurement, the calorimeter was warmed up for 1 hour and then calibrated with a reference gas mixture of 95% oxygen and 5% carbon dioxide. In agreement with the bedside nurse, measurements were performed during a period without stressful procedures. In some cases (change in FiO₂, physiotherapy, agitation or suction of the endotracheal tube), the measurement was stopped and restarted at least 30 minutes after the event. At the end of the measurement, data for each minute were exported with the CaloDatex software (Datex-Ohmeda, Helsinki, Finland) into Excel (Microsoft Office, USA) and analysed in detail.

REE was calculated with the modified Weir's equation¹³, using measured values of oxygen consumption (VO₂) and carbon dioxide production (VCO₂). The first 10 minutes were discarded for all patients to exclude artefacts. A steady state was defined by a coefficient of variation of VCO₂ ≤ 10% for at least 25 consecutive minutes.¹⁴ For five measurements, steady state was not achieved and these REE and TUN measurements were excluded from the analysis. During the study period, 51 REE and TUN

measurements were missed on day one and 16 on day two, mainly because informed consent had not yet been acquired or because the investigators were unavailable.

2.3. Calculation of nitrogen and energy balances

Nitrogen balance was calculated daily as: nitrogen intake minus total nitrogen losses. Nitrogen intake was obtained from total enteral and parenteral feeding from data in a clinical data management system (MetaVision by iMDsoft, Tel Aviv, Israel). Total nitrogen losses included TUN and faecal/miscellaneous (skin, tegumental) losses estimated according to the WHO recommendations⁶ as follows: total nitrogen losses (g/d) = TUN (g/d) + faecal losses (0.021 g/d * body weight) + miscellaneous losses (0.001 g/d * body weight). To convert the nitrogen into protein, the usual nitrogen-to-protein conversion factor of 6.25 was used.

Energy balance was calculated daily as: energy intake minus energy requirements. Energy intake was obtained from total enteral and parenteral feeding, including intravenous glucose solutions for medication. Propofol was not used as a continuous infusion for sedation. Energy requirements were considered at REE measured by indirect calorimetry, without adding a factor for physical activity.

When available, the difference between the weights at admission and on the last studied day (or the day after) was calculated, and the change in weight and the cumulative energy balance was analysed. The cumulative energy balance was calculated by the summation of daily balances.

2.4. Determination of the minimum intake of protein and energy for nitrogen equilibrium

The minimum intake of protein needed to achieve nitrogen equilibrium (i.e. zero nitrogen balance) was calculated from the estimated regression line for longitudinal data¹⁵ between protein intake and nitrogen balance as described by the WHO⁶. The same regressions between energy intake and nitrogen balance were used to determine the energy intake needed to achieve nitrogen equilibrium.

2.5. Recommended values

Values obtained from the regressions were compared to the A.S.P.E.N. recommendations specifically developed for critically ill children⁴ and the DRIs for healthy children². For protein, the A.S.P.E.N. recommendations are 2–3 g/kg/d in 0–2 year olds and 1.5–2 g/kg/d in 2–13 year olds. The DRI for protein, expressed in g/kg/d, are 1.52 in 0–6 month olds, 1.2 in 7–12 month olds, 1.05 in 1–3 year olds and 0.95 in 4–8 year olds. For energy, the A.S.P.E.N. does not provide recommended intakes. The DRIs for energy are calculated by the factorial method using age and weight, as well as the physical activity level in children 3 years of age and older. The lowest physical activity level of 1 corresponding to a sedentary lifestyle was used.

2.6. Statistical analysis

Results are expressed as the mean $\pm$ standard deviations or with a 95% confidence interval (95% CI) and as median [interquartile range (IQR)], as appropriate. Data were analysed for the entire sample and for age groups using age intervals similar to those of the DRIs²: 0–6 months, 7–12 months, 1–3 years and 4–8 years. Differences in demographic data (age, weight, height, days in PICU, days on mechanical ventilation, PRISM II score, CRP max) between the four age groups were tested by analysis of variance (ANOVA) or the Kruskal-Wallis test for non-normally distributed variables (age, CRP, length of PICU stay and PRISM score). TUN, REE, and protein and energy intakes/balances were compared between the four age groups by using generalized linear models for longitudinal data with an autoregressive structure.¹⁵ Equality of the four means was tested by the Wald test. The analysis of TUN was adjusted for energy intake, nitrogen intake, and PRISM score. The analysis of nitrogen balance was adjusted for energy intake. No adjustment was performed for the other outcomes. Similar analyses were performed to compare the means across the studied day of the six variables. The Wald test was also used to test conditions that may affect TUN and REE, including diagnosis type (surgical/nonsurgical), sepsis status, cardiopulmonary bypass status, neuromuscular blocking drugs use and fever status (body temperature > 37.5°C). For all comparisons, a *P*-value < 0.05 was considered statistically significant. Statistical analyses were performed by using Stata 13.1 for Windows (StataCorp LP, College Station, TX, USA).

3. RESULTS

3.1. Clinical characteristics

Seventy-four patients (42 boys and 32 girls) with a median age of 21 [4–35] months were included (Table 1). There were 54 surgical diagnoses (cardiac, digestive and ear-nose-tracheal surgeries) and 20 medical diagnoses (respiratory, medical emergencies and one burn). Thirty-one patients received neuromuscular blocking drugs during the first days. The median value of CRP was 25 [8–69] mg/l on day 1 and 48 [20–76] mg/l over the study period. No patient died during the course of the study, the patients were stable and the PRISM II score at admission was 6 [4-9]. The duration of mechanical ventilation was 6 [4-8] days and the length of PICU stay was 11 [7-16] days. The oldest patient included in the study was 8.6 years old. At admission, 38 children (51%) had a normal nutritional status, 15 suffered from chronic malnutrition, 12 from acute malnutrition and 9 from both chronic and acute malnutrition. Weight at discharge was not available for 11 patients. For the other 63 patients, the mean body weight was 9.0 ± 5.2 kg at admission and 9.2 ± 5.1 kg at discharge ($P > 0.05$). A deterioration of nutritional status was observed in one patient who had a normal nutritional status at admission and acute malnutrition at PICU discharge.

3.2. Nutritional support

Nutritional support began within 22 [11–27] hours after admission. During their PICU stay, 69 (92%) children received exclusive enteral nutrition, mainly post-pyloric. Three children received enteral and parenteral nutrition and two children with short PICU stays received only intravenous glucose-containing solutions without nutritional support. Fourteen children received mother's milk, 19 received infant formulae and 39 received enteral formulae. Mean carbohydrate intake was 4.8 ± 1.4 mg/kg/min.

3.3 TUN, nitrogen intake and balance

On the basis of 402 measurements in 74 patients, the mean TUN was 0.20 (95% CI: 0.19, 0.22) g/kg/d. The TUN was stable during the first 4 days of the study at 0.18 (95% CI: 0.16, 0.20) g/kg/d (Fig. 1) and was higher on days 6 and 7 compared to the others days ($P < 0.001$). TUN was not affected by the different clinical conditions tested. In comparing the age groups, the TUN was higher in children aged 4–8 years with 0.25 (95% CI: 0.21, 0.29) g/kg/d than in younger children ($P < 0.001$) (Table 2). Nitrogen balance was lower on day 1 compared to the other days ($P < 0.001$), and it became positive on day 8 (Fig. 1). The maximal negative cumulative balance of -2.9 g/kg/d of protein was observed on day 7. Nitrogen balance was more negative in the 4–8 year olds than in younger children ($P < 0.001$) (Table 2).

3.4 REE, energy intake and balance

On the basis of 402 indirect calorimetry measurements (median of 6 per patient), the mean REE was 55 (95% CI: 54, 57) kcal/kg/d with a respiratory quotient of 0.84 ± 0.1 . The mean body temperature at the time of measurement was $37.0 \pm 0.7^{\circ}\text{C}$. The REE was stable for the first 10 days and increased slightly, but not significantly, thereafter (Fig. 2). The REE was only affected by the use of neuromuscular blocking drugs, which decreased it by 6% ($P = 0.031$), and by body temperature, which increased it by 8% per degree ($P = 0.003$). Across age groups, the REE was lowest in children aged 4-8-years ($P < 0.001$) (Table 2). Energy balance was strongly negative on the first two days and became positive at day 7 on average (Fig. 2). The maximal negative cumulative energy balance of -76 kcal/kg/d was observed on day 7.

Body weights at admission and on the last studied day were available for a subgroup of 35 patients with a median age of 11 [1-24] months. Clinical characteristics of these patients were similar to those of the entire group: the median duration of mechanical ventilation was 6 days and the length of PICU stay was 11 days. Their median REE over the course of the study was 56 kcal/kg/d and their median cumulative energy deficit during the studied period was -55 kcal/kg. The median weight at admission was 7.1 [3.8-9.8] kg and did not differ from weights on the last studied day (7.2 [3.9-9.9] kg) and at PICU discharge (7.3 [4.0-10.3] kg).

3.5. Minimum protein and energy intakes to achieve nitrogen equilibrium

Using the regression line between protein intake and nitrogen balance, we determined that a minimum intake of 1.5 (95% CI: 1.4, 1.6) g/kg/d of protein was needed to achieve nitrogen balance in the entire group (Table 3). The following protein intakes were required to achieve nitrogen balance in each age group: 1.3 (95% CI: 1.0, 1.6) g/kg/d in the 0-6 month olds, 1.5 (95% CI: 1.2, 2.3) g/kg/d in the 7-12 month olds, 1.5 (95% CI: 1.3, 1.6) g/kg/d in the 1-3 year olds and 1.9 (95% CI: 1.7, 2.3) g/kg/d in the 4-8 year olds (Fig. 3). The energy intake required to achieve nitrogen equilibrium was 58 (95% CI: 53, 63) kcal/kg/d in the entire group. For the different age groups, it was: 54 (95% CI: 40, 69) kcal/kg/d in the 0-6 month olds, 66 (95% CI: 55, 87) kcal/kg/d in the 7-12 month olds, 56 (95% CI: 50, 63) kcal/kg/d in the 1-3 year olds and 56 (95% CI: 49, 75) kcal/kg/d in the 4-8 year olds.

The energy and nitrogen intakes and PRISM scores were significantly lower in children aged 4-8 years; thus, their effects on TUN were analysed by the Wald test. After adjustment for these variables, the difference in TUN between age groups remained significant: 0.15 (95% CI: 0.11, 0.19) in 0-6 months-old; 0.16 (95% CI: 0.10, 0.22) in 7-12 months; 0.18 (95% CI: 0.15, 0.22) in 1-3 years old and 0.24 (95% CI: 0.20, 0.28) g/kg/d in 4-8 years old ($P < 0.001$). Similarly, the energy intake was lower in children aged 4-8 years compared to younger age groups. After adjustment for energy intake, the mean nitrogen balance remained more negative in older children: 0.01 (95% CI: -0.02, 0.04) in the 0-6 months; -0.01 (95% CI: -0.07, 0.04) in the 7-12 months; -0.01 (95% CI: -0.04, 0.01) in the 1-3 years old and -0.09 (95% CI: -0.14, -0.05) g/kg/d in the 4-8 years old ($P = 0.003$).

3.7. Comparisons with recommended values

For the entire group, the A.S.P.E.N recommendations for protein intakes in critically ill children, which are higher than those for healthy children, were higher than the minimum protein intake that we determined were needed to achieve nitrogen equilibrium (Table 3). In contrast, the DRIs were lower than

our calculated minimum values. For energy, the DRIs were in excess of the energy intakes required to achieve energy equilibrium in all age groups.

4. Discussion

The primary aim of this study was to assess how much protein and energy are needed to achieve nitrogen and energy balances in ventilated critically ill children. More than 400 TUN measurements by chemiluminescence and REE measurements by indirect calorimetry were made in 74 critically children. Minimum intakes of 1.5 g/kg/d of protein and 58 kcal/kg/d were required in the entire group, although large variations among the different age groups were found.

In this study, we used the four age groups defined by the DRIs, i.e. 0-6 month olds, 7-12 month olds, 1-3 year olds and 4-8 year olds. In the three younger age groups, TUN ranged between 0.17 and 0.21 g/kg/d. These losses are at least three times higher than those in healthy children⁶, as previously reported.¹⁶⁻²¹ Older children had even higher nitrogen losses consistent with findings in previous studies.^{17, 19, 21} These elevated losses were observable from the first day of ventilation and remained quite stable during the first week on ventilation in all age groups.

To achieve nitrogen equilibrium and considering non-urinary losses according to the WHO⁶, minimum protein intakes of 1.3–1.5 g/kg/d in younger children and 1.9 g/kg/d in children aged 4–8 years were required. Except for older children, our results are in agreement with the recent systematic review by Bechara et al. that recommended a minimum protein intake of 1.5 g/kg/d in critically ill children.⁵ These authors noted the inconsistency in the methodology of nitrogen balance and the urgent need for longitudinal studies including measurements of both nitrogen and energy balances. Out of nine studies, only two cross-sectional studies measured TUN.¹⁷ In others, TUN was estimated from the urinary urea nitrogen content, which may be very variable in critically ill patients.⁷ In addition, because REE was not measured by indirect calorimetry in all studies, it was not possible to examine the influence of energy balance on nitrogen balance. In the current study, using statistical models for longitudinal data, a protein

intake of 1.5 g/kg/d was required, with a narrow confidence interval (1.4, 1.6), except in older children. The acquisition of repeated data increases the precision of confidence intervals and accounts for the variability of TUN and REE measurements and of protein and energy intakes during the PICU stay.

A few studies that determined the minimal protein intake to achieve nitrogen equilibrium found quite similar results. Coss-Bu et al. determined a minimal protein intake of 2.8 g/kg/d in 19 children with a mean age of 8 years¹⁷ and 33 children with a mean age of 5 years.²¹ Joosten et al. found a value of 1.4 g/kg/d in 36 infants with a median age of 10 months.²⁰ In the present study, older children received lower energy intakes than younger children, which could have influenced TUN and nitrogen equilibrium. However, after adjustment for potential confounders (i.e. energy and nitrogen intakes and PRISM scores), the elevated TUN and protein requirements in older children could not be explained by an insufficient energy intake. Considering that protein needs decrease with age in healthy children², the higher protein requirements in older children were not expected. We might hypothesise that at this age when growth is minimal and when the body composition has a larger skeletal muscular mass, older children may show an adult behaviour, with increased hydrolysis of skeletal protein as demonstrated by Plank in critically ill adults.²²

In contrast to TUN, REE has been measured by several authors. As already demonstrated by Chawls in 1988²³ and others^{16, 24}, REE is much lower in ventilated critically ill children compared to healthy children and remains quite stable during the period of mechanical ventilation.^{25, 26} Several factors, such as sedation, analgesia, mechanical ventilation, reduced growth and physical activity, have been described to explain this decrease in energy needs.^{4, 24} In their systematic review, Bechard et al. recommended an intake of 57 kcal/kg/d to achieve nitrogen equilibrium.⁵ This value is in agreement with the current work. Only in the group of older children who had high TUN and low REE values did we find that the minimum energy intake required to equilibrate nitrogen balance was 22% higher than the REE. In the three younger age groups, this value was close to the REE.

Despite the deficits of the first few days, due to fluid restriction and the use of intravenous medications,^{27, 28} most patients in our study maintained their weight during their PICU stay. In a subgroup

of 35 children, the cumulative caloric deficit during the 6 days on mechanical ventilation was -55 kcal/kg and did not negatively affect their body weight. Using the recommended dietary allowance as the caloric goal, Hulst et al. observed that a cumulative caloric deficit of -100 kcal/kg was associated with a decrease in the z-score of weight-for-age in preterm and term neonates as well as in older children.²⁹ Only one study measured the total energy expenditure with doubly labelled water and REE with indirect calorimetry in critically ill children during and after weaning from mechanical ventilation.²⁶ The authors found that REE was 20% lower than the total energy expenditure with large inter-individual variations of physical activity measured by accelerometer. In our patients on mechanical ventilation, sedation and analgesia, we used measured REE without adding a factor for estimating the total energy expenditure and we did not observe a change in body weight during the PICU stay.

The secondary aim of this study was to compare the protein and energy required to achieve equilibrium with the A.S.P.E.N. guidelines⁴ and the DRIs². Compared to our results and those of Bechard et al.⁵, the DRIs² developed for healthy children strongly underestimate the protein needs of critically ill children. By contrast, the A.S.P.E.N. guidelines⁴ were close to our results, except for older children aged 4-8 years. The use of DRIs would highly overestimate energy needs in ventilated critically ill children.

This study has some limitations. First, the number of older patients was low, similar to the population in most PICUs, although 54 measurements could be performed. Second, these results can be applied to critically ill children on mechanical ventilation, sedation and enteral nutrition, but not to spontaneously breathing children. The heterogeneity of the sample with various ages and diagnoses may be a limitation, but it is a good representation of the PICU population. Furthermore, the number of patients decreased over the study period and the most severely ill children had longer stays and more measurements. Finally, the methodology of nitrogen balance has some limitations, but remains the most accessible method to determine protein needs.³⁰ The WHO's calculation of extra-renal nitrogen losses could be inappropriate in some groups of patients. The use of stable isotopes, a recent technique, is probably more accurate. However, this method is expensive, invasive and may be rejected by parents in this vulnerable population.

In conclusion, this longitudinal study shows that TUN is elevated whereas REE is reduced in critically ill children during the entire period of mechanical ventilation. Minimum intakes of 1.5 g/kg/d of protein and 58 kcal/kg/d could equilibrate nitrogen and energy balances in children up to 4 years of age and a higher protein intake may be required in older children.

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Statement of authorship

The authors' responsibilities were as follows: CJC conducted the research, analysed the data and wrote the manuscript; JLD designed the research, conducted the research and revised the manuscript; DL and MHP assisted in clinical work and revised the manuscript; PT made the statistical analyses; JC designed the research, conducted the research, analysed the data, wrote the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript.

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Legends to the figures

Figure 1: Evolutions of TUN, nitrogen intake and nitrogen balance over the study period. TUN is presented as a dotted line, nitrogen intake is shown as a solid line and nitrogen balance is depicted as black bars. Data are displayed as the mean with a 95% confidence interval.

Figure 2: Evolutions of REE, energy intake and energy balance over the study period. REE is presented as a dotted line, energy intake is shown as a solid line and energy balance is depicted as black bars. Data are displayed as the mean with a 95% confidence interval.

Figure 3: Regression between protein intake and nitrogen balance for each age group: (A) 0–6 months, (B) 7–12 months, (C) 1–3 years and (D) 4–8 years. The regression line is estimated using generalized linear models for longitudinal data. The dotted vertical line shows the amount of protein needed to achieve a zero nitrogen balance.

Table 1Demographic and clinical variables for the entire group and by age group. ^a

Variable	Group	0-6 months	7-12 months	1-3 years	4-8 years	P-value
<i>n</i> patients	74	22	8	34	10	
Age (months)	21 [4–35]	1 [0.2–4]	9 [8–11]	25 [21–35]	65 [56–80]	
Weight (kg)	9 [4–13]	4 [3–4]	8 [7–9]	11 [9–13]	17 [16–22]	< 0.001 ^b
Height (cm)	77 [60–93]	51 [48–58]	69 [68–71]	84 [79–94]	109 [101–117]	< 0.001 ^b
Length of PICU stay (days)	11 [7–16]	11 [7–17]	11 [8–19]	9 [6–17]	9 [7–13]	0.824 ^c
Mechanical ventilation (days)	6 [4–8]	6 [4–9]	6 [5–8]	5 [4–8]	7 [3–9]	0.957 ^b
PRISM II score	6 [4–9]	8 [5–8]	8 [4–15]	6 [4–8]	5 [2–8]	0.046 ^c
CRP max (mg/l)	92 [38–159]	86 [39–127]	83 [22–133]	108 [30–180]	108 [66–171]	0.578 ^b

^a All values except for *n* are median and interquartile range [P25-P75].^b Differences in age groups were tested by ANOVA.^c Differences in age groups were tested by the Kruskal–Wallis test

Table 2Nitrogen and energy balance measurements for the entire group and for each age group.^a

Variable	Group	0-6 months	7-12 months	1-3 years	4-8 years	P-value^b
<i>n</i> patients	74	22	8	34	10	
<i>n</i> measurements	402	126	43	179	54	
TUN (g/kg/d)	0.20 (0.19, 0.22)	0.18 (0.15, 0.20)	0.19 (0.14, 0.24)	0.20 (0.18, 0.23)	0.25 (0.21, 0.29)	< 0.001
Total nitrogen losses	0.24 (0.23, 0.25)	0.21 (0.19, 0.24)	0.22 (0.17, 0.27)	0.23 (0.21, 0.26)	0.29 (0.25, 0.33)	< 0.001
Nitrogen intake	0.19 (0.19, 0.21)	0.21 (0.16, 0.25)	0.18 (0.09, 0.26)	0.18 (0.14, 0.21)	0.13 (0.06, 0.21)	< 0.001
Nitrogen balance	-0.05 (-0.05, -0.03)	0.00 (-0.04, 0.04)	-0.04 (-0.11, 0.03)	-0.05 (-0.08, -0.02)	-0.15 (-0.21, -0.09)	< 0.001
REE (kcal/kg/d)	55 (54, 57)	57 (53, 61)	60 (52, 67)	56 (53, 59)	46 (40, 52)	< 0.001
Energy intake	48 (46, 51)	54 (47, 61)	51 (38, 64)	46 (40, 52)	30 (19, 41)	< 0.001
Energy balance	-7 (-8; -5)	-3 (-9, 3)	-9 (-19, 4)	-10 (-15, -5)	-16 (-25, -5)	< 0.001

^a All values except for *n* are the mean with a 95% confidence interval.^b Differences in the age groups were tested by the Wald test.

Table 3

Comparison between the minimal protein and energy intakes required to equilibrate nitrogen and energy balances in this study and the intake recommended by the DRIs and A.S.P.E.N.

Variable	Group	0-6 months	7-12 months	1-3 years	4-8 years
<i>n</i>	74	22	8	34	10
Minimal protein intake (g/kg/d) ^a	1.5 (1.4, 1.6)	1.3 (1.0, 1.6)	1.5 (1.2, 2.3)	1.5 (1.3, 1.6)	1.9 (1.7, 2.3)
DRIs ^b	1.2	1.52	1.2	1.05	0.95
A.S.P.E.N. ^c	1.5-3	2-3	2-3	1.5-3	1.5-2
REE (kcal/kg/d) ^a	55 (54, 57)	57 (53, 61)	60 (52, 67)	56 (53, 59)	46 (40, 52)
DRIs ^a	84 (81, 88)	100 (93, 108)	78 (76, 80)	81 (79, 82)	67 (59, 88)

^a Values are the mean with a 95% confidence interval.

^b Values are the mean.

^c Values are the range.

Figure 1

Figure 2

Figure 3