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# Nutritional-metabolic factors affecting nitrogen balance and substrate utilization in the critically ill

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**Abstract.** To determine clinical, anthropometric, metabolic, and nutritional factors affecting nitrogen balance somatic protein status and substrate utilization in critically ill children measured energy expenditure (MEE) was measured by indirect calorimetry within 24 hr of an acute illness, solid organ transplantation, or cardiovascular surgery. Predicted basal metabolic rate was calculated using the Schofield equation. Somatic protein was estimated by the creatinine-height index. Nitrogen balance (NB) was calculated by subtracting the total nitrogen input from output. The net substrate (fat, carbohydrate, and protein) oxidation rates were calculated using the Weir formula modified by Frayn. Sixty-eight NB studies, indirect calorimetric and anthropometric measurements performed in 37 patients. Nitrogen balance was worse when the MEE/Predicted basal metabolic rate ratio was $< 0.9$ or $> 1.1$. The incidence of negative NB was 91% when the caloric intake was less than MEE and 9% when it was equal to or greater than MEE ($P < 0.05$). On day 1, 27% had mild to moderate somatic protein depletion and 5.4% had severe somatic protein depletion. Only the persistence of stress and co-morbidity were associated with the creatinine-height index ($P < 0.001$). Without Multiple Organ System Failure (MOSF), there was a trend toward positive nitrogen balance by day 7 while with MOSF, negative nitrogen balance persisted even by day 7 ($P < 0.05$). When caloric intake was less than MEE, mean substrate utilization was 48.6% from lipid, 37.1% from carbohydrate, and 14.3% from proteins. But, when caloric intake was greater than MEE, mean substrate utilization was 83.3% from carbohydrate and 16.7% from protein. Significant negative nitrogen balance and somatic protein depletion develops in critically ill pediatric patients, especially when they are inadequately fed, develop MOSF, or have previous chronic illness. Caloric intake and MOSF independently affect substrate utilization.

Keywords: Nitrogen balance, multiple system organ failure, pediatric risk of mortality score, energy expenditure, substrate utilization

## 1. Introduction

Critical illness is often accompanied by muscle protein breakdown and increased nitrogen excretion [1]. Significant malnutrition can occur in critically ill children within 48 hr of admission to a pediatric intensive care unit (PICU) [2,3]. Malnutrition increases the risk of infection, which may prolong hospital stay and increases morbidity and mortality in adults [4] and critically ill children [5]. Tissue protein breakdown can lead to negative nitrogen balance (NB) if adequate and optimal nutrition is not provided [6]. In addition, a futile cycle of proton pumping and proton leak, which accounts for 26% ± 7% of the total oxygen consumption rate and up to 38% of the basal metabolic rate in an animal model, might retain the ability to allow rapid switching of flux from leak to ATP turnover [7].

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Accordingly, patients with low metabolic rates had slower protein breakdown and decreased glucose clearance compared to patients with high metabolic rates [8]. The predominance of hypometabolism, however, associated with inaccuracy of standard equations, may well lead to overfeeding [9], lipogenesis, and increased carbon dioxide production [10]. This practice may further lead to liver dysfunction, especially when parenteral nutrition is employed, and worsens the prognosis of these patients [11].

It is not yet known which factors related to an acute and/or chronic stress state might independently affect the NB and the somatic protein status in critically ill children. We have previously shown that achievement of positive protein and energy balance in relation to the basic metabolic rate using an aggressive early enteral nutrition protocol improves NB during the acute phase of stress in 2/3 of critically ill children [12]. This prospective study was undertaken to determine stress-related factors affecting the nitrogen balance, the somatic protein status, the substrate utilization rates in critically ill children, and to examine the relation between NB and anthropometric, clinical, metabolic and nutritional indices.

## 2. Materials and methods

### *2.1. Patients*

This study was approved by the Institutional Review Board and informed consent was obtained from the parents of children recruited to the study. All patients who were admitted to the PICU at Children's Hospital of Pittsburgh and mechanically ventilated for an acute illness, or within the first 24 hr of transplantation or a major operative procedure during a 6 mo period were eligible for the study. All patients were ventilated using the Siemens-Elema Servo 900C ventilator (Siemens-Elema, Solna, Sweden). All patients who were intubated with cuffed endotracheal tubes and those with uncuffed endotracheal tubes without a significant air leak were included in the study. The absence of significant air leak was confirmed by both the absence of an audible air leak by clinical examination and the difference between the inspired and expired tidal volume <10%. Exclusion criteria were: 1) children with dysmorphic syndromes, 2) children with severe brain injury being evaluated for brain death, 3) children with acute renal failure, 4) mechanical ventilation < 24 hr, 5) uncuffed tubes with leak >10%, 6) fraction of inspired oxygen (FIO2) > 0.6, 7) inspiratory gas containing gases other than nitrogen, oxygen, and carbon dioxide such as helium or nitric oxide, 8) the presence of chest tubes, and 9) the presence of a bronchopleural fistula. All patients were hemodynamically stable before the study period and throughout during the indirect calorimetry measurement. Only continuous feeding was used but the judgment and decisions regarding nutritional repletion were made by the physician in charge of the patient and were made entirely independent of the study. The physician in charge was blinded to any of the data collected for the study.

### *2.2. Data collection*

Data collected included demographic data, clinical diagnosis, and vital signs. Patients were classified a priori into the following diagnostic categories: Sepsis, Brain injury, Respiratory failure, Transplant, and Postoperative Cardiac Surgery. Sepsis, septic shock, and systemic inflammatory syndrome were defined as described by the American College of Chest Physicians / Society of Critical Care Medicine consensus [13]. The mode of feeding (enteral or parenteral), the nature of nutrition, and the amount of caloric intake or repleted energy (RE) were also recorded. The nutritional status of all children was evaluated for the presence of protein-energy malnutrition. Nutritional status was classified as normal, chronic protein-energy malnutrition (CPEM), and acute protein-energy malnutrition (APEM) as defined by the Waterlow's stages [14]. Anthropometric measurements included midarm circumference (MAC), cutaneous triceps skin-fold thickness (TSF), mid-arm muscle diameter, mid-arm muscle area (MMA), and mid-arm fat area (MFA) [15]. Fat stores were assessed by measurements of TSF and MFA. Somatic protein stores were assessed by mid-arm muscle diameter and MMA. Fat and protein stores were classified as normal, nutritionally at risk, or deficient as defined by Frisancho [16], Ryan and Martinez [17]. Standards of the National Center of Health Statistics were used for the assessment of weight for length and length for age [18]. Standards of the Ten-State Nutritional Survey were used for TSF, MAC, MMA, and MFA [19]. Only first day anthropometric measurements were recorded because of technical and accuracy issues on subsequent days (edema, central catheters, various interventions, etc). The severity of illness was assessed by the Pediatric Risk of Mortality Score [20], the Therapeutic

Intervention Scoring System modified for children [21], and indices of organ failure. Organ failure was assessed using the multiple organ dysfunction score as defined by Marshall et al. [22] and the modified multiple organ system failure (MOSF) as defined by Wilkinson et al. [23].

### 2.3. Metabolic recording

Patients were studied from day 1 to day 14 or until discharge (or death). Measurements were repeated on days 3, 7 and 14 in those patients still in PICU. Oxygen consumption, carbon dioxide production, and the respiratory quotient (RQ) were measured by 30 min-indirect calorimetry using the deltatrac metabolic monitor (sensor medics). The metabolic monitor was calibrated before each measurement using a two-point calibration with room air and a commercially available special gas mixture containing 4% carbon dioxide and 96% oxygen [24,25]. Measured energy expenditure (MEE) was calculated using the Weir [26] formula modified by Frayn [27]. Predicted basal metabolic rate (PBMR) was calculated using the Schofield [28] equation. The ratio of MEE/PBMR was calculated to determine the pattern of energy expenditure. Patients were classified as hypermetabolic, normometabolic, and hypometabolic when the measured resting energy expenditures were > 110%, 90–110% and, < 90% of the PBMR, respectively. Caloric intake was calculated on the same day of MEE measurement and the caloric intake/MEE ratio was calculated to determine the pattern of feeding. Recommended Dietary Allowances were estimated using the tables proposed by the food and nutrition board national research council [29] and by Kerner [30]. Oxygen consumption, carbon dioxide production, MEE, PBMR, and caloric intake were indexed to body surface area.

### 2.4. Nitrogen balance and substrate utilization

Many of our patients had substantial losses through a stoma or other drainage sites. In some transplant patients, the overall contribution of the non-urine nitrogen to the nitrogen output was as high as 67% of the total amount of nitrogen losses. Therefore, we chose to include nitrogen losses from all potential sources in the calculation of nitrogen balance. Accordingly, 24 hr urine collections, blood samples, fecal losses and other body fluid samples were started with the first indirect calorimetric measurement, within 12 hr of admission to the PICU and repeated on days 3, 7 and 14 along with MEE measurements. In all patients, 0.5 g. per day of nitrogen was added to the output to account for nitrogen lost through skin [31]. Nitrogen in urine, stool, and all drainages was determined by manual micro-Kjeldahl digestion and by high-resolution liquid chromatography [32]. NB was calculated by subtracting the total nitrogen input from output. Total nitrogen output (g/day) was calculated as the sum of total urinary nitrogen, the change in blood urea nitrogen, nitrogen in fecal and other fluid losses, and 0.5 g for cutaneous losses. The change in blood urea nitrogen was calculated as the 24 hr change in blood urea nitrogen multiplied by 60% of body weight, assuming that urea was distributed uniformly in body water [33]. The creatinine-height index (CHI) [34], which is an indirect measure of somatic protein status, was calculated as the fraction of the 24-hr urine creatinine excretion to the creatinine excretion of normal individuals of same height and sex multiplied by 100. The somatic protein status was classified as follows: CHI 60–80% = mild to moderate depletion of somatic protein status and ≤ 60% severe depletion of somatic protein status. The non-protein RQ and the net substrate (fat, carbohydrate, and protein) oxidation rates were calculated using the Weir [26] formula modified by Frayn [27]. Substrate utilization was calculated by the following formulas: Protein (g/day) = $6.25 \times$ total urine nitrogen; fat (g/day) = $([1.689 \times VO2] - [1.689 \times VCO2]) - (1.943 \times$ total urine nitrogen); and carbohydrate = $([4.115 \times VCO2] - [2.909 \times VO2]) - (2.539 \times$ total urine nitrogen).

### 2.5. Statistical analysis

Normally distributed data are expressed as mean ± SD, while non-normally distributed data are given as median and range. Statistical analysis was performed with a two-tailed t-test for normally distributed paired data after Levene's correction for equality of variances or by Mann-Whitney *U*-Wilcoxon rank sum W test for non-normally distributed data. Comparisons between nominal data were made by chi-square statistic with continuity correction when applicable. Differences among various disease groups and among subcohorts were tested by analysis of variance. Repeated measurements over time within and between groups were tested by analysis of variance for repeated measures with a Student-Newman-Keuls post-hoc range test. Multivariate stepwise regression analysis was used to analyze the contribution of the various clinical factors,

the caloric intake, substrate intake, substrate utilization, presence or absence of MOSF, and severity of illness to the variation in the nitrogen balance and CHI. These same data were also analyzed by multiple logistic regression (forward stepwise method) for dichotomous outcomes to identify those factors that were independently associated with negative nitrogen balance, abnormal CHI or MOSF. All analyses were done using the Statistical Package for the Social Sciences for Windows (release 17, Statistical Package for the Social Sciences, Chicago, IL) [35].

## 3. Results

### 3.1. Patients' characteristics

Sixty-eight nitrogen balance studies, indirect calorimetric and anthropometric measurements were performed in 37 patients. The patients ranged in age from 1 to 210 mo (median age 80 mo). Male to female sex ratio was 1.85:1. The mean length of mechanical ventilation was 6.8 ± 1.2 days, (range 1 to 30 days), while the mean length of stay in PICU was 10.0 ± 1.6 days (range 1 to 41 days), and the mean hospital stay was 24.7 ± 4.3 days (range 3 to 133 days). Eighty-six percent of patients were Caucasian, with African American and Hispanics, comprising 8% and 5% of the total study population, respectively. Twenty-four patients (65%) had chronic illnesses. Of all patients, eight had undergone solid organ transplantation (21.6%), six were cardiac surgical patients (16.2%), nine had sepsis (24.3%), five respiratory failure (13.5%), five metabolic coma (13.5%), three head trauma (8.1%) and one patient suffered from severe Guillain-Barré syndrome (2.7%). The first day incidence of CPEM was 21.6% and APEM 8.1% (Waterlow's stages 2–3). An additional 7.7% was recorded to be at risk for APEM and 15.6% for CPEM (stage 1). Four patients died (11%), all with preexisting diseases.

Regarding main ventilator settings, $FiO_2$ ranged between 0.25 to 0.6 (mean 0.4 ± 0.01), respiratory rate was 31.5 ± 14, tidal volume 9.8 ± 1.8, and positive end expiratory pressure 5 ± 1.2.

### 3.2. Metabolic and nutritional characteristics

The RQ ranged from 0.73 to 1.07 (mean 0.89 ± 0.01) and MEE from 100 to 1879 kcal/day (17 to 63 kcal/kg/day). The main clinical, anthropometric, and metabolic, characteristics of patients are given in table 1. Expressed in proportional terms, the mean RE was averaging at 46% of the mean Recommended Dietary Allowances, and 90% of the mean PBMR. The RE-MEE difference varied between −40 and +62 kcal/kg/day (median −3 kcal/kg/day), while the RE-PBMR difference ranged between −38 and +59 kcal/kg/day (median −7.7 kcal/kg/day). The non-controlled by the protocol mode of feeding differed during the course of the study; on day 1 only 16.2% and 5.4% received total parenteral or enteral nutrition, respectively, compared to day 3 (58% and 10.5%), day 7 (50% and 50%), and day 10

Table 1
Clinical, anthropometric, and metabolic characteristics of study patients

| Variables | Mean ± SE | Median | Abnormal (%) |
|---|---|---|---|
| Pediatric risk of mortality score | 8.89 ± 0.76 | 9 | |
| Therapeutic intervention scoring system | 36.1 ± 1.7 | 36 | |
| Systemic inflammatory syndrome (%) | | | 48.6 |
| Multiple organ system failure (%) | | | 51.4 |
| Pediatric risk of mortality score probability of death (%) | 9.7 ± 2.4 | 7.4 | |
| Multiple organ system failure probability of death (%) | 7.8 ± 1.6 | 4.9 | |
| Midarm fat area ($mm^{2}$)/(stage 2) | 1294.4 ± 100.6 | 1107.5 | 11.7 |
| Midarm muscle area ($mm^2$)/(stage 2) | 1833.6 ± 184.7 | 1494.3 | 28.6 |
| Acute protein-energy malnutrition (stage 2–3) | | | 8.1 |
| Chronic protein-energy malnutrition (stage 2–3) | | | 22.1 |
| Respiratory quotient | 0.89 ± 0.01 | 0.87 | |
| Measured energy expenditure (kcal/kg) | 38.9 ± 1.28 | 40.77 | |
| VO2/body surface area ($mL/min/m^2$) | 141.5 ± 3.2 | 145.3 | |
| VCO2/body surface area ($mL/min/m^2$) | 125.9 ± 3.08 | 129.5 | |
| Nitrogen output (g/kg/day) | 0.26 ± 0.01 | 0.27 | |
| Nitrogen intake (g/kg/day) | 0.21 ± 0.02 | 0.19 | |
| Nitrogen balance (g/kg/day)/negative | −0.1 ± 0.02 | −0.11 | 73.5 |
| Creatinine-height index (mg/day) / < 80% | 88.29 ± 3.51 | 83.95 | 47 |

(68% and 33%) of the study ($P < 0.0001$). Table 2 presents average nutritional characteristics of studied patients (days 1, 3, 7, 10 and 14). First day nutrition strategies were dominating by the hypofeeding pattern which was over 50% (hypofeeding: 20/37 (54.1%), normofeeding: 14 (37.8%), hyperfeeding: 3 (8.1%)). On the first day of stress 10.8% of children were hypermetabolic, 48.6% hypometabolic, and 40.5% normometabolic.

### 3.3. Nitrogen balance and somatic protein status

In the hypometabolic pattern NB was −2.3 ± 4.9, in the normometabolic, −0.3 ± 4.8 and in the hypermetabolic −4.8 ± 6.9 g/kg/day, respectively ($P = 0.033$). On day 1, 10/37 (27%) had mild to moderate somatic protein depletion and 2/37 (5.4%) had severe somatic protein depletion. The initially negative mean NB attained a positive value by the 14th day of stress (−0.12 vs. 0.05 g/kg/day). Patients with a previous chronic illness had a higher relative risk of an abnormal CHI (83%), than previously healthy patients (17%) ($P < 0.001$, relative risk 5.4, 95% confidence interval: 1.9 to 15). Only the persistence of stress and co-morbidity were significantly negatively associated with the CHI.

### 3.4. Relation of NB and CHI to clinical data

There was no significant difference in nitrogen balance, CHI, or MEE between the various disease groups or between medical and surgical patients. Only the repleted protein combined with a reduced protein oxidation rate were independently associated with a positive nitrogen balance, while presence of concurrent or pre-existing illness was the only variable independently associated with an abnormal protein store depletion (estimated relative risk 5.4 (95% confidence interval: 1.9 to 15). There was no difference in somatic protein depletion with or without MOSF. Without MOSF, there was a trend toward positive NB by day 7 while with MOSF, negative NB persisted even by day 7 ($P < 0.05$). This difference was not due to differences in protein intake or RE in the two groups (Table 3).

### 3.5. Nutrition

A caloric intake less than MEE was associated with a higher incidence of negative nitrogen balance than when caloric intake was equal to or greater than MEE (91% vs. 9%, respectively, $P < 0.05$). A caloric intake greater than MEE was associated with a higher incidence of positive nitrogen balance compared to caloric intake less than MEE (56% vs. 22%, respectively, $P < 0.05$). Caloric intake relative to MEE did not affect the risk for an abnormal CHI. Multiple organ system failure patients had much lower MEE by day 7 but had a higher carbohydrate intake and a much lower lipid intake (Table 2).

### 3.6. Substrate repletion and utilization

The average during the study period carbohydrate, lipid, and protein intake was 7.0 ± 0.6, 0.7 ± 0.1, and 1.3 ± 0.1 g/kg/day, respectively. Seventy-two

Table 2
Nutritional characteristics of study patients

| Variables | Mean ± SE | Median | Abnormal (%) |
|---|---|---|---|
| Repleted energy (kcal/kg) | 37.27 ± 2.88 | 33.49 | |
| Hypofeeding (%) | | | 31.2 |
| Hyperfeeding (%) | | | 14.3 |
| Repleted carbohydrate (kcal/kg/day) | 25.64 ± 1.99 | 21.05 | |
| Repleted lipid (kcal/kg/day) | 6.3 ± 1.09 | 0.01 | |
| Repleted protein (kcal/kg/day) | 5.33 ± 0.48 | 4.75 | |
| Utilized carbohydrate (kcal/kg/day) | 19.96 ± 1.33 | 19 | |
| Utilized lipid (kcal/kg/day) | 9.38 ± 1.48 | 9.89 | |
| Utilized protein (kcal/kg/day) | 6.21 ± 0.3 | 6.38 | |
| Given/utilized carbohydrate (%) | 2.27 ± 0.66 | 1.29 | |
| Given/utilized lipid (%) | −0.56 ± 0.58 | 0 | |
| Given/utilized protein (%) | 1.03 ± 0.14 | 0.8 | |
| Non-protein kcal/1g N2 | 211.18 ± 22.02 | 154.18 | |
| Non-feed protein (% given protein) | 48.8 ± 5.46 | 44.94 | |

Table 3
Nitrogen balance, energy expenditure, and caloric and nutrient intakes with and without multiple organ system failure

| Variables | No-multiple organ system failure | | | Multiple organ system failure | | |
|---|---|---|---|---|---|---|
| | Day 1 (n = 18) | Day 3 (n = 12) | Day 7 (n = 6) | Day 1 (n = 19) | Day 3 (n = 7) | Day 7 (n = 4) |
| Nitrogen balance (g/kg/day)* | −2.7 | −0.88 | 0.12 | −2.1 | −1.44 | −3.71 |
| Measured energy expenditure (kcal/kg)* | 40.9 | 42.1 | 44 | 40 | 40.8 | 29.9 |
| Repleted energy (kcal/kg)/ measured energy expenditure (kcal/kg) | 0.43 | 0.89 | 1.1 | 0.6 | 1.33 | 1.3 |
| Protein intake (g/kg) | 0.53 | 1.3 | 1.3 | 1.1 | 1.4 | 2.2 |
| Carbohydrate intake (g/kg)* | 3.9 | 6.7 | 8.1 | 4.3 | 9.3 | 11.7 |
| Lipid intake (g/kg)* | 0 | 0.9 | 1.7 | 0 | 0.1 | 0.1 |

All values are expressed as medians.
*$P < 0.05$ no-multiple organ system failure vs. multiple organ system failure by Friedman's ANOVA.

percent of the caloric intake was derived from carbohydrates, 12% from lipids, and 16% from proteins. The substrate utilization was 57% from carbohydrates, 23% from lipids, and 20% from protein. The difference between repleted and utilized carbohydrate was 5.5 ± 1.4 kcal/kg, between repleted and utilized lipid was −3.6 ± 1.9 kcal/kg and between repleted and utilized protein was −0.8 ± 0.5 kcal/kg. The repleted/utilized ratio was 2.3 ± 0.7 for carbohydrate, −0.56 ± 0.6 for the lipid and 1.0 ± 0.1 for the protein. When caloric intake was less than MEE, mean substrate utilization was 48.6% from lipid, 37.1% from carbohydrate, and 14.3% from proteins. Compared to normo- or overfeeding groups of patients this group exhibited a shift toward fat oxidation in (17 vs. 5.6 kcal/kg/day, $P < 0.004$). When caloric intake was greater than MEE, mean substrate utilization was 83.3% from carbohydrate and 16.7% from protein. In group we found a high rate of lipogenesis (−2 vs. 11 kcal/kg/day, $P < 0.01$), combined with a high rate of carbohydrate oxidation (30 vs. 18 kcal/kg/day) without any effect on the protein oxidation rate. Utilized lipid was negative in patients whose caloric intake was higher than MEE suggesting a high ratio of lipogenesis.

## 4. Discussion

The major findings from this study are as follows: 1) Critically ill pediatric patients develop significant negative NB and somatic protein depletion, 2) Negative NB is more likely to occur and is higher in patients when caloric intake is less than MEE, with hyper-or hypo-metabolic pattern, with MOSF, when repleted protein is inadequate, and when the protein oxidation rate is low, 3) Somatic protein depletion is associated with concurrent or pre-existing illness, 4) Substrate utilization differs according to the caloric intake, and 5) MOSF is associated with higher carbohydrate intake, lower lipid intake, persistent hypometabolism, and negative nitrogen balance.

### *4.1. Energy expenditure and nitrogen balance in stress states*

Because protein oxidation represents 8–12% of the total energy expenditure, it has been speculated that changes in NB may be associated with changes in MEE. Benotti and Blackburn [36] proposed that an estimation of MEE could be made from measurement of urea excretion and NB. However, in the current study, in which patients were receiving mixed nutritional regimens, we could not find any correlation or independent association of NB with MEE.

It is speculated that the degree of catabolism of the acutely ill child reflects the degree of stress of the individual, since with more stress there is more neurohumoral activation, more nitrogen loss, and more muscle proteolysis [37]. However, in this study, we did not find any relationship between NB with any of the traditional indices of illness severity such as Pediatric Risk of Mortality and Therapeutic Intervention Scoring System. We also did not find any relationship between MEE and the indices of severity of illness. This finding is in accordance to results of previous studies showing that there is no correlation between severity of illness scores [38], clinical characteristics or

ventilatory parameters [39] and MEE to explain the wide range of metabolic alterations [40] and inaccuracies of energy expenditure estimates in different types of lesion in critically ill children [41] and adults [42].

### 4.2. Relation to the metabolic patterns

The incidence of hypermetabolism was low and associated with a higher degree of negative NB compared to other metabolic patterns. It has been postulated that thermogenesis in these critically ill patients may be related to the accelerated protein turnover occurring as a functioning redistribution of body cell mass, in support of host defense and tissue repair [29]. Moreover, we found that even patients with a hypometabolic pattern had a higher incidence of negative NB than those with a normal pattern. These findings are in agreement to those of our previous studies suggesting that the predominant hypometabolic pattern is a marker for disease severity and it is associated with an increased risk of protein loss and mortality [43]. It has been also previously shown that a true serial progressive pattern of hypometabolism may imply impending septic shock, organ failure, and increased mortality [44]. Using whole-body $CO_2$ production as an index of substrate oxidation and energy expenditure Kao et al. [8] showed that hypometabolism was associated with mortality and changes in protein and glucose metabolism in septic patients. We further showed that although neither IL-6 nor IL-10 was associated with a hypometabolic pattern, hypometabolism was associated with $VO_2$ and $VCO_2$ changes, predominated the acute phase of stress and was associated with increased mortality [45]. Increased glucocorticoid production induced by inflammatory cytokines was shown to further increase nitrogen wasting without increasing the metabolic rate [46].

### 4.3. Malnutrition and somatic protein status

When decreased protein intake and decreased synthetic production are coupled with increased tissue catabolism, the net tissue balance is severely negative, leading to a rapid depletion of body tissue stores and critical elements such as functional protein. Therefore, patients are at risk for acute or chronic malnutrition and somatic and fat stores depletion if their caloric and protein requirements are not met [2]. During their first PICU day, half of our patients were underfed, over 30% had mild to severe somatic protein depletion, 1/5 had already developed CPEM and 1/6 were at risk or had already developed APEM. In a previous study, we found that 16.9% were at risk for CPEM and 21.1% for APEM, whereas 4.2% and 5.6% already had chronic and acute, respectively protein– energy malnutrition [5]. Using the SD score for weight for height or equivalent criteria, the prevalence of APEM over the last 10 yr in hospitalized children in Germany, France, the UK and the USA varied between 6.1 and 14%, whereas in Turkey up to 32% of patients with malnutrition were reported [47]. Furthermore, because of the diversity of medical conditions and syndromes in hospitalized children and the impossibility to correctly measure weight and height in an intensive care setting, it was recently suggested that assessment of nutritional status and interpretation of anthropometric data need a tailored approach [48]. Thus, on day 1, all children with severe traumatic brain injury admitted to an intensive care had negative balances for protein and phosphate which became positive when protein intake was ≥ 1 g/kg/day and energy intake was ≥ 50% of estimated energy expenditure [49]. Creatinine excretion rate was negatively correlated with protein balance showing an abrupt rise in the presence of sepsis [39]. A high rate of inadequate energy intake and negative NB was also recently shown in adult critically ill patients, suggesting that an attempted equilibrium between energy intake and energy expenditure might improve NB [50].

### 4.4. Relationship to MOSF

We found that MOSF was associated with a higher protein loss and a negative NB despite the fact that total protein intake was higher in this group of patients. In a previous study, using an aggressive early enteral nutrition protocol, we also showed that MOSF was negatively correlated with the NB [12]. In another study, in medical patients with MOSF, neither hypercaloric nor isocaloric nutritional support prevented protein catabolism; in contrast, patients enhanced the metabolic burden, thermogenesis, urea production rate, and glucose and lactate levels [51]. It seems, therefore, that the altered metabolic milieu during stress prevents effective use of exogenously delivered protein.

In our study, MOSF patients were more likely to be associated with hypometabolism and with higher carbohydrate intake or lower lipid intake than those without MOSF. Serial muscle biopsy data indicated that intravenously fed patients were wasting away and the

maintenance of MAC was due to fluid retention [52]. In addition, energy and nitrogen balance in the group in which arm circumference decreased had no apparent effect on the rate of wasting [49]. These and our results are in agreement to findings of earlier studies showing that early enteral nutrition was not as effective among patients with refractory septic shock or MOSF as it was among other groups of critically ill patients [53–55]. Thus, in a recent study, children identified by criteria for targeted indirect calorimetry, had a high incidence (72%) of metabolic instability with a predominance of hypometabolism; however, no correlation was found between RQ and energy balance [9].

### *4.5. Relation to nutrition*

During the first few days of illness, insufficient caloric intake and protein intake coupled with an increased protein loss may result in depletion of somatic protein and fat stores [56–58]. Therefore, the goal of nutritional metabolic support in these critically ill patients is to minimize the net negative tissue protein and energy daily balances, which accumulate into net negative tissue protein and fat store balances over time, by providing substrate to at least partially offset the obligatory catabolic losses and to provide fuel for oxidative purposes.

In this study, only the repleted protein combined with a reduced protein oxidation rate were independently associated with a positive nitrogen balance. Providing carbohydrates did not appear to spare protein losses. Several studies in adults have shown that the nitrogen loss during critical illness is not affected by the amount or type of protein provided despite producing a positive nitrogen balance [59,60]. Thus, increased dietary protein maintained NB during exercise-induced energy deficit, but this did not impact resting whole-body protein turnover [61].

Improved nitrogen retention may, therefore, reflect increased synthesis rather than decreased losses. There is considerable controversy regarding the type and composition of fuel to administer in critically ill patients. Enteral feeding, started early, seems to be beneficial in improving nitrogen balance [41]. Even provision of as little as 25% of total calories enterally improved nitrogen balance and reduced bacterial translocation in stressed patients, in a dose dependent fashion [42]. We have recently shown that early enteral feeding is safe and may be beneficial in critically ill children [62]. Early administration of a protein and energy-enriched formula in critically ill infants is well tolerated, promotes a more adequate nutrient intake and improves energy and nitrogen balance without adverse effects [63].

### *4.6. Substrate utilization related to nutrition and calorimetry*

In stressed patients, carbohydrate metabolism is characterized by increased gluconeogenesis that is suppressed poorly by glucose or insulin infusion and increased glucose synthesis from lactate via the Cori cycle [64]. Findings of our study further suggest that in critically ill children the substrate utilization can be affected by the type and amount of fuel provided. Thus, when caloric intake was less than MEE, there was a shift toward fat oxidation with protein contributing less than 20% of the total $CO_2$ production. When patients were hyper-fed, a high rate of lipogenesis combined with a high rate of carbohydrate oxidation was found, without any effect on the protein oxidation rate.

Acute hormonal stress responses may explain the shift toward fat oxidation and either gluconeogenesis or impaired peripheral carbohydrate uptake, without affecting protein or energy expenditure. However, protein is used as a fuel source under stressed conditions peripherally with a propensity for skeletal muscle [56]. Oxidation of amino acids in muscle is stimulated by fasting, sepsis, stress, hormonal influence, and other conditions associated with negative nitrogen balance. However, whether providing adequate amounts of lipid and carbohydrate can decrease protein catabolism and oxidation in critically ill children remains to be established.

In conclusion, in this study, we found that significant negative nitrogen balance and somatic protein depletion develops in critically ill pediatric patients, especially when they are inadequately fed. Patients who develop MOSF have a higher negative nitrogen balance and somatic protein depletion than patients who do not develop MOSF. Previous chronic illness has a profound effect on somatic protein depletion. Caloric intake and MOSF independently affected substrate utilization. Future studies are necessary to establish the optimal nutritional support in these patients.

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