Aspects of protein metabolism in children in acute and chronic illness

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Citation for published version (APA):

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General introduction and outline
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AA</td>
<td>amino acid</td>
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<tr>
<td>APE</td>
<td>atom percent excess</td>
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<tr>
<td>A.S.P.E.N.</td>
<td>American Society for Parenteral and Enteral Nutrition</td>
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<tr>
<td>BCAA</td>
<td>branched-chain amino acid</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>BUN</td>
<td>blood urea nitrogen</td>
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<tr>
<td>CF</td>
<td>cystic fibrosis</td>
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<td>CHD</td>
<td>congenital heart defect</td>
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<td>CPB</td>
<td>cardiopulmonary bypass</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>EN</td>
<td>enteral nutrition</td>
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<td>FFA</td>
<td>free fatty acid</td>
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<tr>
<td>FSR</td>
<td>fractional synthesis rate</td>
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<td>HP</td>
<td>high protein</td>
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<td>I</td>
<td>infusion rate</td>
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<td>ICU</td>
<td>intensive care unit</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor-1</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>IRMS</td>
<td>isotope ratio mass spectrometry</td>
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<tr>
<td>KIV</td>
<td>α-ketoisovalerate</td>
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<tr>
<td>LBM</td>
<td>lean body mass</td>
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<tr>
<td>LOD</td>
<td>limit of detection</td>
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<td>LOS</td>
<td>length of stay</td>
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<td>LP</td>
<td>low protein</td>
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<tr>
<td>MODS</td>
<td>multiple organ disease syndrome</td>
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<td>MPE</td>
<td>mass percent excess</td>
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<td>NOD</td>
<td>non oxidative disposal</td>
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<td>NP</td>
<td>normal protein</td>
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<td>NST</td>
<td>nutritional support team</td>
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<td>PEM</td>
<td>protein energy malnutrition</td>
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<td>PICU</td>
<td>pediatric intensive care unit</td>
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<td>PIM</td>
<td>predicted index of mortality</td>
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<td>PN</td>
<td>parenteral nutrition</td>
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<tr>
<td>RDA</td>
<td>recommended daily allowance</td>
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<tr>
<td>Ra</td>
<td>rate of appearance</td>
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<tr>
<td>REE</td>
<td>resting energy expenditure</td>
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<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
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<tr>
<td>TTR</td>
<td>tracer to tracee ratio</td>
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<tr>
<td>VCO₂</td>
<td>carbon dioxide production</td>
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<tr>
<td>VO₂</td>
<td>oxygen consumption</td>
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<tr>
<td>WBPB</td>
<td>whole-body protein breakdown</td>
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<td>WBPS</td>
<td>whole-body protein synthesis</td>
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Clinical relevance of negative protein balance

In contrast to carbohydrates and fat, the human body has no storage pool of non-functional proteins that can be recruited in situations of extra demand. Proteins are present as structural compounds (*e.g.* organ tissue, muscle) or in a soluble form (*e.g.* hormones, acute phase proteins). Degradation of body proteins for the recruitment of amino acids (AAs) causes subsequent loss of organ function or of the organism as a whole if they are not restored. If loss of total body protein mass exceeds 40%, death is inevitable (1, 2).

In both acute and chronic disease states, negative protein balance with loss of lean body mass (LBM, *i.e.* the fat-free fraction of total body mass) has detrimental effects on short-term and/or long-term clinical outcome. In critically ill patients, cumulative negative protein-energy balance with loss of LBM is associated with an increased incidence of infections, less ventilator-free days, a longer time of recovery to normal physiological functions resulting in increased length of stay (LOS) in the intensive care unit (ICU) (3, 4). Conversely, a malnourished state increases the risk of respiratory infections, hospitalization or long-term institutional care, and is associated with an increase in economic burden (5, 6). Also in pediatric ICU patients, ongoing proteolysis and loss of protein mass is associated with a higher risk of infections, persisting critically ill condition, and increased LOS in the PICU (7, 8). Infants and children are particularly susceptible to developing negative protein balance, due to greater baseline requirements for linear growth and development of visceral organs (9). The effects of negative protein balance during PICU stay can be long lasting in children; anthropomorphic consequences of clinical malnutrition can be measured even up to 6 months following discharge from a PICU (10).

Also, in chronic disease states, protein-energy malnutrition (PEM) with subsequent loss of LBM has a negative effect on outcome (11, 12). This is increasingly relevant to the pediatrician, since successful therapeutic strategies have improved overall mortality rates of specific childhood diseases at the expense of a growing population of chronically ill children. Approximately 500,000 children in the Netherlands are diagnosed as being chronically ill (13). Cystic fibrosis (CF) is a typical example of one such chronic disease, where improved care has resulted in a tremendous decrease in mortality rate during childhood. Over the past 10–20 years, the mean survival of patients with CF has increased from 20, to over 40 years (14). According to a 2005 report of the CF Foundation approximately a quarter of children with CF are below the 10th percentile weight-for-age and sex (15). Twenty-five to thirty percent of children with CF have depleted LBM with loss of muscle mass (16). In these children, malnutrition has been shown to have adverse effects on respiratory muscle strength, lung function, infection rate, and immune defense mechanisms (17). Emphasis on increasing LBM, rather than body mass index (BMI) by high energy strategies, can improve lung function in pediatric pulmonary disease (18).
Nutritional status in chronic illness affects clinical outcome of critical illness, especially considering that approximately a quarter of the PICU population are already undernourished on admission (10, 19). This is not only the result of nutritional deterioration during the acute illness itself, but is in increasing numbers also a consequence of the chronic underlying disease state (20). Therefore, improvement of protein balance and LBM in children with either critical or chronic illness is paramount, since both disease states become increasingly interlinked in modern medical practice.

**Physiological regulation of protein metabolism**

In the 1930s, stable isotope studies by Schoenheimer *et al* showed for the first time that proteins are continually broken down and resynthesized, and hence that proteins in the body have a dynamic rather than a static nature (21). In healthy children and adults, protein breakdown occurs at a rate of more than double that of daily dietary protein intake (6.7 g/kg/d in newborns and 3.5 g/kg/d in adults, respectively) (22). Approximately 80% of whole-body protein synthesis involves the recycling of AA derived from endogenous protein breakdown (23). Thus, only about 15–20% of protein synthesis results from the exogenous AA in the daily intake. Although this recycling of AA is an energy wasting process, requiring approximately 35% of resting energy expenditure (REE) to fuel basal protein turnover (24), it has the important beneficial effect of amplification of control of protein remodeling with more flexibility in dealing with changing clinical demands (*Figure 1.1*).

![Schematic diagram of amplification effect of recycling of proteins](image)

*Figure 1.1*  Schematic diagram of amplification effect of recycling of proteins. In this example, at higher rates of synthesis and breakdown in B versus A, 50% reduction of breakdown has 3-times amplified effect on balance gain. S, synthesis; Br, breakdown, Bal, balance. Units are arbitrary.
The protein synthesis- and breakdown rates are not equal for all tissues. In fact, there are drastic (up to 30-fold) differences in fractional synthesis rates of proteins between different organs and tissues, and between the cytosolic, nuclear, and mitochondrial compartments of cells (25, 26). The sum of these individual rates is expressed as whole-body protein synthesis or breakdown. It is the overall balance between these determinants that defines loss (catabolism) or gain (anabolism) of total protein mass of an organism (23). Thus a catabolic state can result from an increase in breakdown, a decrease in synthesis, or a combination of these two responses.

Protein synthesis and degradation are affected by various conditions, such as fasting, feeding, exercise, and aging. In vivo, these conditions are closely regulated by hormonal, nutritional, neural, inflammatory, and other influences. Within this extremely complex and dynamic interplay, availability of AAs and the serum concentration of insulin play a dominant role in the regulation of protein metabolism.

The most important driving force behind protein synthesis is the availability of AAs; muscle and visceral tissue protein synthesis is stimulated in a linear correlation within the normal diurnal plasma concentrations of AAs from the direct postprandial to the postabsorptive state (27–29). The maximal anabolic effect of AAs appears between 30 min and 2 hrs following a meal, or subsequent to the start of an in vitro AA infusion in the postabsorptive state (28). The metabolic fate of surplus AAs, exceeding maximal muscle protein synthesis capacity, is oxidation resulting in ureagenesis (28).

As a key anabolic hormone, insulin potently inhibits whole-body, splanchnic, skeletal and heart muscle proteolysis, probably via the ATP-dependent ubiquitin-proteasome proteolytic pathway (25, 30). It appears that in older children and adults proteolysis is more sensitive than protein synthesis to small changes in plasma insulin concentration within its physiological range (25). Whereas in healthy subjects proteolysis is diminished by even a modest increase in serum insulin concentration, protein synthesis is stimulated only by higher concentrations, provided sufficient AAs are present (31–34). In vitro research demonstrates that insulin above a higher threshold concentration stimulates protein synthesis. This occurs via an increased muscular blood flow and hence AA supply, and also by promoting AA-induced mRNA translation (35–37). This effect has been observed in various different types of muscles, but not in visceral tissues (38). The proteolysis-inhibiting effect of insulin diminishes with age (39).

In vitro, insulin and AAs can independently regulate protein synthesis and degradation; however, their respective roles in vivo are more complexly interrelated. As an example, insulin induces hypoaminoacidemia, potentially depriving protein synthesis of substrate, whilst some
AAs or their derivatives (e.g. arginine, lysine, phenylalanine, ornithine, alanine, leucine and isoleucine) can in turn stimulate insulin release (40, 41). Also, sufficient availability of AAs enhances both the proteolytic suppressive effect of insulin and the sensitivity of protein synthesis to insulin (25, 42, 43).

Other hormones also play a role in the complex endocrine regulation of protein homeostasis, of which insulin-like growth factor-1 (IGF-1) and growth hormone (GH) are the most important. In contrast to insulin, IGF-1 stimulates protein synthesis in muscle and whole-body in lower concentrations than those necessary for effects on glucose homeostasis, whereas higher concentrations are required for inhibition of proteolysis (44, 45). GH can stimulate muscle protein synthesis independent of IGF-1, and is essential for linear growth during childhood (46, 47). During fasting, GH inhibits proteolysis and thus preserves body protein (48). The complexity of the endocrine regulation of protein homeostasis is also demonstrated by the fact that GH has both insulin-like (e.g. phosphorylation of the insulin receptor substrate 1), and insulin-antagonistic (mitigation of the anti-proteolytic effect of insulin) properties. These are dependent on the individual’s nutritional status, serum glucose concentration and availability of AAs (25).

Finally, also glucagon, thyroid hormones, glucocorticoids, and gonadal steroids modulate protein metabolism, but these will not be discussed here.

**Stress response to critical illness and surgery**

The overall effect of the acute stress response to injury is to preserve cardiovascular homeostasis, maintain fluid volume, and protect the body from invasion by pathogens. Also, substrates and energy for the synthesis of acute phase proteins, inflammatory peptides and gluconeogenesis are mobilized. In evolutionary terms, it seems likely that this ‘hypermetabolic response’ to stress developed as a survival mechanism which allowed injured animals to sustain themselves without food by using stored body fuels and retaining water and salt until healing had occurred (49). In modern medicine, it is questionable whether this ancient response is beneficial in all circumstances, since ongoing catabolism with negative protein balance contributes to a worsened clinical status (50).

In critical illness and following trauma or surgery, there are three major systems that protect the organism against internal and external insults: the central nervous system, the endocrine system, and the immune system (51). The first two are usually combined and referred to as the neuroendocrine system.
The neuroendocrine response to illness and injury consists of sympathetic autonomic nervous system induced adrenal medullar production of catecholamines and activation of the renin-angiotensin-aldosterone system in the renal juxtaglomerular cells (52). Pituitary-synthesized corticotropin (adreno-corticotrophic hormone, ACTH), via the hypothalamic pituitary-adrenal (HPA) axis, stimulates the adrenal cortical secretion of cortisol, which also increases vascular tone and promotes renal salt retention (50, 52, 53). The posterior pituitary produces arginine vasopressin (AVP) which has a major role in the preservation of circulatory volume as anti-diuretic hormone (ADH) (49).

In the acute phase of the systemic inflammatory response syndrome (SIRS), pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukins (IL) 1, 6 and 8, play a major role in the early (2–4 hrs) response to sepsis and injury (49, 54). They modulate inflammation by inducing production of acute phase proteins, such as C-reactive protein (CRP), fibrinogen, and α2-macroglobulin (49). IL-10 is the key anti-inflammatory cytokine that selectively blocks expression of pro-inflammatory genes and simultaneously enhances expression of anti-inflammatory molecules (e.g. the IL-1 receptor antagonist, via competitive inhibition of the pro-inflammatory IL-1 cytokine receptor) (55).

Also in pediatric cardiac surgery, like in all surgical and trauma patients, SIRS is one of the endogenous factors that can complicate post-operative recovery. In addition to the effect of surgery, during most cardiac surgical procedures there is additional use of the cardiopulmonary bypass (CPB) circuit, which has been shown to further induce complement activation, endotoxin release, leukocyte activation, and cause the release of many pro-inflammatory mediators (49, 56, 57).

The neuroendocrine and immune systems are interrelated through a bidirectional network of hormones and neuropeptides that affect immune function and, in turn, immune responses that are triggered by neuroendocrine changes (Figure 1.2). As an example, TNF-α, IL-1 and IL-6 directly induce activation of the HPA axis, whereas hypothalamic corticotrophin-releasing hormone itself has proinflammatory properties (49, 50, 58). On the other hand, catecholamines and cortisol protect against excessive inflammation via direct anti-inflammatory properties, such as inhibition of the synthesis of prostaglandins (47, 49, 50).

**Metabolic response to critical illness**

The hypermetabolic stress response is characterized by mobilization of nutrients and energy via lipolysis, proteolysis, glycolysis and gluconeogenesis (7, 59, 60). The latter two, together with inflammation-induced insulin resistance and poor carbohydrate utilization,
lead to hyperglycemia, which is associated with increased morbidity and mortality in critically ill adults and children (61–65). The metabolic response to illness and surgery differs from starvation in that it is an insulin-resistant, high-energy state, in contrast to the conservation of LBM and energy during starvation (Table 1.1).

In critically ill adults, REE is increased to fuel the hypermetabolic stress response with sufficient energy. Classification of adult patients into three categories (non-SIRS, non-septic SIRS, and septic SIRS) has been shown to be a valid predictor of metabolic stress, with increasingly elevated REE between groups (66). In critically ill children however, the presence of a hypermetabolic response to stress is less evident. There are studies in different populations of critically ill children that report REE to be 15–50% higher than expected for age and body

Figure 1.2 Immune neuroendocrine interactions. Reproduced with permission from (50).
composition during the peak of the hypermetabolic response (67–69). More recent studies report an absence of increased REE, or even a hypometabolic response to critical illness in children, with a possible correlation between metabolic stress and severity of disease scores and LOS (70–75). In surgical newborns and infants, the hypermetabolic response is only short-lived or is altogether absent, with return to baseline levels within 12–24 hrs (59, 76–79). As an explanation, it has been proposed that in acutely ill or postoperative children, energy may be diverted from growth to fuel the stress response, thus avoiding an overall excessive increase in energy expenditure (78, 79).

In response to trauma and sepsis, increased proteolysis occurs (predominantly in skeletal muscle but also of visceral proteins), for the recruitment of sufficient AAs that can subsequently be metabolized into acute phase proteins, glucose, free fatty acids (FFAs) or ketone bodies (49). In pediatric surgical patients, plasma concentrations of gluconeogenic AAs (alanine and glutamine) are low, whereas the branched-chain AAs (BCAAs; leucine, isoleucine and valine) concentrations are high, suggesting that gluconeogenesis is the driving force behind muscle protein breakdown (9, 80). In critically ill adult patients with multiple organ disease syndrome (MODS), studies using sequential muscle biopsies have shown that proteolysis causes up to 1.5% of muscle fiber area to disappear per day (81). In children who undergo

### Table 1.1  Summary of hormones and substrate response to illness and surgery compared to starvation [adapted from (9)]

<table>
<thead>
<tr>
<th></th>
<th>Starvation</th>
<th>Illness or surgery</th>
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<tbody>
<tr>
<td>Glucagon</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Insulin</td>
<td>↓</td>
<td>↓ then ↑</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>↓</td>
<td>↑ - ↑↑</td>
</tr>
<tr>
<td>Cortisol</td>
<td>↓</td>
<td>↑ - ↑↑</td>
</tr>
<tr>
<td>Skeletal protein breakdown</td>
<td>↑ then ↔/↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>Amino acid oxidation</td>
<td>↑ then ↔/↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>Skeletal protein synthesis</td>
<td>↓↓</td>
<td>↑</td>
</tr>
<tr>
<td>Protein balance</td>
<td>↓</td>
<td>↓ - ↓↓</td>
</tr>
<tr>
<td>Glucose turnover</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Fatty acid turnover</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Ketone body synthesis</td>
<td>↑</td>
<td>↓</td>
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</tbody>
</table>
surgery, urinary nitrogen secretion is increased as a result of protein degradation, and may remain elevated for up to 4 to 5 days following the operation (80). In children with severe burns, muscle breakdown has been observed for even up to 9 mos (82).

In the liver, synthesis rates of acute phase proteins such as CRP and fibrinogen are increased, at the expense of decreased synthesis of selected proteins (e.g. albumin, transferrin, prealbumin, retinol-binding protein, and fibronectin) (83, 84). Simultaneously, protein synthesis in muscles is reduced during critical illness due to low intramuscular precursor concentration and decreased activity of the translation initiation pathway (83, 85). During the metabolic stress response the increase of whole-body proteolysis is greater than the increase in protein synthesis, resulting in a net negative protein balance (69).

Due to increased synthesis of acute phase proteins and immune cells, some conditionally essential AAs such as arginine and glutamine, can become depleted in critical illness and after injury, including burns (86, 87). Arginine is involved in several T-lymphocytic functions, including IL-2 production (88). Glutamine serves as a metabolic substrate for enterocytes and immune cells, thus supporting intestinal barrier function and immune responses. It also serves as a messenger to switch on genes involved in immune regulation (89). Additionally, the aromatic AAs tyrosine has been shown to be conditionally indispensable in critically ill infants and young children (90).

**Negative protein balance in the PICU: causes and therapeutic options**

Critically ill children usually receive less than the recommended protein intake, with 50% of cumulative deficits developing in the first 48 hrs of admission on a PICU (91, 92). As a result, their LBM can deteriorate even further during the admission period, this superimposed on the fact that approximately a quarter of these children are already undernourished on admission (10).

Furthermore, the majority of PICU patients have a negative cumulative energy balance at discharge (91, 93). This is partially a result of incorrectly prescribed calories due to prescriptions often being based on notoriously unreliable predictive requirement equations instead of the actual needs as measured by indirect calorimetry (94–98). Additionally, medications typical in an ICU setting such as inotropic agents, sedatives and muscular blocking drugs, further complicate accurate predictions of the patient’s actual caloric needs (99–103). Other causes of clinical malnutrition are: fluid volume restriction in patients with SIRS or cardiac surgery, partial delivery to the patient of prescribed diets, procedural interruption for which the patient has to be fasted (e.g. in- and extubation, surgery, radiological studies
outside the PICU), gastrointestinal intolerance to feeding, mechanical problems (e.g. feeding tube displacement, pump dysfunction), and absence of a dedicated nutrition support team (104–107). It has become clear that malnourishment during PICU stay can be reduced by early recognition of patients that are at risk, by means of prompt, regular and comparative nutritional assessments (108).

There is a vast body of evidence that stresses the importance of tailoring energy intake in critically ill children based on actual measured, as opposed to predicted total EE to avoid overfeeding, which may lead to diet-induced thermogenesis, increased carbon dioxide production and fatty deposition in the liver (96, 109–113). Measurement of the respiratory quotient ($\frac{V_{CO2}}{V_{O2}}$) can be helpful in differentiating under- and overfeeding, and adequacy of dietary macronutrient composition (113, 114).

Although it is generally agreed that macronutrient utilization in this population differs from that in healthy children, recommended quantities of protein for critically ill neonates and children are not so clear and are based on limited data (115, 116). A recent systematic review, that reviewed 6 interventional trials and 3 observational studies of energy and protein intake on protein balance in critically ill children, has reported that a minimum intake of 57 kcal/kg/d and 1.5 g/kg/d were required to achieve positive protein balance (Table 1.2) (117). The latest international guideline for critically ill children of various age groups advises a protein intake as follows: 0–2 yrs, 2–3 g/kg/d; 2–13 yrs, 1.5–2 g/kg/d; and 13–18 yrs, 1.5 g/kg/d (116). There is evidence that significantly higher protein intake (up to 3 g/kg/d) via an aggressive feeding strategy correlates with better protein balance in children with acute illness (Figure 1.3) (69, 118). To date, it is unknown if these outcome data can be improved with an even higher protein intake.

The role of insulin infusion in improving the negative protein balance in critically ill patients is still under debate. In burn victims, insulin can stimulate muscle protein synthesis, and improve LBM in children (119–121). In children with sepsis however, high AA intake enhances protein synthesis, whilst insulin infusion-induced hyperinsulinemia does not have an additional positive effect on protein balance (122). Insulin, as opposed to glucose, has anti-inflammatory effects, and can, both via direct action and by reversing hyperglycemia, mitigate inflammation and thus catabolism. Hyperglycemia induces mitochondrial dysfunction with subsequent organ failure (123). In a large study of critically ill infants and young children in a mixed PICU with 85% surgical patients, insulin-induced tight glycemic control reduced inflammation as expressed by plasma concentrations of CRP, LOS in the PICU, and overall mortality by 3% in the study group, compared to the controls (124). However, the authors observed severe hypoglycemia < 2.2 mmol/L in 25% of patients in the tight glycemic control group, compared to
Table 1.2 Overview of protein balance studies, interventional trials

<table>
<thead>
<tr>
<th>Study, design, class/rating</th>
<th>Number of subject, population, age group, feeding route</th>
<th>Intervention</th>
<th>Energy intake, kcal/kg/day</th>
<th>Protein intake, g/kg/day</th>
<th>Protein balance, g/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botran et al, 2011 Randomized controlled trial</td>
<td>n = 41 73% postcardiac surgery Median age 7 mos (75% &lt; 1 y) EN</td>
<td>Protein-enriched vs standard age-appropriate formula over 5 d</td>
<td>Day 1 median S, 61.9; median HP, 65.1  Day 3 median S, 68.4; median HP, 74.2  Day 5 median S, 67.5; median HP, 76.6</td>
<td>Day 1* median S, 1.5; median HP, 2.6  Day 3* median S, 1.7; median HP, 2.7  Day 5 median S, 1.5; median HP, 3.1</td>
<td>Day 1 median S, -1.2; median HP, 0.6  Day 3 median S, -0.1; median HP, -0.2  Day 5 median S, -0.4; median HP, 0.5</td>
</tr>
<tr>
<td>Briassoulis et al, 2005 Randomized controlled trial</td>
<td>n = 50 Respiratory failure, sepsis, severe head injury Age: 103 (48) mos EN</td>
<td>EN with glutamine, L-arginine, ω-3 fatty acids, fiber, vitamin E, betacarotene, Zn, Cu, Se, protein vs standard formula</td>
<td>After 5 d: HP, 58 (35); S, 64 (30)</td>
<td>After 5 d: HP, 2.6 (2); S, 2.2 (0.8)</td>
<td>After 5 d: HP, 0.44 (2.19); S, -0.38 (1.25)</td>
</tr>
<tr>
<td>Briassoulis et al, 2006 Randomized controlled trial</td>
<td>n = 40 Severe head injury Age: 120 (51) mos EN</td>
<td>EN with glutamine, L-arginine, ω-3 fatty acids, fiber, vitamin E, betacarotene, Zn, Cu, Se, protein vs standard formula</td>
<td>Mean after 5 d: HP, 57; S, 62</td>
<td>Mean after 5 d: HP, 2.5; S, 2.2</td>
<td>Mean after 5 d: HP, 0.44; S, -0.38</td>
</tr>
<tr>
<td>Chaloupecky et al, 1997 Randomized controlled trial</td>
<td>n = 37 Congenital heart surgery Age: 6.7 (3.4) mos PN</td>
<td>PN at postoperative day 1 vs stand IV fluids</td>
<td>Nonprotein kcal: PN, 33 (9); S, 25 (15)</td>
<td>PN, 0.8 (0.1) S, 0</td>
<td>At postoperative day 1*: PN, -0.71 (0.51); S, -1.53(0.54)</td>
</tr>
<tr>
<td>Van Waardenburg et al, 2009 Randomized controlled trial</td>
<td>n = 20 RSV Age: 2.9 (1.7) mos EN</td>
<td>Protein-enriched infant formula vs standard formula over 5 days</td>
<td>On day 5*: HP, 112 (37); S, 82 (13)</td>
<td>On day 5*: HP, 2.8 (0.8); S, 1.5 (0.3)</td>
<td>On day 5*: HP, 1.86 (0.73); S, 0.77 (0.46)</td>
</tr>
<tr>
<td>Briassoulis et al, 2002 Noncontrolled trial</td>
<td>n = 71 25% sepsis, 41% brain injury, 13% respiratory failure, 10% neuromuscular, 11% burns Median age 54 mos (range, 2-204 mos) EN</td>
<td>EN starting within 12 hrs after admission</td>
<td>Paired samples*:  Day 1, 22 (9.3); Day 5, 66 (22.8)</td>
<td>Paired samples*:  Day 1, 0.69 (0.25); Day 5, 1.9 (0.59)</td>
<td>Paired samples*:  Day 1, -1.63 (1.06); day 5, 0.19 (1.06)</td>
</tr>
</tbody>
</table>

EN, enteral nutrition; HP, high protein; RSV, respiratory syncytial virus; S, standard group. All values are presented as mean (SD), unless otherwise noted.*p < 0.05. [From (117)].
1% in the usual care group (124). At 4 years follow-up, there were no differences in mortality or neurocognitive development between groups (125). In adult critically ill patients, insulin-induced tight glycemic control, although potentially beneficial for a subgroup of surgical patients, significantly increased the risk of hypoglycemia and conferred no overall mortality benefit (126). Therefore, glucose infusion-induced hyperinsulinemia, as opposed to infusion of insulin, might be a safer option. At present, there is no evidence that this strategy can improve protein balance, prevent hypoglycemia, and mitigate inflammation at the same time.

Within the realm of nutritional therapy of critically ill children, there is no compelling evidence for immunonutrition, i.e. the modulation of the inflammatory response by immune-modulating nutritional components. In this field, supplementation to critically ill children of the semi-essential AAs glutamine, arginine, glycine and omega-3 polyunsaturated fatty acids and nucleotides have been studied most extensively, since they are involved in the immune response (127–132). All studies showed various immune-modulating properties of these nutritional components, but without effect on clinically relevant outcome parameters, partly due to poor study design (133). The latest A.S.P.E.N. guideline on nutrition in critically ill children, based on the available pediatric data, does not recommend the routine use of immunonutrition or immune-enhancing diets/formulas (116).

![Graph](image.png)

**Figure 1.3** Protein balance associated with corresponding level of protein intake in critically ill children (Spearman $r = 0.729$; $p = 0.011$). Reproduced with permission from (117).
Cystic fibrosis as a model of chronic protein malnutrition

As stated earlier, 25 to 30% of children with CF have LBM depletion with loss of muscle mass (16). Historically, since the classical 1980s observational study of CF patients in Toronto and Boston that reported that individuals with high-calorie-high-fat diets were taller and weighed more, emphasis of nutritional strategies in CF has been on caloric rather than protein intake (134). Even to date, ‘high-fat-high-energy’ diets with use of pancreatic enzyme replacement therapy in pursuit of better control of malabsorption is considered the standard of care (14). High-energy intake in CF patients can improve weight and BMI, and is associated with better pulmonary function (135, 136). Also, greater weight-for-age in the peripubertal period is associated on average with improved tempo and timing of pubertal linear growth (137).

Although weight gain per se is important, maintenance of normal muscle mass may be closely connected with both normal growth and good pulmonary function in CF children (138). There are several reports of increased protein catabolism in stunted children with CF (139–141). In infants, protein malnutrition can even be the presenting symptom (142). Some studies show that improvement of LBM, as opposed to fat mass, improves respiratory function and outcome (18, 143). Therefore, pediatric CF is both a relevant and suitable model for studying the effects on protein balance of different levels of dietary protein intake.

Despite the fact that oral protein energy supplements are widely prescribed for patients with CF to improve energy intake and nutritional status, there are no evidence-based recommendations for optimal daily protein intake (135, 144). A multicentre randomized controlled trial in 102 moderately malnourished children with CF failed to show any effect on nutritional status or linear growth after 1-y use of oral protein energy supplements, despite a trend towards increased mid-arm muscle circumference in the supplemented group (145). The supplements provided 18% in excess of usual diet, but the authors did not report actual daily protein intake. Only recently, a pediatric study using a stable isotope technique in a limited number of children with CF showed increased whole-body protein synthesis and balance after feeding with a leucine-rich essential AA mixture, compared to a isonitrogenous balanced AA mixture (146). To date, in pediatric CF, there are no dose-effect studies of the relationship between normal to high protein intakes and protein synthesis, breakdown and net balance.

Stable isotope technique [based on (23) and (147)]

Dynamic assessment of whole-body protein turnover can be performed by kinetic tracer analysis, by using stable isotopes of precursors (AAs) or end products (e.g. urea) of protein metabolism. In contrast to mere examination of the nitrogen balance (the difference between
nitrogen intake and mostly urinary output), stable isotope technique is able to quantify separate protein synthesis and breakdown rates.

Isotopes are chemically identical to the original compound with equal biological properties, but have a higher molecular weight due to one extra neutron (e.g. $^{15}$N-glycine versus $^{14}$N-glycine). Stable isotopes have no spontaneous decay and are therefore not radioactive, and are thus safe and ethically justifiable for use in clinical studies and also in children (148). Depending on the isotope, there is a certain percentage of natural abundance of stable isotopes in our biosphere (background enrichment). For research purposes commercially available synthetic stable isotopes are used.

Isotopic tracers are administered as either a very low dose single bolus or a continuous infusion, after which they are assumed to take part in all metabolic pathways of the original compound (tracee) with the same metabolic rate. Thus, in the case of a continuous tracer infusion, isotopic equilibrium will be achieved over time if the rate of appearance of tracer/tracee into the pool is equal to the rate of disappearance from the pool (usually blood). Once this isotopic equilibrium is achieved, the various other pools become irrelevant in a stochastic model, in which all metabolic pools other than blood are considered as one intracellular pool.

In a state of isotopic equilibrium samples from the blood pool can be obtained and tracer to tracee ratio (TTR) can be measured using mass spectrometry (MS) and isotope ratio mass spectrometry (IRMS), with a respective precision of 0.2% and 0.002% (149). In studies with single bolus administration the same general principles apply as when constant infusion is used, but in this case isotopic enrichment is described as a single exponential function of clearance of the tracer from the pool.

In studies of whole-body protein synthesis and breakdown, usually stable isotopes of essential AAs (e.g. $^{13}$C-valine, $^{13}$C-leucine or $^{13}$C-phenylalanine) are used. When the tracer and tracee of an essential AA appear in the blood compartment, this can only be the result of invasion from an external pool (intravenous infusion or enteral absorption from the diet), or endogenous protein breakdown (B), since the body is unable to newly synthesize essential AAs:

$$\text{Ra} = \text{I} + \text{B} = \text{Q} \quad [1]$$

In this equation Ra is rate of appearance (synonyms: turnover or flux; Q). In the case of parenteral nutrition (PN), I is equal to the infusion rate of the studied AA. In enterally fed subjects (enteral nutrition, EN) AAs are taken up by the gut, and then either retained by the gut, released by the gut and taken up by the liver on the first pass, or released into the systemic circulation. Since access to the portal vein is usually not possible in human studies,
it is not possible to distinguish between retention of AAs in the gut and first-pass clearance of AAs by the liver. In this situation, I (equation 1) represents the net resulting appearance of AAs in the circulation after this so called first-pass splanchnic uptake.

Thus, endogenous protein breakdown equals the rate of appearance of the studied AA minus its exogenous infusion (PN) and/or absorption (EN):

\[ B = Ra - I \]  

In an isotopic state of equilibrium the rate of appearance equals the rate of disappearance (Rd) of the tracer and tracee from the pool:

\[ Ra = Rd \]

AAs can leave the blood pool via the pathway of degradation, with formation of urea (and to a lesser extent ammonia) from the amino group, and oxidation of the carboxyl group with the formation of carbon dioxide, or via the pathway of protein synthesis.

By using a so-called end product isotope (\(^{13}\)C-urea or \(^{15}\)N2-urea), urea formation can be measured and a nitrogen balance can be calculated by subtracting measured urea-nitrogen production from known dietary nitrogen intake. By using a \(^{13}\)C-tracer, the amount of exhaled \(^{13}\)CO\(_2\) and with the formation of a \(^{13}\)C-ketoacid (e.g. \(\alpha\)-ketoisocaproate in case of leucine) as the first irreversible step in AA oxidation can be measured, and thus the total amount of oxidation of the studied AA can be calculated (Figure 1.4). Before using this technique, the large bicarbonate pool of the body needs to be primed with \(^{13}\)C-bicarbonate, in order to

**Figure 1.4** Model for calculating whole-body synthesis and breakdown using the infusion of \(^{13}\)C-leucine and measurement of \(\alpha\)-ketoisocaproate enrichment as a reflection of intracellular leucine enrichment and as the precursor for leucine oxidation. Reproduced with permission from (23).
detect the production of $^{13}\text{CO}_2$ above plateau. Also, a correction factor is needed to adjust for $^{13}\text{CO}_2$ retention in the body (e.g. in the gluconeogenesis pathway).

Thus, the contribution of an essential AA to whole-body protein synthesis can then be defined by its non-oxidative disposed (NOD) portion:

$$S = Rd - \text{NOD} \quad \text{[4]}$$

Net balance between protein synthesis and breakdown, relative to AA contribution to these processes, can be calculated by:

$$\text{Balance} = S - B \quad \text{[5]}$$

Finally, when the average relative contribution of the studied AA to the whole-body protein pool is known, whole-body protein synthesis and breakdown rates can be calculated.

The major drawback of whole-body studies is that a certain stimulus or clinical condition might induce opposite reactions in different tissues or proteins, leading to the lack of a net observed effect on the whole-body level (149).

**Outline of this thesis**

The main objective of this thesis was to investigate if whole-body protein synthesis and net balance in both acutely and chronically ill children can be improved with a much higher (5 g/kg/d, high protein; HP) than normal (2 g/kg/d, normal protein; NP) daily protein intake. Additionally, we studied the effects of insulin and protein intake on the inflammatory response in these patient groups. As a homogenous population of acutely ill children, we selected infants following cardiac surgery for congenital heart disease that had been supported by a CPB circuit during surgery. We intentionally did not include other conditions of critical illness, as we wanted to standardize our study population as much as possible. As a model for a pediatric chronic disease state with protein malnutrition, we selected prepubertal patients with CF in a stable phase of their disease.

For reference, in **Chapter 2** we mimic the common practice of limited intake of surgical patients by high-carbohydrate/low-protein (LP) diets in the direct post-surgical phase of pediatric cardiac surgery. We report the effect on whole-body protein synthesis, breakdown and balance of a carbohydrate-induced hyperinsulinemia in these patients. In **Chapter 3** we describe the results of a study in this pediatric cardiac surgical population after the effects of short-term HP versus NP/iso-carbohydrate intake on whole-body protein balance and the neuroendocrine and inflammatory response.
In Chapter 4 we show the results of a study of the effects on whole-body protein synthesis, breakdown and net balance of LP, NP and HP/iso-carbohydrate intake in children with CF. We also discuss the effects of these diets on the neuroendocrine response to illness in this group.

In anticipation of the finding that it is important to deliver adequate protein intake in a timely fashion to critically ill children, we studied prescription and delivery of calories and protein in our tertiary PICU (Chapter 5), and the effect of the institution of a nutritional support team on actual energy and macronutrient intake in our patients (Chapter 6).

In Chapter 7, the results of the studies presented in this thesis are summarized and arranged in a broader perspective, with suggestions for future research.
REFERENCES


