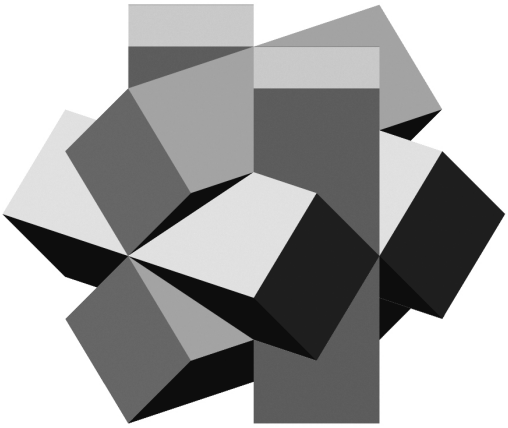
PROTEIN ANABOLISM IN CRITICALLY ILL CHILDREN

Pathophysiological aspects and interventional challenges

Carlijn de Betue



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The study as presented in chapter 6 and 7 and a part of chapter 3 was financially supported by Nutricia Advanced Medical Nutrition, Zoetermeer, The Netherlands. The grant supplier had no involvement in the study design, collection, analysis and interpretation of data and decision to submit reports for publication.

Printing of this thesis has been financially supported by
Erasmus Universiteit Rotterdam
J.E. Jurriaanse Stichting
Nutricia Advanced Medical Nutrition
Nestlé Nutrition
Covidien Nederland B.V.

ISBN: 978-94-6169-278-8

Cover and graphic design by Roeland de Betue
Layout and printing by Optima Grafische Communicatie, Rotterdam, The Netherlands

PROTEIN ANABOLISM IN CRITICALLY ILL CHILDREN

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EIWITANABOLISME IN KRITISCH ZIEKE KINDEREN

Pathofysiologische aspecten en zoektocht naar geschikte interventies

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
donderdag 6 september 2012 om 13.30 uur

door

Carlijn Tini Ireen de Betue

geboren te Leidschendam

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. D. Tibboel
	Prof.dr. N.E.P. Deutz
Overige leden:	Prof.dr. A.B.J. Groeneveld
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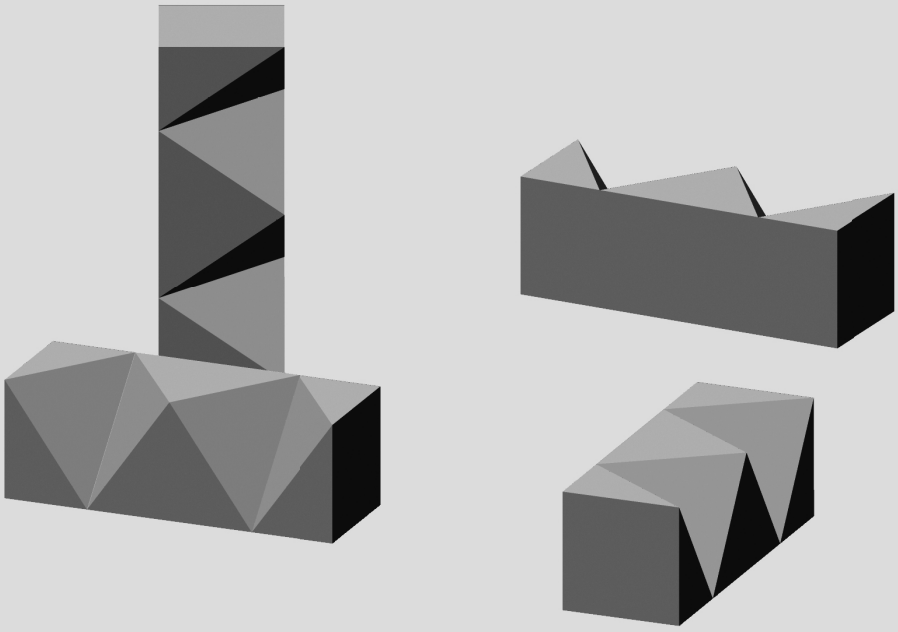
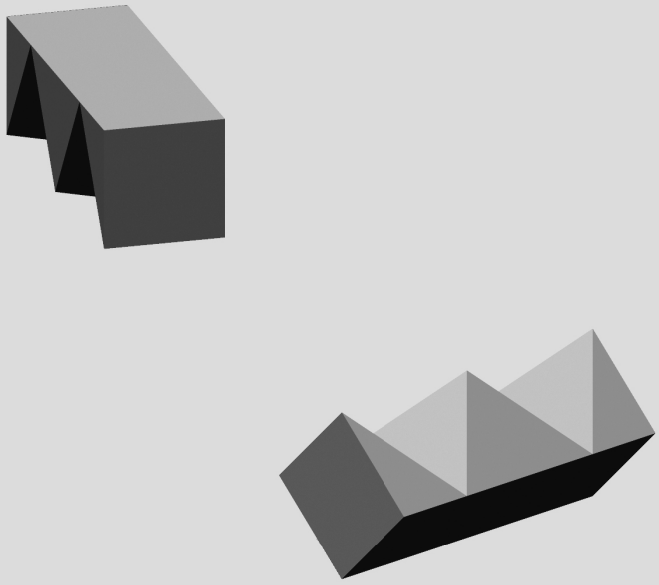


PART 1

GENERAL INTRODUCTION

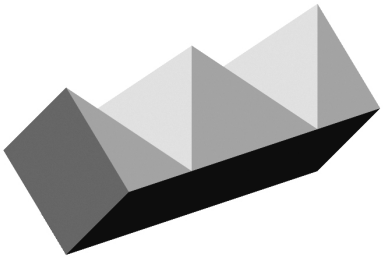


Let food be thy medicine
and medicine be thy food.

Hippocrates (460-377 BC)

chapter 1

GENERAL INTRODUCTION & AIMS



LIST OF ABBREVIATIONS

ICU	Intensive Care Unit
N	Nitrogen
N-balance	Nitrogen balance
NO	Nitric oxide
PICU	Pediatric Intensive Care Unit
RQ	Respiratory Quotient
SIRS	Systemic inflammatory response syndrome
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption
WHO	World Health Organization

METABOLIC CHANGES DURING CRITICAL ILLNESS

Critical illness can be defined as "a life threatening medical or surgical condition usually requiring intensive care unit (ICU) level care" [1]. It mostly results from infection, sepsis and trauma (including surgery and burns). These conditions are accompanied by similar physiological and biochemical responses, which have been termed the systemic inflammatory response syndrome (SIRS) [2]. The associated major metabolic changes are also known as the acute stress response. From an evolutionary point of view these responses are required for the "fight, flight, fright" reaction when encountering a thread, to mobilise fuels for tissues that are activated [3-5]. A key feature is increased sympathetic nervous system activity, resulting in increased levels of adrenaline and glucocorticoids. Subsequently, immune cells are activated and pro-inflammatory cytokines secreted, which trigger further metabolic changes. In addition, insulin secretion is increased as well as the counter regulatory hormones glucagon, cathecholamines, cortisol and growth hormone. As a result, glucose production is increased *via* increased glycogenolysis and gluconeogenesis and insulin resistance develops, leading to hyperglycemia. Also, fat is mobilised (lipolysis) and fat oxidation and ketone body formation are increased, while muscle protein breakdown is stimulated to provide amino acids for protein synthesis in proliferating cells, the production of acute phase proteins and other peptides (e.g. cytokines) and for gluconeogenesis. Thus, protein turnover is increased, with both increased protein breakdown and protein synthesis. Protein synthesis, however, is stimulated to a lesser extent than protein breakdown, resulting in net protein loss, i.e. protein catabolism. In addition, the increased substrate cycling results in increased energy expenditure, because both protein synthesis and breakdown consume ATP.

Whereas the acute stress response is needed to overcome a threat in the initial phase, prolongation of the stress response may become detrimental to the individual. Hyperglycemia damages tissues, especially those with insulin-independent glucose uptake, such as the central nervous system [3]. In clinical studies, hyperglycemia has been associated with increased morbidity and mortality in critically ill children [4-6]. To limit these detrimental effects, (intensive) insulin therapy is nowadays used in the pediatric intensive care unit (PICU), although discussion remains about what the targeted blood glucose levels should be. A large randomized controlled trial in a PICU showed that intensive insulin therapy with tight blood glucose targets (2.8-4.4 mmol/L in infants and 3.9-5.6 mmol/L in children) led to decreased morbidity and mortality [7], but at the expense of potentially dangerous episodes of hypoglycemia (≤ 2.2 mmol/L), which were found in 25% of the patients. Our research group, as well as others therefore advocates less tight blood glucose targets, starting insulin therapy at blood glucose levels of ≥ 8.0 mmol/L [8, 9].

The prolonged catabolic state of protein and fat metabolism is unfavourable and will result in loss of body protein and body fat. Major losses of protein are associated with increased morbidity and mortality in critically ill children [10]. Loss of body muscle mass is associated with profound respiratory muscle weakness and may lead to difficulties to wean patients from

mechanical ventilation [11]. Also, protein-energy malnutrition in children is associated with reduced cardiac muscle mass and impaired cardiac function [12], and the metabolic alterations during critical illness may contribute to ICU acquired weakness of skeletal muscle [13]. In addition, malnourished critically ill children may become more susceptible to infection [14] and protein malnutrition may lead to delayed healing of wounds [15, 16]. It is essential to provide sufficient nutrients in an optimal mixture to support critically ill children under these conditions.

NUTRITIONAL SUPPORT DURING CRITICAL ILLNESS IN CHILDREN

Importance of adequate nutritional support

In light of the metabolic changes with increased and specific needs during critical illness and detrimental effects of prolonged catabolism, adequate nutritional support is an important aspect of critical care in children. In childhood, an anabolic state is required for growth and brain development. Malnutrition in critically ill children has been associated with increased morbidity and mortality [17]. In non-critically ill children a history of malnutrition, especially in the first 2 years of life, is associated with shorter status and both short term and longer-term cognitive problems, (aggressive) behavioural problems and impaired work habits [18-22]. One of the most important aims of nutritional support in children at the intensive care therefore should be to limit protein catabolism as much as possible and to achieve protein anabolism as early as possible to prevent malnutrition and to diminish delays in growth and neurodevelopment.

Not only does malnutrition result from catabolic processes during intensive care admission and inadequate nutritional intake, in critically ill children upon admission to the ICU, acute or chronic malnutrition is already present in 24% of the children and is more common in children with an underlying disease [23]. In order to facilitate catch-up growth proportionally higher protein intakes as compared to energy intakes are required [24].

Components of nutritional support

As long as nutritional intake is not established, endogenous glucose production is essential to maintain glucose homeostasis to provide glucose dependent tissues with fuel. If endogenous glucose production is not sufficient and no exogenous glucose is provided, there is a high risk to develop hypoglycemia. In children, especially in the young, this is a potential serious complication, which may impair neurological outcome [25, 26]. Endogenously, glucose is provided from glycogenolysis - the mobilisation of glucose from its storage as glycogen in liver and muscle - and gluconeogenesis, which is the formation of glucose from other sources (lactate, amino acids and glycerol). However, glycogen stores in children are limited and gluconeogenesis may not meet glucose utilization in young children [27]. Hence, it is standard care to provide glucose-containing fluids in critically ill children. As second purpose glucose

delivers energy, which, although by far not sufficient to meet energy needs, provides initial nutritional support until (enteral) nutritional intake can be constituted. On the other hand, providing intravenous glucose during the acute stress response may iatrogenically contribute to the stress-response-induced hyperglycemia. This in turn, may require insulin therapy to reduce blood glucose levels, with hypoglycemia as potential serious side effect. Indeed in the large randomized controlled trial on intensive insulin therapy in critically ill children mentioned above, substantial amounts of glucose were provided (~5-7 mg/kg/min on average), hyperglycemia was highly prevalent and insulin therapy was required in the conventional group (> 11.9 mmol/L) in 46% of patients and in the intensive insulin therapy group (> 4.4 mmol/L in infants and > 5.6 mmol/L in children) in 99% of patients. When intensive insulin therapy was used, hypoglycemia was frequently observed [7]. Guidelines on glucose intake in children are based on limited data [28] and may not be appropriate for hyperglycemic states. Another approach could be to provide a lower exogenous glucose intake during the initial phase of the stress response, e.g. 2.5 mg/kg/min. However, as less energy is provided this may result in increased protein catabolism to provide amino acids as energy source. To date, no studies have investigated whether this approach would be feasible in critically ill children to prevent both hyper- and hypoglycemia and what the effects on protein metabolism are.

Whenever enteral nutrition is tolerated, it is the preferred route of nutrition. It supports both the functional and the structural integrity of the gut, through maintaining tight junctions between intra-epithelial cells, stimulating blood flow, inducing release of trophic endogenous agents, maintaining villous height, supporting the mass of secretory IgA-producing immune cells of the gut-associated lymphoid tissue (GALT) and contributing to mucosal-associated lymphoid tissue (MALT) [29]. Very early enteral nutrition (within hours) has been proposed to benefit the acutely injured patient, initiating an early anabolic response and leading to improved outcome as shown by animal studies [30, 31] and in human subjects with burns [32]. Guidelines recommend to start enteral nutrition as soon as possible after admission, preferably within 24-28h after admission [33].

When enteral nutrition is contra-indicated or insufficiently tolerated, parenteral nutrition may be used to supplement or replace enteral nutrition [33]. Recently in adults a large randomized controlled study evaluated whether early parenteral nutrition to supplement enteral nutrition if energy goals are not met, was more beneficial than initiating supplemental parenteral nutrition after 1 week [34]. It appeared that the late initiation resulted in reduced morbidity as compared to the early initiation. No such comparisons have been done in children, but results may differ in children, because they have less energy reserves and a shorter acute stress response [35]. However, this study [34] generated much critical perspective and discussion remains whether it is of benefit to meet energy requirement goals with both enteral and parenteral nutrition [36-41]. Critical in this discussion is the fact that protein intake should be sufficient. Guidelines do not specifically recommend when parenteral nutrition should be initiated in critically ill children, because no data is available in this age group [28, 33].

PROTEIN REQUIREMENTS

Definition and currently available data on protein balance in critically ill children

In general, dietary protein requirements are defined by the World Health Organization (WHO) as "*the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health*" [24]. Thus in adults the minimal protein requirement is the amount of protein needed to achieve a neutral protein balance. However, in children the minimal protein requirement is the protein intake that results in a neutral protein balance but does not allow growth. The optimal protein requirement in children is the amount of protein intake that results in an anabolic state with a normal growth rate and optimal organ (e.g. muscle) function. [42]. In the recovery phase after (critical) illness, protein requirements will be even higher to achieve catch-up growth. It is generally accepted that protein requirements to achieve protein accretion in critically ill children are higher than in healthy children [33]. However, no clear recommendations are made in guidelines for critically ill children, because of insufficient data [28, 33]. In **Table 1.1** recommendations of American and European (parenteral) guidelines are summarized. These guidelines are partly based on the few studies with only small numbers of subjects from which protein or nitrogen balance (N-balance) at different protein intake levels are available.

Table 1.2 summarizes these studies on the effect of protein intake on protein metabolism in critically ill children. Most studies used nitrogen balance to determine whether protein intake was sufficient and stable isotopes have been used sporadically. Furthermore, the majority of the studies were observational and did not comprise randomized protein intake.

Table 1.1 – Recommendations on protein intake.

A.S.P.E.N. [33] Guidelines on nutrition support in critically ill child		ESPGHAN/ESPEN/ESPNIC [28] Guidelines on parenteral nutrition in children	
Age group	Protein (g/kg/d)	Age group	Parenteral amino acids (g/kg/d)
		Preterm infants	1.5-4.0
0-2 years	2.0-3.0	Term neonates	1.5-3.0
2-13 years	1.5-2.0	2 months – 3 years	1.0-2.5
13-18 years	1.5	3-18 years	1.0-2.0
		Critically ill children	Up to 3.0 g/kg/d

A.S.P.E.N., American Society for Parenteral and Enteral Nutrition; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; ESPEN, Europen Society for Clinical Nutrition and Metabolism; ESPNIC, European Society of Paediatric and Neonatal Intensive Care.

Table 1.2 – Studies on protein intake and protein balance in critically ill children.

Study	Patient population	Protein intake	Benefit	Method
Coss-Bu 1998 [45] and Coss-Bu 2001 [20]	Critically ill children (n=33, 0.4-17 years)	1.74 ± 0.78 g/kg/d 2.8 vs 1.7 g/kg/d	Increased energy intake associated with increased N-balance. Positive balance at 2.8 g/kg/d; negative balance at 1.7 g/kg/d protein intake	N-balance
De Klerk [46]	Critically ill children (n=18; 0.3-4.4 year)	1.9 g/kg/d	Equilibrates N-excretion	N-balance
Joosten [47]	Critically ill children (n=36; 0.02-13 year)	2.2 vs 0.9 g/kg/d	Positive vs negative N-balance	N-balance
Briassoulis 2005 [48]	Critically ill children (n=50; 8.6 ± 0.8 (SEM) year)	2.6 (Immunonutrition) vs 2.2 (Standard) g/kg/d	Positive N-balance	N-balance
Briassoulis 2006 [49]	Severe head injury (n=40; 10 (6-10.5) year)	2.5 (Immunonutrition) vs 2.2 (Standard) g/kg/d	More patients achieved positive N-balance with immunonutrition	N-balance
Chaloupecky [50]	Cardiac surgery (n=37; 0.17-1 year)	0.8 vs 0 g/kg/d (PN)	Less negative N-balance	N-balance
Botran [51]	Cardiac surgery (73%), airway surgery, medical (n=41; median 7 (IQR 3-13) months)	Day 5: 3.1 vs 1.5 g/kg/d	Positive vs. negative N-balance at day 5	N-balance
Reynolds [52]	Critically ill post-surgery (gastroschisis) (n=13; 2-3 days)	2.5 vs 1.5 g/kg/d (PN)	Higher N- and protein balance	N-balance, stable isotopes (^{13}C-Leu)
Verbruggen [53]	Critically ill adolescents (n=9; 15 ± 1.2 (SD) year)	High AA PN (3.0 g/kg/d) vs Low AA PN (1.5 g/kg/d)	Higher protein balance	Stable isotopes (^{13}C-Leu)

^{13}C-Leu, L-[1-^{13}C]Leucine; AA, amino acids; N-balance, nitrogen balance; PN, parenteral nutrition.

In addition, no studies on the effect of different protein intakes on clinical outcome parameters have been conducted in critically ill children. These studies are warranted though.

Bechard et al. performed a systematic literature search on the effect of protein intake on protein or N-balance in mechanically ventilated children and found 9 studies reporting protein balance in children requiring mechanical ventilation [43]. Positive N-balance was reported in 6 of the included studies, with a wide range of associated energy and protein intakes. Measures of central tendency for daily energy and protein intakes were significantly correlated with positive protein balance. A minimum intake of 57 kcal/kg/day and 1.5 g protein/kg/day were required to achieve positive protein balance. It was concluded that no uniform protocol has been used to determine N-balance and that there is a paucity of interventional studies.

Methods to assess protein requirements

Different methods exist to study protein requirements on a biochemical level. The most frequently used methods, which are also used in this thesis, will be summarized below.

Plasma amino acid concentrations

Plasma amino acid concentrations are relatively simple to assess by measurement in blood. Amino acids appear in plasma (rate of appearance) from dietary protein intake, amino acid release from body protein breakdown and *de novo* amino acid synthesis. Amino acids disappear from plasma (rate of disappearance) by utilization for protein synthesis, (irreversible) oxidation, conversion to other components such as neurotransmitters and loss from the body e.g. in urine and feces (the latter losses are negligible) [44].

Plasma amino acid concentrations are the resultant of disposal capacity in relation to the rate of appearance into the plasma pool. Decreased or increased plasma amino acid concentrations usually only indicate alterations in the protein metabolic status, but do not have any relation with the flux of amino acids in or out of the plasma pool. It should be kept in mind that the plasma amino acid pool is very small as compared to the intracellular amino acid pool, which in turn is small as compared to the protein-bound amino acid pool [44]. Hence, plasma amino acid concentrations are often considered to be difficult to interpret. It has been proposed, though, that as long as the physiologic situation is taken into account that plasma amino acid concentrations can provide valuable information [44].

Nitrogen balance

Nitrogen (N) balance studies involve determination of the difference between the intake of nitrogen and the amount excreted in urine and feces and obligatory losses from sweat and other routes. In most studies only the N content of the diet, urine, and feces are directly measured and obligatory losses estimated [54]. Therefore precautions are warranted for interpretation of results. The adequacy of this method has also been questioned as N-balance adapts slowly to changes in protein intake; therefore, a long period of study diet intake is required.

Furthermore, there is much discussion about the estimations of obligatory losses of nitrogen [55]. N-contents of protein vary, but in average 16% of protein mass is nitrogen, therefore the conversion factor is 6.25 to convert nitrogen to protein mass.

Stable isotope tracer studies

In recent decades stable isotope tracer methods have become the reference method to determine protein balance. Stable isotopes are naturally abundant non-radioactive variations of atoms, containing a higher number of neutrons in the core than the "normal" variation and therewith have a higher mass than the "normal" variation. During stable isotope tracer studies, a molecule of interest (e.g. glucose or an amino acid) containing the stable isotope (the "tracer") is administered to the subject in a higher amount than the natural abundance. It is assumed that the stable isotope does not influence the metabolic characteristics of the molecule. Hence the tracer will participate in all metabolic processes similar to the molecule of interest (the "tracee"). The ratio of the tracer to the tracee is determined in plasma or urine with mass spectrometry, using its characteristic of a higher mass. Since the tracer is administered at a known rate, fluxes of the tracee can be calculated using specific models and equations.

To determine protein requirements with stable isotopes, protein balance studies are required that use an stable isotope form of an amino acid. These methods rely on the components of the rate of appearance and rate of disappearance of amino acids into the plasma pool, as described above. The basic concept is that for the amino acid that is used as a tracer (an essential amino acid, usually leucine or phenylalanine; these are not synthesized *de novo* in the body) the rate of appearance consists of protein intake and release of amino acids from protein breakdown and the rate of disappearance consists of amino acid oxidation and utilization for protein synthesis. Under steady state conditions, the rate of appearance is equal to the rate of disappearance and stable isotope tracer experiments are usually conducted during these steady-state conditions. The rate of appearance can be calculated from the tracer-to-tracee ratio and the known infusion rate of the tracer. Since protein intake is a known variable (and usually the experimental intervention), the rate at which a specific amino acid enters the plasma pool from protein breakdown can be calculated. In order to also calculate the rate of utilization of the amino acid for protein synthesis, the rate of oxidation needs to be known. Most often L-[1-^{13}C]Leucine [56] is used as a tracer. Leucine oxidation can be determined by measuring $^{13}CO_2$ production in expired breath. An alternative is the use of a phenylalanine tracer (e.g. L-[$^{2}H_5$]Phenylalanine). Phenylalanine is not directly oxidized, but first hydroxylized into tyrosine at the 3-position of the ring structure. The rate of this irreversible conversion is a substitute for phenylalanine "oxidation", which can then be used in calculations of protein kinetics as with the leucine method. The advantage of the phenylalanine method is that it does not require additional breath sampling methods. When the rate of utilization for protein synthesis is known, the rate of release from protein breakdown can be subtracted and the net bal-

ance of this specific amino acid results. This balance can then extrapolated to protein balance, using a value for the average content of this amino acid in body protein.

In the 2007 WHO report on human protein and amino acid requirements 24-hour studies [57-60] are considered the reference method to determine protein requirements, since these account for the entire day and may comprise both fed and fasted states [24]. For specific amino acid requirements within the diet, the indicator amino acid method is considered the reference method [24, 54]. It is based on the principle that when an essential amino acid is not sufficiently provided in the diet, it will be the rate limiting factor for protein synthesis, provided that all other essential amino acids are sufficiently available. Because amino acids cannot be stored other than in body protein, the excess of the other amino acids will be oxidized and irreversibly lost. In this method different essential amino acid than the test amino acid is labeled with a ^{13}C-label: the indicator amino acid, usually phenylalanine. The oxidation rate of the indicator amino acid is measured at different intake levels of the test amino acid within the same subject [54], or in different subjects [61]. When the intake of the test amino acid is increased, oxidation of the indicator amino acid decreases, until the optimal intake level of the test amino acid is achieved. The oxidation rate of the test amino acid will then not further increase. The test amino acid intake level at the breakpoint at which the decreasing oxidation rate is passed into the stable oxidation rate is assumed to be requirement of this amino acid. Currently, amino acid requirements for healthy infants are re-assessed with this method [61, 62].

24-Hour studies have only been conducted in healthy adults. The study designs used are too invasive for use in healthy children [54]. Neither have these techniques been used in critically ill children. It is common to use short-duration studies of several hours (4-8 hours). However, as these short duration tracer protocols have not been validated against 24-hour protocols, it is not known whether these short duration protocols represent 24-hour protein metabolism adequately.

Whole body protein metabolism versus organ protein metabolism

Above described methods focus on whole body protein metabolism, which is the net protein state of the body that results from the individual protein balances across tissues [63]. Meanwhile, protein metabolism across individual organs may substantially differ. For instance, during critical illness in skeletal muscle protein metabolism is characterized by net protein breakdown, to mobilise amino acids for protein synthesis elsewhere in the body. In the splanchnic area (intestines and liver) though, a net protein synthetic state is achieved, as a result of the acute phase proteins that are synthesized [63]. Inter-organ metabolism measurements require blood sampling before and after the organ, i.e. in arterial and venous vessels draining the organ. In human subjects, this can be done at the femoral level to measure skeletal muscle metabolism [44]. Studies across intra-abdominal organs are mostly unethical in human subjects, since it requires an intra-abdominal surgical intervention, except for patients who already undergo abdominal surgery. In animal models these studies are more feasible. Either arterial-venous plasma amino

acid concentration changes can be measured, using a measure to determine blood flow, but only arterial-venous amino acid balances are then determined. To also determine rates of protein synthesis and breakdown in the organ stable isotope tracer methodology is required [63].

As amino acids are retained in the splanchnic area at first pass after enteral intake, for whole body protein metabolism calculations in the fed state it is important to know which proportion of the ingested amino acid does not enter the plasma pool. Otherwise the proportion of the rate of appearance in the plasma pool of the amino acid that is provided from dietary protein intake is overestimated and the protein breakdown rates is consequently underestimated. The retained proportion of ingested amino acids in the splanchnic area is called splanchnic extraction. This proportion can be assessed using both an enteral and an intravenous stable isotope tracer (dual tracer method) and measurement of their enrichment in plasma [64]. This method to determine splanchnic extraction is, in contrast with measurement of organ metabolism, well feasible in humans and has been used in different age groups [65, 66]. In contrast to organ metabolism, it does not require blood sampling across organs.

SPECIFIC AMINO ACIDS DURING CRITICAL ILLNESS IN CHILDREN

Apart from the amount of protein that is required, the composition of the provided protein or amino acid mixture is of importance. Optimal protein quality is a composition of amino acids that matches the amino acids that are needed for protein synthesis and optimal organ function. During critical illness and/or recovery this may be a different composition than during health [24]. If low quality protein is provided, one of the amino acids will be the limiting factor for protein synthesis and the other amino acids are oxidized in excess. For instance during critical illness, skeletal muscle is used as endogenous source to provide amino acids for acute phase proteins. As such, for adults it has been estimated that for the synthesis of 1 gram of acute phase protein 2 gram of muscle protein is required, to supply the required amino acids [67].

The two most important and most studied amino-acids during critical illness are glutamine and arginine, since they both have a role in immune function. Other important amino acids are the branched chain amino acids (leucine, isoleucine, valine), which are most abundant in skeletal muscle. Especially leucine possesses protein anabolism stimulating properties [68]. Since we did not specifically study branched chain amino acids in this thesis, these will not further be discussed here.

Glutamine

Glutamine is the most abundant amino acid in plasma and tissues. It is an important nitrogen carrier and has several functions besides being incorporated into body protein. It is the precursor of glutathione, which is an antioxidant, it is an important fuel for immune cells, it preserves cell membrane integrity through enhancement of heat shock expression, it is an important fuel for

enterocytes and may protect gut wall integrity, it is the precursor of purine and pyrimidine and hence is important for DNA and RNA synthesis, it diminishes cytokine release and the activation of nuclear factor-kappa B, thus has anti-inflammatory purposes [69, 70]. Under normal circumstances glutamine is a non-essential amino acid, because it can be endogenously synthesized mainly from skeletal muscle. However, glutamine plasma concentrations are reduced in different disease states, such as in sepsis, trauma, after surgery, and in cancer. In critically ill patients reduced plasma glutamine levels are associated with worse outcome [71]. In adult patients after surgery, beneficial effects on the immune function have been shown of glutamine supplementation [72, 73] and on the basis of these observation, glutamine supplementation is also advocated in critically ill patients [41]. In premature neonates enteral glutamine supplementation is associated with a reduced number of septic episodes [74]. In 80 critically ill newborns and infants after major gastro-intestinal surgery though, no effect of glutamine-supplemented parenteral nutrition as compared to standard parenteral nutrition was found on number of septic episodes and other outcome measures such as intestinal permeability, N-balance and length of stay [75]. In critically ill children not enough evidence is available to be able to recommend glutamine in (subgroups) of critically ill children [33].

Glutamine is the precursor of citrulline. In enterocytes, glutamine can be converted to citrulline. Subsequently, from citrulline, which is a non-protein amino acid, arginine is synthesized *de novo* in the kidney. Hence, a link exists between arginine and glutamine, with an overlap in their specific function, i.e. in immune function [69].

Arginine

Arginine is an important amino acid for the immune system, because it is a fuel for leukocytes. Also, it is the precursor of nitric oxide (NO), which is needed, among others, for killing of bacteria [76, 77]. In addition, it has a role in wound healing and cell regeneration, and in vasodilatation as precursor of NO [78]. During sepsis, trauma and after surgery the plasma arginine concentration is severely reduced [77, 79]. It has been postulated in critically ill adults and children that arginine becomes an essential amino acid. We have previously shown that plasma arginine concentrations were severely decreased proportional to the severity of inflammation in critically ill children [80]. If arginine indeed becomes essential during critical illness in children one might speculate that a beneficial effect of arginine supplementation or increased content in nutrition can be expected. In critically ill children, only one study has been conducted on arginine kinetics though, with healthy young adults as control subjects [81]. The results of this study were not entirely in agreement with studies in critically ill adults [82-84], because in these septic children citrulline production and NO synthesis were found to be increased, whereas in septic adults citrulline production was severely reduced and NO synthesis was decreased as well. The results in the studies of adults are more in line with the decreased plasma citrulline concentrations that were meanwhile found; in the septic children plasma concentrations of arginine and citrulline were not assessed. Arginine kinetics studies

in critically ill children are thus needed to re-assess these discrepancies and to get more insight into the pathophysiological changes during critical illness in childhood.

At least after surgery supplementation of arginine in adults results in decreased post-operative infection rates [79]. Supplementation of arginine during sepsis is controversial, because of possible adverse effects [85]. Arginine has been often applied in both adults and children as ingredient of immunonutrition, which usually also contains omega-3-fatty acids and glutamine [48, 85]. Requirements of arginine during critical illness, when it becomes an essential amino acid, are not known and no evident beneficial effects of immunonutrition have been identified in children [33]. Therefore immunonutrition is not recommended in guidelines for critically ill children [33] as it is in adults. It is not known what the effects of increased protein intake are on arginine metabolism in critically ill children. Since the absolute arginine intake increases with increased protein intake, we hypothesized that arginine availability would improve in critically ill children when higher protein intake is provided.

Measurement of arginine metabolism

Arginine is part of a complex pathway involving the amino acids glutamine, proline, citrulline, ornithine and the nitrogen end product urea (**Figure 1.1**). Several fluxes in these pathways can be determined with stable isotope tracers of arginine, in combination with citrulline and urea tracers [86]. Also, plasma or serum amino acid concentrations are used to identify changes in arginine metabolism. The latter are, however, a static measurement and do not give insight into the fluxes and conversion rates of arginine into its metabolic products.

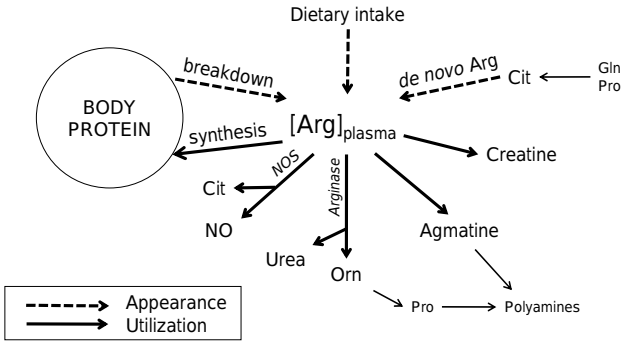


Figure 1.1 – Routes of appearance and utilization of plasma arginine.

Arginine appearance in the plasma pool results from dietary intake, *de novo* arginine synthesis from citrulline (which is produced in the gut from glutamine and proline) in the kidney and arginine release from body protein by protein breakdown. Plasma arginine is utilized for body protein synthesis, nitric oxide synthesis via nitric oxide synthetases, urea and ornithine production via arginases and production of agmatine and creatine.

$[Arg]_{plasma}$, plasma arginine concentration; Cit, citrulline; *de novo* Arg, *de novo* arginine synthesis; Gln, glutamine; NO, nitric oxide; NOS, nitric oxide synthases; Orn, ornithine; Pro, proline.

ENERGY REQUIREMENTS

Definition and measurement of energy requirements

Because of increased substrate cycling to mobilize and use energy sources, energy expenditure is commonly increased during critical illness. Hypermetabolism with high total energy expenditure is observed in patients with burns and sepsis. On the other hand mechanically ventilated and sedated patients may have a lower total energy expenditure than healthy children, due to decreased activity [33]. There is also a large inter-individual variability in total energy expenditure during critical illness. The best way to assess energy expenditure is to use indirect calorimetry, which measures resting energy expenditure. With indirect calorimetry oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory quotient ($RQ=VCO_2/VO_2$) are measured in in- and expiratory air using a metabolic monitor either connected to the ventilator or to a ventilated hood if a patient is not ventilated. Subsequently, measured energy expenditure is calculated with the modified Weir formula [87]. Also specific substrate utilization (protein, fat, carbohydrate) can be calculated from the measured VO_2 and VCO_2. RQ gives an indication of the accuracy of feeding. If RQ > 1, this indicates lipogenesis, which is the result of carbohydrate overfeeding with subsequent storage of carbohydrates as fat.

However, it is often impossible to perform indirect calorimetry in every patient. An alternative is to estimate resting energy expenditure using standard equations, such as the Schofield formula [88], although several studies revealed that these predictions do not accurately match measured resting energy expenditure [89-92]. Stress or activity correction factors that are used to correct the estimated total energy expenditure for critical illness are likely not accurate for all patients. Hence, the chance on under- or overfeeding exists, if nutritional intake is based on these standard equations.

Protein synthesis is an energy consuming process. It is therefore important to provide adequate energy to ensure that protein can be used for protein synthesis optimally and that it is not used as energy source instead. The recommended protein-energy ratio by the WHO is 9-11.5% for acutely malnourished children and 11.5-15% for chronically malnourished children [24]. The optimal ratio for critically ill children is not known. Whether caloric intake is derived from carbohydrates or fat intake does not seem to affect the protein balance [24].

Measurement of glucose metabolism

As outlined above glucose homeostasis is affected during critical illness, with hyperglycemia being prevalent. Although it is standard practice to provide intravenous glucose in otherwise fasted critically ill children, the optimal glucose intake in this patient population is not known. Normoglycemia should be aimed for, while avoiding hyperglycemia and hypoglycemia. Guidelines are based on only limited data [28, 93], hence there is need for more data on glucose metabolism in critically ill children. To study the effect of different glucose intakes on glucose metabolism during critical illness, stable isotope glucose tracers can be used. In

combination with a ^2H_2O tracer the rate of the components of endogenous glucose production can be estimated: gluconeogenesis and glycogenolysis [94].

CHALLENGES TO PROVIDE ADEQUATE NUTRITIONAL SUPPORT IN CRITICALLY ILL CHILDREN

Besides the absence of clear evidence on protein and energy requirements and hence difficulty to provide adequate nutritional intake, there are many factors during intensive care admission that result in inadequate nutritional intake. Fluid restriction, protein restriction, procedural interruptions of feeds, gastro-intestinal intolerance are some of the problems encountered [95]. Malnutrition is already present at admission in 24% of critically ill children [23]. And inadequate nutritional intake may further contribute to major cumulative protein- and energy deficits, which have been associated with deterioration in anthropometrics [96]. However, in many studies evaluating nutritional intake during PICU admission a large proportion of the population did not achieve nutritional goals [97-101].

Hence, it remains a challenge for physicians taking care of critically ill children to provide adequate nutritional support with the ultimate aim to achieve protein anabolism and improve clinical outcome. There is need for more evidence on protein and energy (glucose) intakes in critically ill children. Also, there is lack of data on the effect of achievement of adequate protein (and energy) intake on clinical outcome parameters.

AIMS AND OUTLINE OF THE THESIS

The ultimate aim of the studies in this thesis is to limit protein catabolism in critically ill children as much as possible and acquire protein anabolism as soon as possible during admission, in order to eventually improve clinical outcome.

To achieve this, first more knowledge is needed on pathophysiological changes in protein metabolism during critical illness in children. This is described in **Part 2** of the thesis. In **chapter 2** 24-hour protein metabolism is assessed using stable isotope tracer techniques in relatively stable critically ill children to determine whether an innate circadian rhythm is present or not. Also an optimal time frame for future protein balance studies with stable isotope tracer techniques is determined. In **chapter 3** pathophysiological changes of the specific amino acids arginine and citrulline in relation to inflammation are assessed with stable isotope tracer techniques. Also the effect of increased protein and energy intake on arginine metabolism is determined.

In **Part 3** and **Part 4** nutritional interventions are sought to limit protein catabolism and promote protein anabolism. In **chapter 4 and 5** the influence of a low glucose intake as compared to a standard glucose intake in the early post-operative phase on hyperglycemia, glucose kinetics

and protein kinetics is assessed after craniofacial and cardiac surgery respectively, using stable isotope tracer techniques. The aim of these studies is specifically to diminish hyperglycemia while preventing hypoglycemia and increased protein catabolism. In **chapter 6**, in the phase when enteral nutritional support is established (day 5 after admission), the safety and efficacy of a protein-energy enriched enteral formula as compared to a standard formula to achieve a positive energy and N-balance is determined. In **chapter 7**, the effect of this protein-energy enriched formula on protein kinetics is assessed with stable isotope techniques in order to assess whether protein anabolism can be achieved as compared with the standard formula. In **chapter 8** we study whether achievement of predefined protein and energy intake goals at day 4 after admission improved clinical outcome parameters in an observational study.

REFERENCES

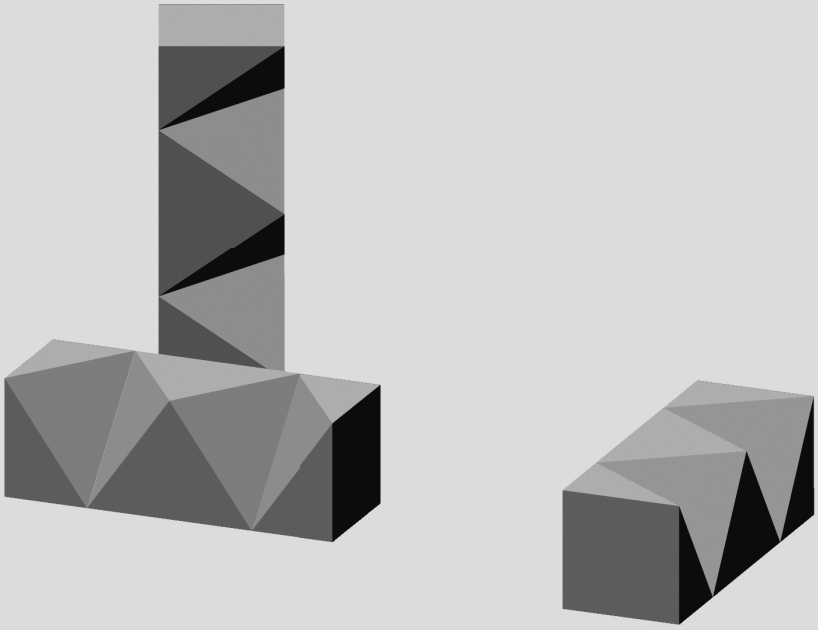
1. Mizock BA. *Immunonutrition and critical illness: an update.* Nutrition. 2010;**26**(7-8):701-7.
2. Bone RC, Balk RA, Cerra FB, Dellinger RP, et al. *Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine.* Chest. 1992;**101**(6):1644-55.
3. Van den Berghe G. *How does blood glucose control with insulin save lives in intensive care?* J Clin Invest. 2004;**114**(9):1187-95.
4. Srinivasan V, Spinella PC, Drott HR, Roth CL, et al. *Association of timing, duration, and intensity of hyperglycemia with intensive care unit mortality in critically ill children.* Pediatr Crit Care Med. 2004; **5**(4):329-36.
5. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, et al. *Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit.* Pediatrics. 2006;**118**(1):173-9.
6. Faustino EV, Apkon M. *Persistent hyperglycemia in critically ill children.* J Pediatr. 2005;**146**(1):30-4.
7. Vlasselaers D, Milants I, Desmet L, Wouters PJ, et al. *Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study.* Lancet. 2009;**373**(9663):547-56.
8. Verbruggen SC, Joosten KF, Castillo L, van Goudoever JB. *Insulin therapy in the pediatric intensive care unit.* Clin Nutr. 2007;**26**(6):677-90.
9. Preissig C, Rigby M. *Glycaemic control in paediatric critical care.* Lancet. 2009;**373**(9673):1423; author reply 1424.
10. Pollack MM, Ruttimann UE, Wiley JS. *Nutritional depletions in critically ill children: associations with physiologic instability and increased quantity of care.* JPEN J Parenter Enteral Nutr. 1985;**9**(3):309-13.
11. Christman JW, McCain RW. *A sensible approach to the nutritional support of mechanically ventilated critically ill patients.* Intensive Care Med. 1993;**19**(3):129-36.
12. Faddan NH, Sayh KI, Shams H, Badrawy H. *Myocardial dysfunction in malnourished children.* Ann Pediatr Cardiol. 2010;**3**(2):113-8.
13. Puthucheary Z, Harridge S, Hart N. *Skeletal muscle dysfunction in critical care: wasting, weakness, and rehabilitation strategies.* Crit Care Med. 2010;**38**(10 Suppl):S676-82.
14. Secker DJ, Jeejeebhoy KN. *Subjective Global Nutritional Assessment for children.* Am J Clin Nutr. 2007;**85**(4):1083-9.
15. Otranto M, Souza-Netto I, Aguila MB, Monte-Alto-Costa A. *Male and female rats with severe protein restriction present delayed wound healing.* Appl Physiol Nutr Metab. 2009;**34**(6):1023-31.
16. Schaffer MR, Tantry U, Ahrendt GM, Wasserkrug HL, et al. *Acute protein-calorie malnutrition impairs wound healing: a possible role of decreased wound nitric oxide synthesis.* J Am Coll Surg. 1997;**184**(1): 37-43.
17. Briassoulis G, Zavras N, Hatzis T. *Malnutrition, nutritional indices, and early enteral feeding in critically ill children.* Nutrition. 2001;**17**(7-8):548-57.
18. Benton D. *The influence of dietary status on the cognitive performance of children.* Mol Nutr Food Res. 2010;**54**(4):457-70.
19. Benton D. *The influence of children's diet on their cognition and behavior.* Eur J Nutr. 2008;**47 Suppl 3**:25-37.
20. Dykman RA, Casey PH, Ackerman PT, McPherson WB. *Behavioral and cognitive status in school-aged children with a history of failure to thrive during early childhood.* Clin Pediatr (Phila). 2001;**40**(2): 63-70.
21. Berkman DS, Lescano AG, Gilman RH, Lopez SL, et al. *Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study.* Lancet. 2002; **359**(9306):564-71.
22. Black MM, Dubowitz H, Krishnakumar A, Starr RH, Jr. *Early intervention and recovery among children with failure to thrive: follow-up at age 8.* Pediatrics. 2007;**120**(1):59-69.

23. Hulst J, Joosten K, Zimmermann L, Hop W, et al. *Malnutrition in critically ill children: from admission to 6 months after discharge.* Clin Nutr. 2004;**23**(2):223-32.
24. *Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation*, 2007, WHO: Geneva, Switzerland.
25. Flykanaka-Gantenbein C. *Hypoglycemia in childhood: long-term effects.* Pediatr Endocrinol Rev. 2004;**1 Suppl 3**:530-6.
26. Vannucci RC, Vannucci SJ. *Glucose metabolism in the developing brain.* Semin Perinatol. 2000;**24**(2): 107-15.
27. Zijlmans WC, van Kempen AA, Serlie MJ, Sauerwein HP. *Glucose metabolism in children: influence of age, fasting, and infectious diseases.* Metabolism. 2009;**58**(9):1356-65.
28. Koletzko B, Goulet O, Hunt J, Krohn K, et al. *1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR).* J Pediatr Gastroenterol Nutr. 2005;**41 Suppl 2**:S1-87.
29. McClave SA, Martindale RG, Vanek VW, McCarthy M, et al. *Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.).* JPEN J Parenter Enteral Nutr. 2009;**33**(3):277-316.
30. Gianotti L, Nelson JL, Alexander JW, Chalk CL, et al. *Post injury hypermetabolic response and magnitude of translocation: prevention by early enteral nutrition.* Nutrition. 1994;**10**(3):225-31.
31. Inoue S, Epstein MD, Alexander JW, Trocki O, et al. *Prevention of yeast translocation across the gut by a single enteral feeding after burn injury.* JPEN J Parenter Enteral Nutr. 1989;**13**(6):565-71.
32. Chiarelli A, Enzi G, Casadei A, Baggio B, et al. *Very early nutrition supplementation in burned patients.* Am J Clin Nutr. 1990;**51**(6):1035-9.
33. Mehta NM, Compher C. *A.S.P.E.N. Clinical Guidelines: nutrition support of the critically ill child.* JPEN J Parenter Enteral Nutr. 2009;**33**(3):260-76.
34. Casaer MP, Mesotten D, Hermans G, Wouters PJ, et al. *Early versus late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(6):506-17.
35. de Groof F, Joosten KF, Janssen JA, de Kleijn ED, et al. *Acute stress response in children with meningococcal sepsis: important differences in the growth hormone/insulin-like growth factor I axis between nonsurvivors and survivors.* J Clin Endocrinol Metab. 2002;**87**(7):3118-24.
36. Felbinger TW, Weigand MA, Mayer K. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1839; author reply 1841-2.
37. Bistrian BR. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1840; author reply 1841-2.
38. Marik PE. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1840-1; author reply 1841-2.
39. O'Leary MJ, Ferrie S. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011; **365**(19):1839-40; author reply 1841-2.
40. McClave SA, Heyland DK, Martindale RG. *Adding supplemental parenteral nutrition to hypocaloric enteral nutrition: lessons learned from the Casaer Van den Berghe study.* JPEN J Parenter Enteral Nutr. 2012;**36**(1):15-7.
41. Singer P, Berger MM, Van den Berghe G, Biolo G, et al. *ESPEN Guidelines on Parenteral Nutrition: intensive care.* Clin Nutr. 2009;**28**(4):387-400.
42. Wolfe RR. *Protein Summit: consensus areas and future research.* Am J Clin Nutr. 2008;**87**(5): 1582S-1583S.
43. Bechard LJ, Parrott JS, Mehta NM. *Systematic Review of Energy and Protein Intake on Protein Balance in Critically Ill Children.* J Pediatr. 2012.
44. Cynober LA. *Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance.* Nutrition. 2002;**18**(9):761-6.

45. Coss-Bu JA, Jefferson LS, Walding D, David Y, et al. *Resting energy expenditure and nitrogen balance in critically ill pediatric patients on mechanical ventilation.* Nutrition. 1998;**14**(9):649-52.
46. de Klerk G, Hop WC, de Hoog M, Joosten KF. *Serial measurements of energy expenditure in critically ill children: useful in optimizing nutritional therapy?* Intensive Care Med. 2002;**28**(12):1781-5.
47. Joosten KF, Verhoeven JJ, Hazelzet JA. *Energy expenditure and substrate utilization in mechanically ventilated children.* Nutrition. 1999;**15**(6):444-8.
48. Briassoulis G, Filippou O, Hatzi E, Papassotiriou I, et al. *Early enteral administration of immunonutrition in critically ill children: results of a blinded randomized controlled clinical trial.* Nutrition. 2005; **21**(7-8):799-807.
49. Briassoulis G, Filippou O, Kanariou M, Papassotiriou I, et al. *Temporal nutritional and inflammatory changes in children with severe head injury fed a regular or an immune-enhancing diet: A randomized, controlled trial.* Pediatr Crit Care Med. 2006;**7**(1):56-62.
50. Chaloupecky V, Hucin B, Tlaskal T, Kostelka M, et al. *Nitrogen balance, 3-methylhistidine excretion, and plasma amino acid profile in infants after cardiac operations for congenital heart defects: the effect of early nutritional support.* J Thorac Cardiovasc Surg. 1997;**114**(6):1053-60.
51. Botran M, Lopez-Herce J, Mencia S, Urbano J, et al. *Enteral nutrition in the critically ill child: comparison of standard and protein-enriched diets.* J Pediatr. 2011;**159**(1):27-32 e1.
52. Reynolds RM, Bass KD, Thureen PJ. *Achieving positive protein balance in the immediate postoperative period in neonates undergoing abdominal surgery.* J Pediatr. 2008;**152**(1):63-7.
53. Verbruggen SC, Coss-Bu J, Wu M, Schierbeek H, et al. *Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin-resistant adolescents with tight glucose control.* Crit Care Med. 2011;**39**(11):2518-25.
54. Pencharz PB, Ball RO. *Different approaches to define individual amino acid requirements.* Annu Rev Nutr. 2003;**23**:101-16.
55. Yudkoff M, Nissim I. *Methods for determining the protein requirement of infants.* Clin Perinatol. 1986; **13**(1):123-32.
56. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, et al. *Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine.* Am J Physiol. 1980;**238**(5):E473-9.
57. el-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, et al. *Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-13C]leucine and [15N-15N]urea.* Am J Clin Nutr. 1994;**59**(5):1000-11.
58. el-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, et al. *The 24-h pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults.* Am J Clin Nutr. 1994;**59**(5):1012-20.
59. Sanchez M, El-Khoury AE, Castillo L, Chapman TE, et al. *Twenty-four-hour intravenous and oral tracer studies with L-[1-13C]phenylalanine and L-[3,3-2H2]tyrosine at a tyrosine-free, generous phenylalanine intake in adults.* Am J Clin Nutr. 1996;**63**(4):532-45.
60. Sanchez M, el-Khoury AE, Castillo L, Chapman TE, et al. *Phenylalanine and tyrosine kinetics in young men throughout a continuous 24-h period, at a low phenylalanine intake.* Am J Clin Nutr. 1995;**61**(3): 555-70.
61. Huang L, Hogewind-Schoonenboom JE, van Dongen MJ, de Groof F, et al. *Methionine requirement of the enterally fed term infant in the first month of life in the presence of cysteine.* Am J Clin Nutr. 2012; **95**(5):1048-54.
62. Huang L, Hogewind-Schoonenboom JE, de Groof F, Twisk JW, et al. *Lysine requirement of the enterally fed term infant in the first month of life.* Am J Clin Nutr. 2011;**94**(6):1496-503.
63. Soeters PB, de Blaauw I, van Acker BA, von Meyenfeldt MF, et al. *In vivo inter-organ protein metabolism of the splanchnic region and muscle during trauma, cancer and enteral nutrition.* Baillieres Clin Endocrinol Metab. 1997;**11**(4):659-77.
64. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, et al. *Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach.* Am J Physiol. 1992;**262**(4 Pt 1):E455-63.

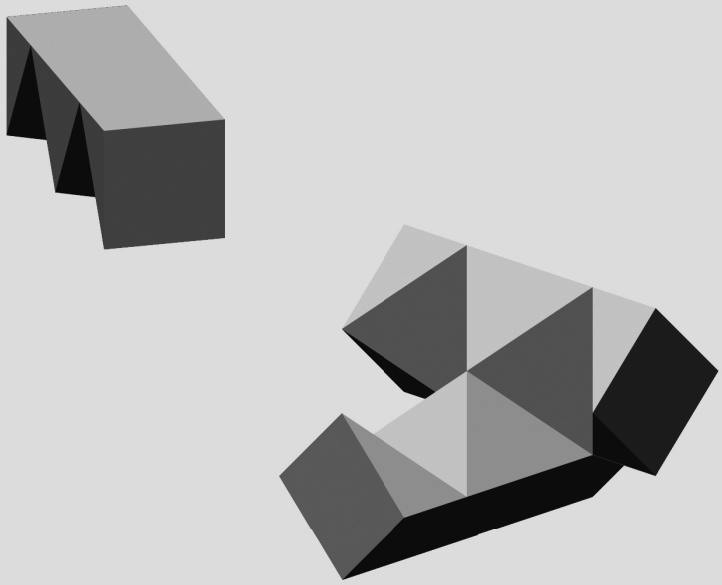
65. van der Schoor SR, Stoll B, Wattimena DL, Buller HA, et al. *Splanchnic bed metabolism of glucose in preterm neonates.* Am J Clin Nutr. 2004;**79**(5):831-7.
66. Engelen MP, Rutten EP, De Castro CL, Wouters EF, et al. *Altered interorgan response to feeding in patients with chronic obstructive pulmonary disease.* Am J Clin Nutr. 2005;**82**(2):366-72.
67. Reeds PJ, Fjeld CR, Jahoor F. *Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states?* J Nutr. 1994;**124**(6):906-10.
68. De Bandt JP, Cynober L. *Therapeutic use of branched-chain amino acids in burn, trauma, and sepsis.* J Nutr. 2006;**136**(1 Suppl):308S-13S.
69. Vermeulen MA, van de Poll MC, Ligthart-Melis GC, Dejong CH, et al. *Specific amino acids in the critically ill patient--exogenous glutamine/arginine: a common denominator?* Crit Care Med. 2007; **35**(9 Suppl):S568-76.
70. Soeters PB, Grecu I. *Have we enough glutamine and how does it work? A clinician's view.* Ann Nutr Metab. 2012;**60**(1):17-26.
71. Oudemans-van Straaten HM, Bosman RJ, Treskes M, van der Spoel HJ, et al. *Plasma glutamine depletion and patient outcome in acute ICU admissions.* Intensive Care Med. 2001;**27**(1):84-90.
72. Avenell A. *Glutamine in critical care: current evidence from systematic reviews.* Proc Nutr Soc. 2006; **65**(3):236-41.
73. Novak F, Heyland DK, Avenell A, Drover JW, et al. *Glutamine supplementation in serious illness: a systematic review of the evidence.* Crit Care Med. 2002;**30**(9):2022-9.
74. Neu J, Roig JC, Meetze WH, Veerman M, et al. *Enteral glutamine supplementation for very low birth weight infants decreases morbidity.* J Pediatr. 1997;**131**(5):691-9.
75. Albers MJ, Steyerberg EW, Hazebroek FW, Mourik M, et al. *Glutamine supplementation of parenteral nutrition does not improve intestinal permeability, nitrogen balance, or outcome in newborns and infants undergoing digestive-tract surgery: results from a double-blind, randomized, controlled trial.* Ann Surg. 2005;**241**(4):599-606.
76. Morris SM, Jr. *Arginine: master and commander in innate immune responses.* Sci Signal. 2010;**3**(135): pe27.
77. Davis JS,Anstey NM. *Is plasma arginine concentration decreased in patients with sepsis? A systematic review and meta-analysis.* Crit Care Med. 2011;**39**(2):380-5.
78. Morris SM, Jr. *Arginine: beyond protein.* Am J Clin Nutr. 2006;**83**(2):508S-512S.
79. Drover JW, Dhaliwal R, Weitzel L, Wischmeyer PE, et al. *Perioperative use of arginine-supplemented diets: a systematic review of the evidence.* J Am Coll Surg. 2011;**212**(3):385-99, 399 e1.
80. van Waardenburg DA, de Betue CT, Luiking YC, Engel M, et al. *Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation.* Am J Clin Nutr. 2007;**86**(5): 1438-44.
81. Argaman Z, Young VR, Noviski N, Castillo-Rosas L, et al. *Arginine and nitric oxide metabolism in critically ill septic pediatric patients.* Crit Care Med. 2003;**31**(2):591-7.
82. Luiking YC, Poeze M, Ramsay G, Deutz NE. *Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production.* Am J Clin Nutr. 2009;**89**(1):142-52.
83. Kao CC, Bandi V, Guntupalli KK, Wu M, et al. *Arginine, citrulline and nitric oxide metabolism in sepsis.* Clin Sci (Lond). 2009;**117**(1):23-30.
84. Villalpando S, Gopal J, Balasubramanyam A, Bandi VP, et al. *In vivo arginine production and intravascular nitric oxide synthesis in hypotensive sepsis.* Am J Clin Nutr. 2006;**84**(1):197-203.
85. Heyland DK, Novak F, Drover JW, Jain M, et al. *Should immunonutrition become routine in critically ill patients? A systematic review of the evidence.* JAMA. 2001;**286**(8):944-53.
86. van Eijk HM, Luiking YC, Deutz NE. *Methods using stable isotopes to measure nitric oxide (NO) synthesis in the L-arginine/NO pathway in health and disease.* J Chromatogr B Analyt Technol Biomed Life Sci. 2007;**851**(1-2):172-85.
87. Weir JB. *New methods for calculating metabolic rate with special reference to protein metabolism.* J Physiol. 1949;**109**(1-2):1-9.

88. Schofield WN. *Predicting basal metabolic rate, new standards and review of previous work.* Hum Nutr Clin Nutr. 1985;**39 Suppl 1**:5-41.
89. Verhoeven JJ, Hazelzet JA, van der Voort E, Joosten KF. *Comparison of measured and predicted energy expenditure in mechanically ventilated children.* Intensive Care Med. 1998;**24**(5):464-8.
90. Hardy CM, Dwyer J, Snelling LK, Dallal GE, et al. *Pitfalls in predicting resting energy requirements in critically ill children: a comparison of predictive methods to indirect calorimetry.* Nutr Clin Pract. 2002; **17**(3):182-9.
91. Mehta NM, Bechard LJ, Leavitt K, Duggan C. *Cumulative energy imbalance in the pediatric intensive care unit: role of targeted indirect calorimetry.* JPEN J Parenter Enteral Nutr. 2009;**33**(3):336-44.
92. Vazquez Martinez JL, Martinez-Romillo PD, Diez Sebastian J, Ruza Tarrio F. *Predicted versus measured energy expenditure by continuous, online indirect calorimetry in ventilated, critically ill children during the early postinjury period.* Pediatr Crit Care Med. 2004;**5**(1):19-27.
93. Kalhan SC, Kilic I. *Carbohydrate as nutrient in the infant and child: range of acceptable intake.* Eur J Clin Nutr. 1999;**53 Suppl 1**:S94-100.
94. Chacko SK, Ordonez J, Sauer PJ, Sunehag AL. *Gluconeogenesis is not regulated by either glucose or insulin in extremely low birth weight infants receiving total parenteral nutrition.* J Pediatr. 2011;**158**(6): 891-6.
95. Hulst JM, Joosten KF, Tibboel D, van Goudoever JB. *Causes and consequences of inadequate substrate supply to pediatric ICU patients.* Curr Opin Clin Nutr Metab Care. 2006;**9**(3):297-303.
96. Hulst JM, van Goudoever JB, Zimmermann LJ, Hop WC, et al. *The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population.* Clin Nutr. 2004;**23**(6): 1381-9.
97. Meyer R, Harrison S, Sargent S, Ramnarayan P, et al. *The impact of enteral feeding protocols on nutritional support in critically ill children.* J Hum Nutr Diet. 2009;**22**(5):428-36.
98. de Neef M, Geukers VG, Dral A, Lindeboom R, et al. *Nutritional goals, prescription and delivery in a pediatric intensive care unit.* Clin Nutr. 2008;**27**(1):65-71.
99. Tume L, Latten L, Darbyshire A. *An evaluation of enteral feeding practices in critically ill children.* Nurs Crit Care. 2010;**15**(6):291-9.
100. Rogers EJ, Gilbertson HR, Heine RG, Henning R. *Barriers to adequate nutrition in critically ill children.* Nutrition. 2003;**19**(10):865-8.
101. Taylor RM, Preedy VR, Baker AJ, Grimble G. *Nutritional support in critically ill children.* Clin Nutr. 2003;**22**(4):365-9.



PART 2

PATHOPHYSIOLOGICAL ASPECTS

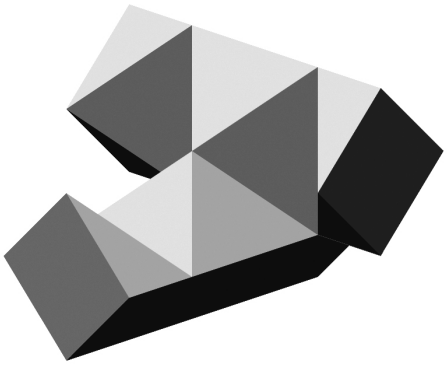


It's a very odd thing, as odd as can be,
that whatever Miss T. eats, turns into Miss T.

From "Miss T." - Walter de la Mare (1873-1956)

chapter 2

24-HOUR PROTEIN, ARGININE AND CITRULLINE METABOLISM IN FED CRITICALLY ILL CHILDREN DOES NOT SHOW A CIRCADIAN RHYTHM – A STABLE ISOTOPE TRACER STUDY



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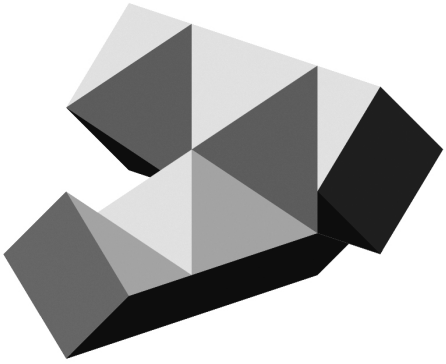
Submitted

chapter 3

ARGININE APPEARANCE AND NO SYNTHESIS IN CRITICALLY ILL CHILDREN CAN BE INCREASED WITH A PROTEIN-ENERGY ENRICHED ENTERAL FORMULA



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Submitted

ABSTRACT

Background: We observed previously that plasma arginine concentrations are reduced proportional to the severity of inflammation in critically ill children. However, underlying arginine kinetics has not been studied. Additionally, the effect of increased protein and energy intake on arginine metabolism is not known.

Objective: To investigate the effect of inflammation and nutrition on arginine kinetics in critically ill children.

Design: A 2-hour stable isotope tracer protocol was conducted in 10 fasted critically ill children with varying grades of inflammation (C-reactive protein (CRP)) and in two groups of fed critically ill infants, receiving either a protein-energy enriched formula (PE-formula, n=8) or a standard formula (S-formula, n=10). Data as mean ± SD.

Results: CRP and plasma arginine concentrations were strongly inversely associated (r=-0.733, p=0.016), as well as CRP and citrulline production (r=-0.818, p=0.004) in fasted children (age 1.71 ± 0.73 years), while *de novo* arginine synthesis was strongly associated with citrulline production (r=0.733, p=0.016). Intake of PE-formula in critically ill infants (age 0.23 ± 0.14 years) resulted in increased arginine appearance (PE-formula: 248 ± 114 vs. S-formula: 128 ± 53 μmol/kg/h; p=0.012) and nitric oxide (NO) synthesis (PE-formula: 1.92 ± 0.99 vs. S-formula: 0.83 ± 0.36 μmol/kg/h; p=0.003), whereas citrulline production and plasma arginine concentrations were unaffected.

Conclusions: In critically ill children citrulline production, hence *de novo* arginine synthesis, is reduced proportional to the severity of inflammation, which contributes to reduced arginine availability. Intake of a protein-energy enriched formula increases arginine appearance and NO synthesis in critically ill children, independent of plasma arginine concentrations.

LIST OF ABBREVIATIONS

AD-group	Children with acute inflammatory disorder in fasted state
Arg	Arginine
Cit	Citrulline
CRP	C-reactive protein
I-FABP	Intestinal fatty acid binding protein
Leu	Leucine
MUMC	Maastricht University Medical Center
NO	Nitric oxide
PE-group	Protein-energy enriched formula fed infants with viral bronchiolitis
Phe	Phenylalanine
PICU	Pediatric intensive care unit
PRISM	Pediatric Risk of Mortality score
Ra	Rate of appearance in plasma (also known as Q or flux)
Rd	Rate of disappearance in plasma (also known as Q or flux)
S-group	Standard formula fed infants with viral bronchiolitis
TTR	Tracer-to-tracee ratio

INTRODUCTION

Arginine is an important amino acid during disease and healing. Arginine is needed for protein synthesis, ureagenesis, the production of agmatine, creatine, polyamines, proline and the signaling molecule nitric oxide (NO) (**Figure 3.1**) [1, 2]. Hence, it has a function in wound healing, cell regeneration, immune function and vasodilatation. Plasma arginine utilized for these functions can be derived from nutrition, released from body protein or newly synthesized primarily in the kidneys from its sole precursor citrulline (*de novo* arginine synthesis) (**Figure 3.1**) [1, 2]. The latter makes arginine a non-essential amino acid under healthy conditions. Its precursor citrulline is a non-protein amino acid that is produced in the intestines, predominantly from glutamine and proline [1, 3]. During conditions with increased metabolic needs, such as critical illness, arginine is considered a conditionally essential amino acid, because the arginine production rate does not meet the increased needs [2]. Arginine deficiency may occur especially during sepsis as shown by reduced arginine plasma concentrations [4], potentially leading to reduced NO synthesis. Likewise, we found that plasma arginine concentrations were decreased in critically ill children proportional to the severity of inflammation as assessed with C-reactive protein (CRP) [5]. In addition, the rate of *de novo* arginine synthesis may decrease during critical illness. Our group was the first to show that citrulline production and consequently *de novo* arginine synthesis was severely decreased in critically ill adults with sepsis as compared to critically ill controls and healthy controls [6].

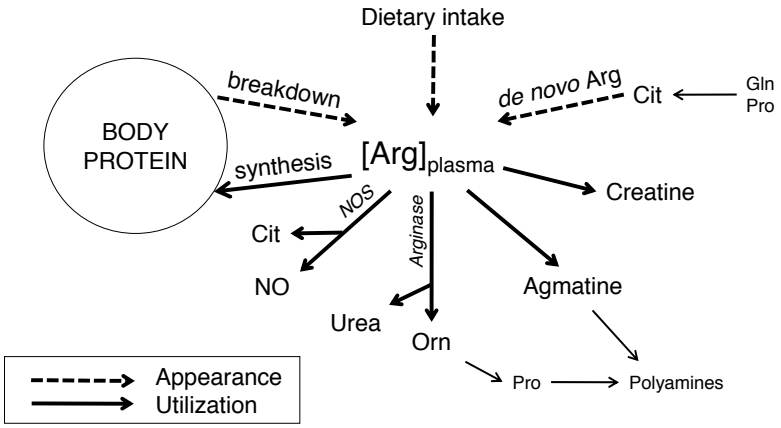


Figure 3.1 – Schematic representation of plasma arginine appearance and utilization.

Arginine appearance in the plasma pool results from dietary intake, *de novo* arginine synthesis from citrulline (which is produced in the gut from glutamine and proline) in the kidney and arginine release from body protein by protein breakdown. Plasma arginine is utilized for body protein synthesis, nitric oxide synthesis via nitric oxide synthetases, urea and ornithine production via arginases and production of agmatine and creatine.

$[Arg]_{plasma}$, plasma arginine concentration; Cit, citrulline; *de novo* Arg, *de novo* arginine synthesis; Gln, glutamine; NO, nitric oxide; NOS, nitric oxide synthases; Orn, ornithine; Pro, proline.

In the septic patients, citrulline and arginine plasma concentrations as well as NO synthesis were decreased as compared to both control groups. We hypothesized that citrulline production, hence *de novo* arginine synthesis, is also reduced in critically ill children proportional to the severity of inflammation and that this may result in arginine deficiency during severe inflammation.

To improve NO synthesis in children in diseases with arginine deficiency and/or insufficient NO synthesis, arginine supplementation has been suggested [7-9]. We have previously shown that a protein-energy enriched formula resulted in a positive protein balance in critically ill children as compared to a standard infant formula [10, 11]. We hypothesized that this formula, containing more arginine due to the higher protein content than the standard formula, would stimulate arginine appearance in plasma and stimulate NO synthesis. In the current study we therefore investigated both the effect of inflammation and the effect of different levels of protein-energy intake on arginine and citrulline kinetics in critically ill children using stable isotope tracer methodology.

SUBJECTS AND METHODS

Subjects and setting

Children with acute inflammatory disorders in the fasted state

Between September 2003 and April 2006 critically ill children, aged 1 year-11 years (< 30 kg body weight) with an acute inflammatory disorder admitted to the Pediatric Intensive Care Unit (PICU) of Maastricht University Medical Center (MUMC), Maastricht, the Netherlands were enrolled if they had an arterial line and a central venous line in place for medical treatment. Exclusion criteria were renal failure with oliguria (urine production < 0.25 ml/kg/h) or a glomerular filtration rate < 0-10 ml/min/1.73 m^2 at the time of the metabolic evaluation; extensive skin necrosis or limb amputation; metabolic diseases or endocrine disorders; dysmorphic syndromes. This group will be further referred to as AD-group (acute inflammatory disorders).

Children with viral bronchiolitis in the fed state

For this part of the study we included critically ill infants, admitted to the PICU of either Maastricht University Medical Center (MUMC), Maastricht, the Netherlands or Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands, who participated in a previously reported randomized controlled trial studying the effect of two levels of protein and energy intake on protein kinetics in infants with respiratory failure due to viral bronchiolitis. Details of the inclusion criteria and study protocol are described elsewhere [10, 11]. During the tracer protocol subjects continuously received either a protein-energy enriched enteral formula

(PE-formula; Infatrini®, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands) or a standard infant formula (S-formula; Nutrilon 1®, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands) in a randomized blinded fashion. The formula was introduced and increased up to the target intake as soon as possible after admission and continuously provided for subsequent days. For full description of the contents of both formulas see previous publications [10, 11].

For both studies ethical approval was obtained from either the local ethical committee and/or The Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands). Written informed consent was obtained from parents or caregivers.

Study design

Body weight was assessed on admission and Pediatric Risk of Mortality (PRISM) [12] scores were calculated in all groups.

In the AD-group a primed-continuous stable isotope tracer protocol was conducted between 24 to 72 hours after admission when patients were hemodynamically stable. Subjects were at least 4-hours fasted and received intravenous dextrose to prevent hypoglycemia. The same stable isotope tracer protocol was conducted in the PE- and S-group in the fed state at day 5 after admission to ensure provision of enteral nutrition at target intake, as previously described [11].

Stable isotope tracer protocol

Before the start of the tracer protocol, a baseline arterial blood sample was taken to determine background isotopic enrichments. Stable isotope tracers were primed and subsequently continuously infused for 2-hours with calibrated syringe pumps. Arterial blood was sampled at 60, 90 and 120 minutes for measurement of isotopic enrichments, in order to determine phenylalanine, arginine and citrulline kinetics. In the baseline sample plasma amino acid concentrations were determined as well in order to get insight into the plasma concentrations of arginine and citrulline and related amino acids. Also, plasma CRP concentrations were measured in the baseline sample as marker of severity of inflammation and plasma intestinal fatty acid binding protein (I-FABP) as marker for enterocyte damage. Samples were transfer-pipetted into heparin and EDTA cups, put on ice and centrifuged (3500×g) for 10 min at 4°C. Plasma for stable isotope analysis and plasma amino acid concentrations was deproteinized with 5% sulfosalicylic acid, snap frozen in liquid nitrogen and stored at −80°C until analysis.

In order to assess arginine metabolism L-[*guanido*-$^{15}N_2$-(5,5)-$^{2}H_2$]arginine ([$^{15}N_2$-$^{2}H_2$]Arg; infusion rate 5.6 μmol/kg/h) and L-[*ureido*-^{13}C]citrulline ([^{13}C]Cit); infusion rate 0.2 μmol/kg/h) tracers were used. However, halfway through the inclusion period [$^{15}N_2$-$^{2}H_2$]Arg was no longer available. For subsequent included subjects L-[*guanido*-$^{15}N_2$]arginine ([$^{15}N_2$]Arg) combined with L-[^{13}C-*ureido*-3,3,4-$^{2}H_3$]citrulline ([^{13}C-$^{2}H_3$]Cit was used instead. Consequently in the AD-group the second set of tracers were used in 50% of patients; in the PE- and S-group

only the first set of tracers was used. For assessment of whole body protein kinetics L-[ring-$^{2}H_{5}$]phenylalanine ([$^{2}H_{5}$]Phe; infusion rate: 4.3 μmol/kg/h) and L-[3,3-$^{2}H_{2}$]tyrosine ([$^{2}H_{2}$]Tyr; prime: infusion rate 1.5 μmol/kg) and L-[$^{2}H_{3}$]leucine ([$^{2}H_{3}$]Leu; infusion rate: 4.5 μmol/kg/h) were used. Prior to continuous infusion, a bolus of L-[ring-$^{2}H_{4}$]tyrosine was infused to prime the pool of [$^{2}H_{4}$]Tyr coming from [$^{2}H_{5}$]Phe. To determine splanchnic extraction in the fed state, in the PE-group and S-group L-[^{13}C]phenylalanine ([^{13}C]Phe; infusion rate: 8.1 μmol/kg/h) was meanwhile infused enterally via a nasogastric tube. Tracers (all > 98% mole percent enrichment) were purchased as sterile pyrogen-free powders from Cambridge Isotope Laboratories, Andover, MA, USA and Buchem B.V., Apeldoorn, the Netherlands. Tracer solutions were prepared by a licensed pharmacist and dispensed by the clinical pharmacy of the MUMC after testing for sterility and pyrogenicity. Intravenous tracers were administered through a 2μm pore filter. A sample of the used tracer solution was stored for determination of tracer concentrations.

Sample analysis

Plasma amino acid concentrations and plasma CRP concentrations were determined as described before [5]. Exogenous amino acid stable isotopomer enrichments (tracer-to-tracee ratios, TTR) were determined on a LC-ESI-MS system (QTrap 5500 MS (AB Sciex, Foster City, CA, USA) with ExpressHT Ultra LC (Eksigent Div., AB Sciex, Foster City, CA, USA). Samples were standardized with 0.1 N HCl containing a stable isotopomer of every amino acid as internal standard. For internal standards stable isotopomers with a high mass were chosen (≥ m+5) to prevent contribution of overlapping isotopomer distributions to isotopomers that were used as a tracer in the experimental protocol. Then, samples were transfer-pipetted onto strong-cation-exchange drip columns. Columns were washed with water and eluted with 2.5 M ammonia. Eluates were desolvated in a centrifugal evaporator. Solid residue tubes were capped for storage in the dark at room temperature. Within 3 days of LC-ESI-MS analysis samples were derivatized with 9-Fluorenylmethoxycarbonyl (Fmoc), subsequently neutralized, whereafter 160 nL of the solution was injected onto a 50 x 0.5 mm HALO C18 column and kept at 35°C. Analytes were eluted with a segmentally linear gradient from 35% to 85% acetonitrile in water supplemented with ammonium acetate to 10 μM and 5% isopropanol. Detection was by electrospray triple quadrupole tandem mass spectrometry in multiple reaction monitoring mode. Fmoc amino acid derivatives were fragmented in the collision cell for detection of either free aminoacyl anions or a fragment larger by 26 atom mass units (coming from the Fmoc derivative), whichever gave highest sensitivity. Thus monitoring occurred for each amino acid, their tracers and internal standards. Tracer-to-tracee ratios (TTR) were determined as tracer (labelled substance) / tracee (unlabelled substance).

Plasma I-FABP concentrations were determined using a highly specific in-house enzyme-linked immunosorbent assay (ELISA) that selectively detects human I-FABP (standard: 12.5-800 pg/ml).

Calculations

Whole body metabolism

Whole body rate of appearance (Ra; flux or *Q*) of plasma arginine, citrulline, phenylalanine, tyrosine and leucine were calculated from arterial isotopic enrichments of [$^{15}N_2$-$^2H_2$]Arg (or [$^{15}N_2$]Arg), [^{13}C]Cit (or [^{13}C-$^2H_3$]Cit), [$^2H_5$]Phe, [^{13}C]Phe, [$^2H_2$]Tyr and [$^2H_3$]Leu respectively using the standard steady state isotope dilution equation [13]:

$$\text{Ra} = Q = I/\text{TTR}\ (\mu\text{mol/kg/h}) \qquad \text{(Eq 1)}$$

where *I* is the rate of tracer infusion. Isotopic enrichment was calculated by correcting the measured TTRs at the plateau phase by subtracting the background TTR, which was determined in the sample obtained before the start of the tracer infusion. In case of arginine and citrulline tracers the contribution of overlapping isotopomer distributions of tracers with lower masses to the measured TTR was accounted for as previously described [14].

Whole body arginine and citrulline metabolism

Calculations are described for the [$^{15}N_2$-$^2H_2$]Arg - [^{13}C]Cit tracers set, but equally apply to the [$^{15}N_2$]Arg - [^{13}C- $^2H_3$]Cit tracers set where both arginine and citrulline tracers can be replaced by one another.

The rate of NO synthesis was calculated as the flux from arginine-to-citrulline [15] with the following equation:

$$\text{NO synthesis} = Q_{\text{Arg}\rightarrow\text{Cit}} = Q_{\text{Cit}} \times \text{TTR}_{[^{15}\text{N-}^2\text{H}_2]\text{Cit}}/\text{TTR}_{[^{15}\text{N}_2\text{-}^2\text{H}_2]\text{Arg}} \qquad \text{(Eq 2)}$$

where Q_{Cit} is the Ra of citrulline as calculated with (Eq 1) from [^{13}C]Cit enrichment.

The rate of *de novo* arginine synthesis was calculated as the flux from citrulline-to-arginine [16] as follows:

$$\textit{de novo}\ \text{arginine synthesis} = Q_{\text{Cit}\rightarrow\text{Arg}} = Q_{\text{Arg}} \times \text{TTR}_{[^{13}\text{C}]\text{Arg}} / \text{TTR}_{[^{13}\text{C}]\text{Cit}} \qquad \text{(Eq 3)}$$

where Q_{Arg} is the Ra of arginine as calculated with (Eq 1) from [$^{15}N_2$]Arg enrichment.

Arginine release from breakdown (ArgRP) was calculated from:

$$\text{ArgRP} = Q_{\text{Arg}} - Q_{\text{Cit}\rightarrow\text{Arg}} \qquad \text{(Eq 4)}$$

Arginine clearance in ml/kg/min was calculated [17]:

$$\text{Arg clearance} = (\text{Ra Arg} / 60) / (\text{Plasma [Arg]} / 1000) \qquad \text{(Eq 5)}$$

where Plasma [Arg] is the plasma arginine concentration in μmol/L.

Whole body protein metabolism

Calculations for whole body protein metabolism during the fed state have been described before [11].

Under steady state conditions Ra of amino acids in the plasma pool is equal to the rate of disappearance (Rd; flux or *Q*). In the fasted state Ra equals the rate at which amino acids are released from protein breakdown; in the case of phenylalanine (Phe) PheRP. In the fed state Ra equals the sum of the amino acid release from protein and the rate at which the amino acid enters the blood pool from the nutrition source. In case of enteral nutrition this is the rate of enteral intake, corrected for the proportion of the amino acid intake that is retained in the splanchnic area during first pass (splanchnic extraction, SPE). Both in the fasted and in the fed state Rd equals the sum of the rate of oxidation or hydroxylation (in case of phenylalanine: PheOH) and the rate at which the amino acid is used for protein synthesis, the non-oxidative or non-hydroxylation disposal (in case of Phe: NHPD).

Plasma phenylalanine-to-tyrosine flux, indicating PheOH was calculated with the following equation:

$$\text{PheOH} = Q_{\text{Phe}\rightarrow\text{Tyr}} = Q_{\text{Tyr}} \times \text{TTR}_{[^2H_4]\text{Tyr}} / \text{TTR}[_{[^2H_5]\text{Phe}} \quad \text{(Eq 6)}$$

where Q_{Tyr} is the Ra of tyrosine as calculated with (Eq 1) from [$^2H_2$]Tyr enrichment.

PheRP was calculated in the fasted state as follows:

$$\text{PheRP} = Q_{\text{Phe}} \quad \text{(Eq 7)}$$

In the fed state dietary Phe via the enteral route needs to be adjusted for SPE_{Phe}, as described above. Splanchnic extraction of Phe was calculated:

$$\text{SPE}_{\text{Phe}} = (1- (Q_{[^2H_5]\text{Phe}} / Q_{[^{13}C]\text{Phe}})) \times 100\% \quad \text{(Eq 8)}$$

where $Q_{[^2H_5]\text{Phe}}$ is the Ra of phenylalanine derived from intravenously infused [$^2H_5$]Phe and $Q_{[^{13}C[\text{Phe}}$ the Ra of phenylalanine derived from enterally administered [^{13}C]Phe.

PheRP in the fed state was then calculated:

$$\text{PheRP} = Q_{\text{Phe}} - (\text{dietary Phe intake} \times (1- (\text{SPE}_{\text{Phe}} \times 0.01) \quad \text{(Eq 9)}$$

NHPD was calculated:

$$\text{NHPD} = Q_{\text{Phe}} - \text{PheOH} \quad \text{(Eq 10)}$$

Phenylalanine balance (PheBal) can then be calculated:

$$PheBal = NHPD - PheRP. \quad \text{(Eq 11)}$$

Whole body protein breakdown, protein oxidation, protein synthesis and protein balance rates (in g/kg/d) can be calculated from PheRP, PheOH, NHPD and PheBal respectively using an average Phe content in human proteins of 280 μmol/g protein [18].

Statistics

Data are presented as mean ± SD. Because of the small sample size non-parametric tests were used. Baseline characteristics were compared between the three groups with the Kruskal Wallis test and Wilcoxon rank-sum tests with post-hoc Bonferroni correction to compare pairs of group. Spearman's rho correlation was used to assess associations between variables. Comparisons between the PE- and S-group were done with Wilcoxon rank-sum test. $P < 0.05$ was considered statistically significant. Since our aim was to provide indications for correlations between variables and for differences between the aforementioned groups of children, no correction for multiple comparisons was applied other than for baseline characteristics. In the figures all three groups are shown, but due to differences in baseline characteristics, differences in results between the fasted patients and the fed patients were not assessed statistically. Analysis was done with IBM SPSS Statistics (version 17, IBM, Armonk, NY, USA).

RESULTS

Subjects

Ten children with acute inflammatory disorders were included to study the effect of inflammation on arginine and citrulline kinetics. Diagnoses were septic shock (n=4), meningitis (n=3), pleural empyema (n=1), pneumonia (n=1) and upper airway obstruction due to bacterial laryngitis (n=1). The stable isotope tracer protocol was conducted 66 ± 27 hours after admission, after 9 ± 7 hours fasting. Energy intake during the tracer protocol was 1.2 ± 0.9 kcal/kg/h. All septic patients received inotropics (norepinephrine and/or dopamine) the other patients did not.

The effect of nutrition on arginine and citrulline kinetics was determined in 18 infants with viral bronchiolitis receiving either protein-energy enriched infant formula (n=8) or standard infant formula (n=10) as previously reported [10, 11]. See **Table 3.1** for the overview of baseline characteristics of the three groups. In the AD-group children were older and plasma CRP concentrations were higher, but PRISM scores were not significantly different between groups. Energy and protein intake were significantly higher in the PE-group, hence arginine intake and intake of it's precursors glutamine and proline, as incorporated in protein, were significantly higher (**Table 3.2**).

Table 3.1 – Baseline characteristics at time of the tracer protocol in three groups of critically ill children.

	AD-group (n=10)	S-group (n=10)	PE-group (n=8)
Male gender – n (%)	9 (90%)	3 (30%)	2 (25%)
Age (years)	1.71 ± 0.73	0.24 ± 0.16 ‡	0.23 ± 0.12 ‡
Weight (kg)	11.4 ± 2.1	4.8 ± 1.2 ‡	4.0 ± 1.0 ‡
PRISM [4]	14.3 ± 11.9	18.6 ± 4.8	20.3 ± 4.6
C-reactive protein (mg/L)	172 ± 110	23 ± 7 †	28 ± 8 †

Data as mean ± SD. Comparisons between all groups with Kruskal-Wallis test and with Wilcoxon rank-sum test for pairs of groups with post-hoc Bonferroni correction. ‡ $p < 0.001$ as compared to AD-group. † $p < 0.01$ as compared to the AD-group. There were no statistically significant differences between the S-group and the PE-group.
AD-group, children with acute inflammatory disorders in fasted state; PE-group, protein-energy enriched formula fed infants with viral bronchiolitis; PRISM, Pediatric Risk of Mortality [12]; S-group, standard formula fed infants with viral bronchiolitis.

Table 3.2 – Nutritional intake during a stable isotope tracer protocol in fed infants with viral bronchiolitis.

	S-group (n=10)	PE-group (n=8)
Energy (kcal/kg/24h)	84 ± 15	119 ± 25 ‡
Protein (g/kg/24h)	1.7 ± 0.2	3.1 ± 0.3 ‡
Arginine (μmol/kg/h)	13 ± 1.7	24 ± 2.8 ‡
Glutamine (μmol/kg/h)	88 ± 12	191 ± 23 ‡
Proline (μmol/kg/h)	47 ± 6	86 ± 10 ‡

Neither formula contained citrulline or ornithine. More information on the composition of both formulas is reported previously [10, 11]. Comparison between groups with Wilcoxon rank-sum test.
‡ $p < 0.001$ as compared to S-group.
PE-group, protein-energy enriched formula fed infants with viral bronchiolitis; S-group, standard formula fed infants with viral bronchiolitis.

Measurements

Plasma amino acid concentrations in fasted children with acute inflammatory disorders

In the fasted state arginine in the plasma pool originates from the conversion of citrulline to arginine (*de novo* arginine synthesis) and arginine release from protein breakdown. In the fed state, arginine also appears in the plasma pool from dietary intake. Together this is called the rate of appearance (Ra Arg, or arginine appearance). Arginine disappears from the plasma pool (rate of disappearance (Rd Arg) or arginine utilization) by use for protein synthesis, NO

synthesis by nitric oxide synthases, conversion of arginine to urea and ornithine by arginases and synthesis of agmatine and creatine (**Figure 3.1**) [19].

In **Figure 3.2** plasma concentrations of arginine, citrulline, ornithine and glutamine of the AD-group are shown. Comparable to our earlier findings an inverse association between plasma arginine concentrations and the severity of inflammation, expressed as plasma CRP concentrations, was found (r=-0.733, p=0.016) (**Figure 3.3 panel A** shows this relation for all groups). There was also an inverse association between CRP and citrulline plasma concentrations, which just failed to reach significance (r=-0.624, p=0.054).

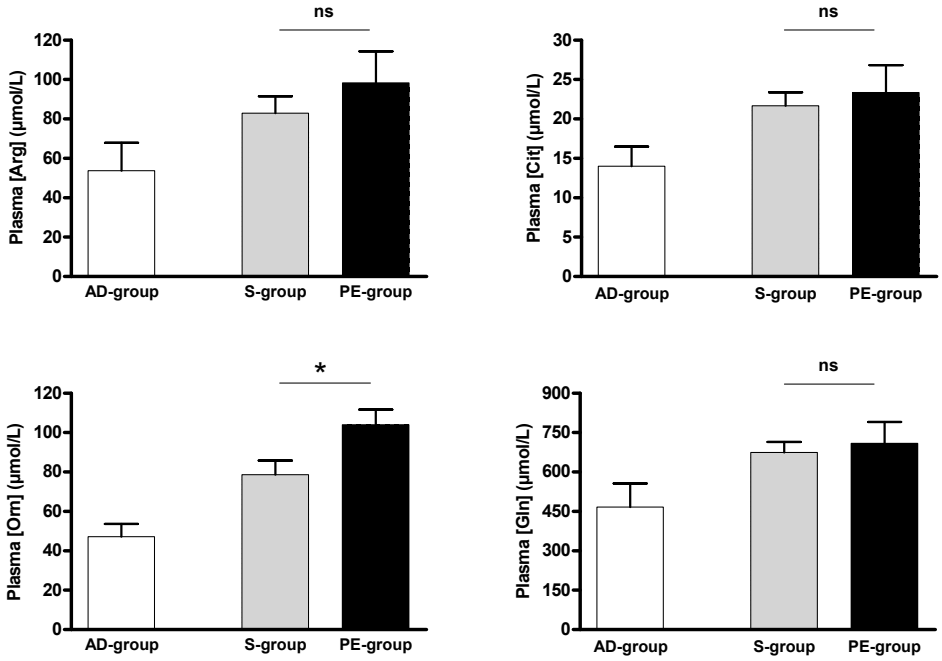


Figure 3.2 – Plasma concentrations of amino acids involved in arginine metabolism in three groups of critically ill children.

Data as mean ± SEM. Comparisons between S-formula (n=10) and PE-formula (n=8) with Wilcoxon rank-sum test; * $p < 0.05$.

AD-group, children with acute inflammatory disorders in fasted state (n=10); PE-group, protein-energy enriched formula fed infants with viral bronchiolitis (n=8); Plasma [Arg], plasma arginine concentration; Plasma [Cit], plasma citrulline concentration; Plasma [Gln], plasma glutamine concentration; Plasma [Orn], plasma ornithine concentration; S-group, standard formula fed infants with viral bronchiolitis (n=10).

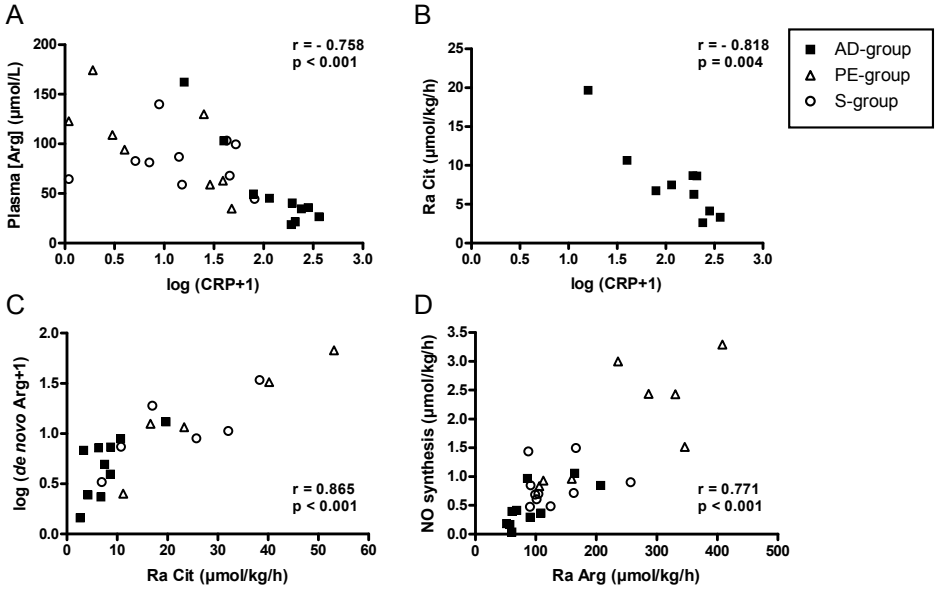


Figure 3.3 – Associations between CRP, arginine and citrulline kinetics and plasma concentrations in three groups of critically ill children.

Associations were assessed with Spearman's rho correlation. CRP and *de novo* Arg were converted to logarithmic values after addition of 1 to achieve positive values.
AD-group, children with acute inflammatory disorders in fasted state (n=10); CRP, C-reactive protein; *de novo* Arg, *de novo* arginine synthesis; NO, nitric oxide; PE-group, protein-energy enriched formula fed infants with viral bronchiolitis (n=8); Plasma [Arg], plasma arginine concentration; Ra Arg, rate of arginine appearance; Ra Cit, rate of citrulline appearance (citrulline production); S-group, standard formula fed infants with viral bronchiolitis (n=10).

Arginine and citrulline kinetics in fasted children with acute inflammatory disorders

In **Figure 3.4** arginine and citrulline kinetics are shown for the AD-group. Although plasma arginine concentrations were strongly inversely correlated to CRP, we could not find a relation between Ra Arg and CRP (r=-0.333, p=0.347), Ra Arg and plasma arginine concentrations (r=0.139, p=0.701) and Ra Arg and *de novo* arginine synthesis (r=0.539, p=0.108). Ra Arg was correlated with Ra Leu and Ra Phe (r=0.782, p=0.008; r=0.661, p=0.038 respectively), as all are released from protein breakdown.

Ra Cit (citrulline production in the gut from glutamine and proline and from arginine as by-product from NO synthesis) was strongly and inversely correlated with CRP (r=-0.818, p=0.004) and strongly positively correlated with *de novo* arginine synthesis (arginine production from citrulline in the kidneys) (r=0.733, p=0.016) (**Figure 3.3 panel B and C**). Although we were not able to find a significant association between *de novo* arginine synthesis and CRP (r=-0.527, p=0.117), these results suggest that citrulline production, hence *de novo* arginine

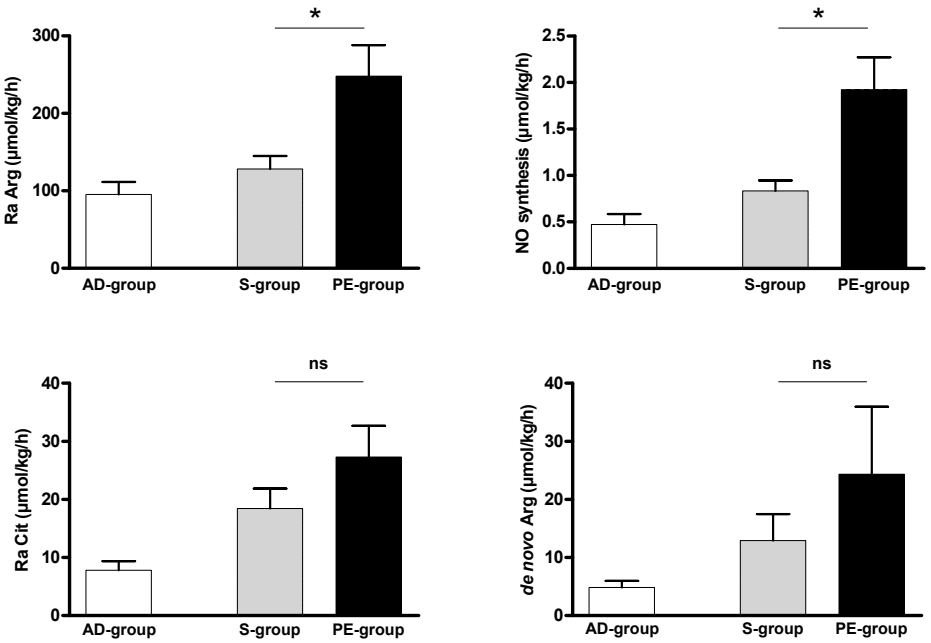


Figure 3.4 – Arginine and citrulline kinetics in three groups of critically ill children.

Data as mean ± SEM. Comparisons between S-formula and PE-formula with Wilcoxon rank-sum test; * $p < 0.05$.

AD-group, children with acute inflammatory disorders in fasted state (n=10); NO, nitric oxide; PE-group, protein-energy enriched formula fed infants with viral bronchiolitis (n=8); Ra Arg, rate of arginine appearance; Ra Cit, rate of citrulline appearance (citrulline production); S-group, standard formula fed infants with viral bronchiolitis (n=10).

synthesis, is reduced with increasing inflammation. In contrast, arginine release from protein was not associated with CRP (r=-0.273, p=0.446), suggesting that protein breakdown was not increased proportional to the severity of inflammation.

Arginine utilization (Rd Arg) was positively correlated with NO synthesis (production of NO from arginine with simultaneous production of citrulline) (r=0.709, p=0.022) (**Figure 3.3 panel D**) and protein synthesis (r=0.697, p=0.025). We did not quantify other fluxes of arginine utilization in this study. Of the different components of arginine appearance, NO synthesis was associated with arginine release from protein (r=0.709, p=0.022) but not with *de novo* arginine synthesis (r=0.055, p=0.881).

Although theoretically the plasma arginine concentration is the result of Ra Arg (arginine appearance) and Rd Arg (arginine utilization) [20], we suggested that plasma arginine concentration relates to arginine clearance capacity [21]. This view is supported by the fact that appearance and utilization during steady state are always equal. We suggest therefore that arginine clearance is a better parameter to compare arginine utilization at different plasma arginine concentrations. In the fasted subjects arginine clearance was 45 ± 42 ml/kg/min on

average and tended to be associated with CRP (r=0.576, p=0.082). The lower plasma arginine concentrations in our subjects may therefore have been primarily caused by increased arginine utilization.

I-FABP concentrations, as marker of enterocyte damage, were available in 8 patients of the AD-group. Mean plasma I-FABP concentration was 556 ± 644 pg/ml (range 54 - 1584 pg/ml). There was no association between I-FABP concentrations and plasma citrulline concentrations (r=0.190, p=0.650) or CRP (r=-0.024, p=0.955).

In **Table 3.3** we have provided an overview of our results in the AD-group and results from available studies in literature on arginine kinetics in critically ill fasted patients. Because these comprised only studies comparing septic patients with control subjects (besides studies in burned patients which are not shown [22-25], results of our septic subjects and other subjects are shown separately for indicative purposes. There were no statistical differences between these two groups, except for CRP (p=0.038) and a tendency for arginine plasma concentrations (p=0.067).

Plasma amino acid concentrations in fed infants with viral bronchiolitis

Figure 3.2 depicts the plasma concentrations of arginine, citrulline, glutamine and ornithine in the PE- and S-group. Plasma arginine, citrulline and glutamine concentrations were not different between both groups, but plasma ornithine concentrations were significantly higher in the PE-group. Even during the fed state plasma arginine concentrations remained associated with CRP (r=-0.479, p=0.044) (**Figure 3.3 panel A**). Plasma arginine, citrulline, glutamine or ornithine concentrations were not associated with arginine, glutamine or proline intake.

Arginine and citrulline kinetics and nutrition in fed infants with viral bronchiolitis

Figure 3.4 depicts arginine and citrulline kinetics in the PE-group and S-group. Ra Arg (total arginine appearance from arginine intake, protein breakdown and *de novo* arginine synthesis) was significantly higher with PE-formula than with S-formula. Ra Cit (citrulline production from glutamine and proline; and from arginine as by-product of NO synthesis) and *de novo* arginine synthesis (arginine production from citrulline in the kidneys) were not different between both groups of infants. The relative contribution of all components of arginine appearance to Ra Arg was not different between both groups (arginine release from protein breakdown: PE-formula 79 ± 6 % vs. S-formula 81 ± 5 %, p=0.429; arginine intake: PE-formula 12 ± 6 % vs S-formula 11 ± 4 %, p=0.897; *de novo* arginine synthesis: PE-formula 8 ± 6 % and S-formula 8 ± 4 %, p=0.931). Concerning arginine utilization, NO synthesis was significantly increased with PE-formula as compared with S-formula (**Figure 3.4**). Also, protein synthesis was significantly increased in the PE-group as compared to the S-group (**Figure 3.5**). As previously reported elsewhere, the increase in protein synthesis was higher than the increase in protein breakdown in the PE-group [11]. Therefore PE-formula resulted in a positive protein balance, whereas

Table 3.3 – Overview of studies on arginine and citrulline kinetics in critically ill children and adults in the fasted state.

Reference	Diagnosis	Age (years)	CRP (mg/L)	Ra Arg (μmol/kg/h)	Ra Cit (μmol/kg/h)	*de novo* Arg synthesis (μmol/kg/h)	NO synthesis (μmol/kg/h)	Ra Leu / Ra Phe (μmol/kg/h)	Plasma [Arg] (μmol/L)	Plasma [Cit] (μmol/L)
Children										
Present study (mean ± SD)	Sepsis (n=4)	1.6 ± 1.1	268 ± 72	93 ± 49	4.7 ± 2.7	3.5 ± 3.0	0.61 ± 0.48	122 ± 39 / 106 ± 53	29 ± 8	10 ± 2
	Other diagnoses (n=6)	1.8 ± 0.5	108 ± 80	97 ± 58	9.9 ± 5.0	5.7 ± 3.9	0.38 ± 0.25	110 ± 16 / 88 ± 13	70 ± 53	17 ± 9
Argaman [28] (mean ± SD)	Sepsis (n=10)	11 ± 4	-	67 ± 21	25 ± 7	9.6 ± 4.2	1.58 ± 0.7	153 ± 13 / -	-	-
	Healthy adults (n=6)	21 ± 3	-	68 ± 10	15 ± 5	9.2 ± 1.4	0.96 ± 0.1	96 ± 21 / -	-	-
Adults										
Kao [17] (mean ± SEM)	Sepsis n=13)	54 ± 10	-	53.0 ± 5.5	4.4 ± 0.5	-	0.20 ± 0.04	130.8 ± 11.8 / -	40.2 ± 3.8	10.2 ± 3.8
	Healthy adults (n=7)	49 ± 6	-	48.7 ± 2.8	10.6 ± 0.8	-	0.15 ± 0.04	89.7 ± 3.0 / -	85.5 ± 3.3	21.4 ± 2.5
Luiking [6] (mean ± SD)	Sepsis (n=10)	56 ± 12	219 ± 123	59 ± 23	4.5 ± 2.1	3.3 ± 3.7	0.8 ± 0.6	- / 62 ± 21	49 ± 12	18 ± 6
	Critically ill controls (n=7)	58 ± 11	85 ± 69	64 ± 19	10.1 ± 2.9	10.9 ± 9.4	1.5 ± 1.0	- / 51 ± 13	69 ± 37	21 ± 10
	Healthy adults (n=16)	61 ± 6	1.4 ± 1.8	72 ± 15	13.7 ± 4.1	11.9 ± 6.6	2.2 ± 1.2	- / 36 ± 9	92 ± 17	41 ± 7
Villalpando [26] (mean ± SEM)	Hypotensive sepsis (n=6)	54 ± 5.2	-	50 ± 7	-	-	-	-	40 ± 11	-
	Healthy adults (n=10)	40 ± 8.6	-	99 ± 8	-	-	-	-	75 ± 8	-

Arg, arginine; Cit, citrulline; CRP, C-reactive protein; Leu, leucine; NO, nitric oxide; Plasma [Arg], plasma arginine concentration; Plasma [Cit], plasma citrulline concentration; Phe, phenylalanine; Ra, rate of appearance.

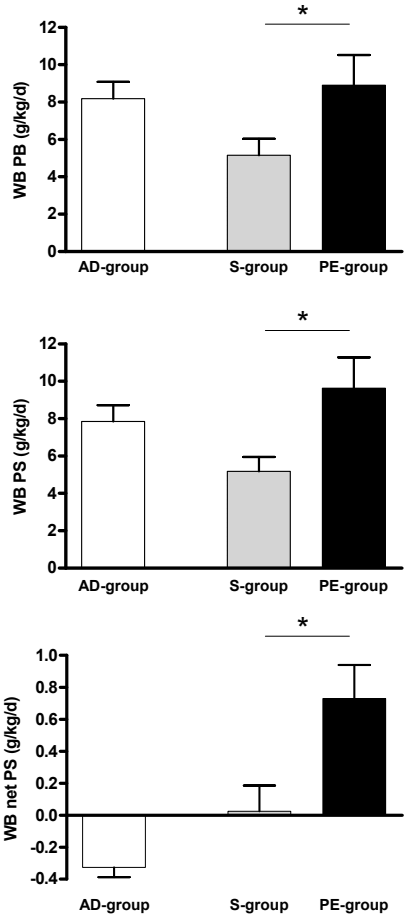


Figure 3.5 – Whole body protein metabolism in three groups of critically ill children.

Data as mean ± SEM. Comparisons between S-formula and PE-formula with Wilcoxon rank-sum test; * $p < 0.05$.

AD-group, children with acute inflammatory disorders in fasted state (n=10); PE-group, protein-energy enriched formula fed infants with viral bronchiolitis (n=8); S-group, standard formula fed infants with viral bronchiolitis (n=10); Wb net PS, whole body net protein synthesis; Wb PB, whole body protein breakdown; Wb PS, whole body protein synthesis.

S-formula resulted in a protein balance that was not significantly different from zero. Arginine clearance was significantly higher in the PE-group than in the S-group (48.3 ± 28.0 vs. 26.3 ± 7.0 respectively, p=0.012) and was associated with protein synthesis (r=0.800, $p < 0.001$) and NO synthesis (r=0.484, p=0.042). I-FABP concentrations were in the normal range, not significantly different between both groups (PE-group (n=6) 283 ± 162 vs. S-group (n=5) 452 ± 222; p=0.177) and not associated with CRP or plasma citrulline concentrations.

During the fed state associations persisted between Ra Cit and *de novo* arginine synthesis (r=0.800, p=0.003) (**Figure 3.3 panel C**), between Ra Arg and NO synthesis (r=0.707, p=0.001) (**Figure 3.3 panel D**) and between Ra Arg and Ra Phe (r=0.893, $p < 0.001$). Ra Arg was associated with arginine intake (r=0.480, p=0.044) and in contrast with the fasted state, also with *de novo* arginine synthesis (p=0.782, p=0.004). The association between Ra Arg and NO synthesis was predominantly caused by an association between NO synthesis and arginine intake (r=0.682, p=0.002) and NO synthesis and arginine release from protein (r=0.664, p=0.026).with a tendency towards an association between NO and *de novo* arginine synthesis (r=0.591, p=0.056).

DISCUSSION

In the current study plasma arginine concentrations were reduced proportional to the severity of inflammation in both fasted and fed critically ill children, as in our previous study [5]. Using stable isotope methodology we now demonstrated that with increasing inflammation citrulline production was reduced and positively associated with *de novo* arginine synthesis, but arginine release from protein breakdown was unchanged. Meanwhile, arginine clearance reflecting arginine utilization, tended to be increased with increasing inflammation. This suggests that plasma arginine concentrations fall with increasing inflammation because of increased arginine utilization, which is not matched by increased arginine appearance. Thus arginine becomes an essential amino acid. Intake of a protein-energy enriched formula resulted in increased arginine appearance as compared to a standard formula, although plasma arginine concentrations did not differ significantly. Moreover it resulted in increased NO synthesis. Our results imply that during severe inflammation increased arginine intake (or of it's precursors) e.g. by a protein-energy enriched formula, may be necessary to improve arginine availability to facilitate important arginine functions. Also, our results suggest that plasma arginine concentrations in the fed state may not accurately reflect its availability for its functions.

Effect of inflammation

Our results are well in line with studies in septic adults by our group and others [6, 17, 26]: arginine appearance was unchanged; citrulline production, *de novo* arginine synthesis and plasma arginine and citrulline concentrations were severely reduced. NO synthesis was either lower [6, 26] or unchanged [17]. The novelty of our study is the direct relationship between changes in arginine metabolism and severity of inflammation. Citrulline production and *de novo* arginine synthesis in our subjects were in the same range as in adults, despite age differences. Thus, inflammation driven changes in arginine metabolism may be age-independent. Decreased precursor availability, altered gut function or enterocyte mass loss may cause reduced plasma citrulline concentrations and reduced citrulline production [21, 27]. We did not find increased I-FABP concentrations as marker of enterocyte damage or an association with plasma citrulline concentrations, leaving the other two factors as a more plausible cause. The only other study in critically ill children showed higher citrulline production and NO synthesis in septic children than in healthy young adults [28]. The reason for these discrepancies is unclear, since study circumstances were similar.

Our subjects had a higher protein turnover rate than septic adults, which is probably an age-related feature as healthy children have a higher protein turnover rate than healthy adults [29]. In contrast with Luiking et al. and Kao et al [6, 17] we could not demonstrate a direct relation between protein breakdown and inflammation. However, we were not able to compare our results with healthy children, for obvious reasons.

Regarding arginine utilization, Kao et al. [17] reported increased arginine clearance in septic patients. Arginine clearance in our subjects was even higher and tended to be associated with inflammation. In general the amino acid clearance rate is higher during sepsis [30] due to increased gluconeogenesis, oxidation for energy supply and (acute phase) protein synthesis in liver and immune cells [6, 30, 31]. This is consistent with the increased arginine oxidation in septic children demonstrated by Argaman et al. [28]. Increased extrahepatic arginase activity, as found by Luiking et al. in septic adults [6], may be one of the major factors determining reduced plasma arginine concentrations during sepsis [32]. Since arginase competes with nitric oxide synthases for arginine it may even result in reduced NO synthesis [32].

Effect of nutrition

We are the first to describe that arginine appearance was increased using a protein-energy enriched formula as compared to a standard formula, and resulted in increased NO synthesis. Yu et al. studied the effect of total parenteral nutrition in burned children and found increased arginine and leucine appearance as compared to a basal state without parenteral amino acid administration [23]. As previously reported the protein-energy enriched formula was safe and well tolerated [10]. Various groups have studied arginine supplementation in NO deficient diseases in children to improve NO synthesis [7-9]. We propose that a well-balanced protein-energy enriched infant formula may be an effective and more physiological tool to achieve this goal. Especially, since the safety of arginine supplementation in patients with severe inflammation has been questioned [33].

Interestingly, the relative contribution of arginine intake, *de novo* arginine synthesis and protein breakdown as components of arginine appearance was similar in the PE-group and the S-group. We expected that protein breakdown would be relatively lower in the PE-group, because increased arginine intake may blunt arginine release from protein. It should be noticed, that we were not able to correct for splanchnic extraction of nutritional arginine, thus underestimating the rate of arginine release from protein. We assume that relative splanchnic arginine extraction was not different between the PE-group and the S-group, since in these groups splanchnic phenylalanine extraction was similar as well [11]. Arginine clearance was increased in the PE-group, possibly in part through increased arginase activity, which is in line with the increased plasma ornithine concentrations.

NO synthesis in relation to arginine plasma concentrations

Although low plasma arginine concentrations may limit NO synthesis [20], we did not find a relationship between the two variables in the fasted nor fed state. NO synthesis seemed rather to be regulated by the total arginine appearance; in the fed state predominantly by arginine intake and protein breakdown. Providing protein-energy enriched formulas might therefore be feasible in diseases with insufficient NO synthesis in children. Remarkably, plasma arginine concentrations did not significantly increase with the protein-energy enriched formula. An

important implication of our results therefore is that plasma arginine concentrations may not be a good predictor of arginine (dis)appearance in the fed state, hence of availability of arginine for its functions. Plasma arginine concentrations are often used as such in the literature [20].

The small sample size and lack of healthy controls is a limitation of our study, but a consequence of ethical considerations. Possibly, we did not find a relationship between CRP and *de novo* arginine synthesis because of the small sample. A larger study in critically ill children with varying grades of inflammation and different amounts of protein and energy intake is warranted to confirm our results. Furthermore, we were obliged to use two different tracer-sets to determine arginine kinetics. Our group compared both sets of tracers previously in a pilot experiment in mice (YC Luiking et al, unpublished observations, 2004) [6]. Measurement of arginine metabolism and NO synthesis with both tracers appeared to be comparable.

In conclusion, arginine becomes an essential amino acid in critically ill children, of which the degree depends on the severity of inflammation. Arginine appearance and NO synthesis can be increased with a protein-energy enriched formula in critically ill infants, despite not affecting plasma arginine concentrations.

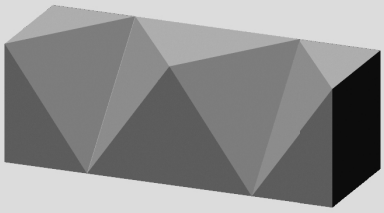
ACKNOWLEDGEMENTS

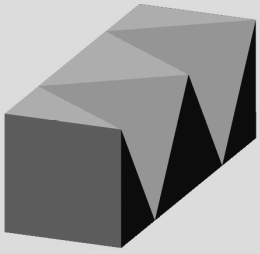
Our gratitude goes out to the children who participated in the study and their parents or representatives. We also thank the PICU nursing and medical staffs for their support in conducting the tracer protocols, John Thaden, Joshua Spore and Marlou Adriaanse for sample analysis and Dimitris Rizopoulos for statistical consultancy.

REFERENCES

1. Wu G, Morris SM, Jr. *Arginine metabolism: nitric oxide and beyond.* Biochem J. 1998;**336 (Pt 1)**:1-17.
2. Morris SM, Jr. *Arginine: beyond protein.* Am J Clin Nutr. 2006;**83**(2):508S-512S.
3. Cynober L, Moinard C, De Bandt JP. *The 2009 ESPEN Sir David Cuthbertson. Citrulline: a new major signaling molecule or just another player in the pharmaconutrition game?* Clin Nutr. 2010;**29**(5):545-51.
4. Davis JS, Anstey NM. *Is plasma arginine concentration decreased in patients with sepsis? A systematic review and meta-analysis.* Crit Care Med. 2011;**39**(2):380-5.
5. van Waardenburg DA, de Betue CT, Luiking YC, Engel M, et al. *Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation.* Am J Clin Nutr. 2007;**86**(5):1438-44.
6. Luiking YC, Poeze M, Ramsay G, Deutz NE. *Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production.* Am J Clin Nutr. 2009;**89**(1):142-52.
7. Amin HJ, Zamora SA, McMillan DD, Fick GH, et al. *Arginine supplementation prevents necrotizing enterocolitis in the premature infant.* J Pediatr. 2002;**140**(4):425-31.
8. Morris CR, Morris SM, Jr., Hagar W, Van Warmerdam J, et al. *Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease?* Am J Respir Crit Care Med. 2003;**168**(1):63-9.
9. Smith HA, Canter JA, Christian KG, Drinkwater DC, et al. *Nitric oxide precursors and congenital heart surgery: a randomized controlled trial of oral citrulline.* J Thorac Cardiovasc Surg. 2006;**132**(1):58-65.
10. van Waardenburg DA, de Betue CT, Goudoever JB, Zimmermann LJ, et al. *Critically ill infants benefit from early administration of protein and energy-enriched formula: a randomized controlled trial.* Clin Nutr. 2009;**28**(3):249-55.
11. de Betue CT, van Waardenburg DA, Deutz NE, van Eijk HM, et al. *Increased protein-energy intake promotes anabolism in critically ill infants with viral bronchiolitis: a double-blind randomised controlled trial.* Arch Dis Child. 2011;**96**(9):817-22.
12. Pollack MM, Ruttimann UE, Getson PR. *Pediatric risk of mortality (PRISM) score.* Crit Care Med. 1988;**16**(11):1110-6.
13. Wolfe RR, Chinkes DL. *Isotope tracers in metabolic research: principles and practice of kinetic analysis.* 2nd ed. ed2004, Hoboken, NJ: John Wiley & Sons
14. Vogt JA, Chapman TE, Wagner DA, Young VR, et al. *Determination of the isotope enrichment of one or a mixture of two stable labelled tracers of the same compound using the complete isotopomer distribution of an ion fragment; theory and application to in vivo human tracer studies.* Biol Mass Spectrom. 1993;**22**(10):600-12.
15. Castillo L, Beaumier L, Ajami AM, Young VR. *Whole body nitric oxide synthesis in healthy men determined from [15N] arginine-to-[15N]citrulline labeling.* Proc Natl Acad Sci U S A. 1996;**93**(21):11460-5.
16. Castillo L, Chapman TE, Sanchez M, Yu YM, et al. *Plasma arginine and citrulline kinetics in adults given adequate and arginine-free diets.* Proc Natl Acad Sci U S A. 1993;**90**(16):7749-53.
17. Kao CC, Bandi V, Guntupalli KK, Wu M, et al. *Arginine, citrulline and nitric oxide metabolism in sepsis.* Clin Sci (Lond). 2009;**117**(1):23-30.
18. Castillo L, Yu YM, Marchini JS, Chapman TE, et al. *Phenylalanine and tyrosine kinetics in critically ill children with sepsis.* Pediatr Res. 1994;**35**(5):580-8.
19. Luiking YC, Poeze M, Dejong CH, Ramsay G, et al. *Sepsis: an arginine deficiency state?* Crit Care Med. 2004;**32**(10):2135-45.
20. Cynober LA. *Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance.* Nutrition. 2002;**18**(9):761-6.
21. Deutz NE. *The 2007 ESPEN Sir David Cuthbertson Lecture: amino acids between and within organs. The glutamate-glutamine-citrulline-arginine pathway.* Clin Nutr. 2008;**27**(3):321-7.
22. Yu YM, Ryan CM, Burke JF, Tompkins RG, et al. *Relations among arginine, citrulline, ornithine, and leucine kinetics in adult burn patients.* Am J Clin Nutr. 1995;**62**(5):960-8.

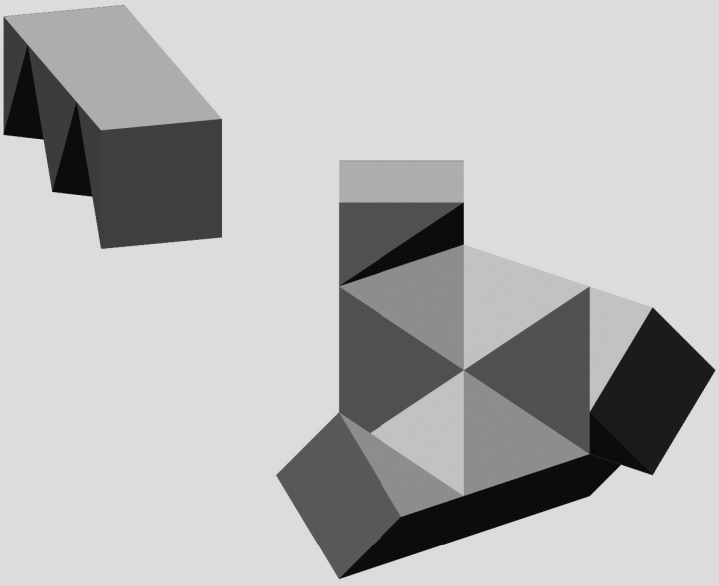
23. Yu YM, Sheridan RL, Burke JF, Chapman TE, et al. *Kinetics of plasma arginine and leucine in pediatric burn patients.* Am J Clin Nutr. 1996;**64**(1):60-6.
24. Yu YM, Young VR, Castillo L, Chapman TE, et al. *Plasma arginine and leucine kinetics and urea production rates in burn patients.* Metabolism. 1995;**44**(5):659-66.
25. Yu YM, Ryan CM, Castillo L, Lu XM, et al. *Arginine and ornithine kinetics in severely burned patients: increased rate of arginine disposal.* Am J Physiol Endocrinol Metab. 2001;**280**(3):E509-17.
26. Villalpando S, Gopal J, Balasubramanyam A, Bandi VP, et al. *In vivo arginine production and intravascular nitric oxide synthesis in hypotensive sepsis.* Am J Clin Nutr. 2006;**84**(1):197-203.
27. Piton G, Manzon C, Cypriani B, Carbonnel F, et al. *Acute intestinal failure in critically ill patients: is plasma citrulline the right marker?* Intensive Care Med. 2011;**37**(6):911-7.
28. Argaman Z, Young VR, Noviski N, Castillo-Rosas L, et al. *Arginine and nitric oxide metabolism in critically ill septic pediatric patients.* Crit Care Med. 2003;**31**(2):591-7.
29. Waterlow JC. *Protein turnover with special reference to man.* Q J Exp Physiol. 1984;**69**(3):409-38.
30. Druml W, Heinzel G, Kleinberger G. *Amino acid kinetics in patients with sepsis.* Am J Clin Nutr. 2001;**73**(5):908-13.
31. Luiking YC, Deutz NE. *Exogenous arginine in sepsis.* Crit Care Med. 2007;**35**(9 Suppl):S557-63.
32. Bansal V, Ochoa JB. *Arginine availability, arginase, and the immune response.* Curr Opin Clin Nutr Metab Care. 2003;**6**(2):223-8.
33. Heyland DK, Novak F, Drover JW, Jain M, et al. *Should immunonutrition become routine in critically ill patients? A systematic review of the evidence.* JAMA. 2001;**286**(8):944-53.





PART 3

INTERVENTIONAL CHALLENGES – GLUCOSE INTAKE

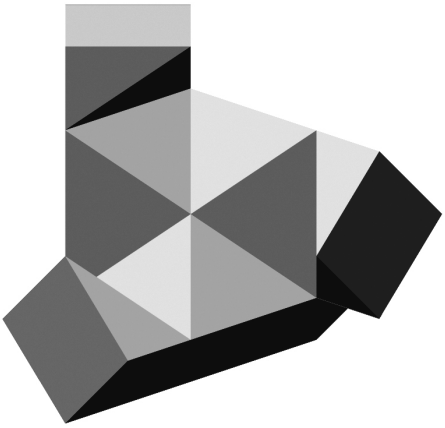


When one does a thing, it appears good, otherwise one would not write it. Only later comes reflection, and one discards or accepts the thing. Time is the best censor, and patience a most excellent teacher.

Frédéric Chopin (1810-1849)

chapter 4

REDUCING GLUCOSE INFUSION SAFELY PREVENTS HYPERGLYCEMIA IN POST-SURGICAL CHILDREN



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*Clin Nutr. 2011;**30**(6):786-92*

ABSTRACT

Background and aims: To investigate the effects of two different glucose infusions on glucose homeostasis and amino acid metabolism in post-surgical children.

Methods: This randomized crossover study evaluated glucose and amino acid metabolism in eight children (age 9.8 ± 1.9 months, weight 9.5 ± 1.1 kg) admitted to a pediatric intensive care unit in a tertiary university hospital after surgical correction for non-syndromal craniosynostosis. Patients were randomized to receive low (LG; 2.5 $mg.kg^{-1}.min^{-1}$) and standard (SG; 5.0 $mg.kg^{-1}.min^{-1}$) glucose infusion in a crossover setting. After a bolus (4 $g.kg^{-1}$) of deuteriumoxide, we conducted a primed, constant, 8 h tracer infusion with [6,6-$^{2}H_{2}$]Glucose, [1-^{13}C] Leucine, [ring-$^{2}H_{5}$]Phenylalanine and [3,3-$^{2}H_{2}$]Tyrosine.

Results: SG resulted in hyperglycemia (defined as > 6.1 $mmol.L^{-1}$), while during LG plasma glucose levels were normoglycemic (5.9 ± 0.6 vs. 7.5 ± 1.7 $mmol.L^{-1}$; LG vs. SG respectively, p = .02). Hypoglycemia did not occur during LG infusion. Endogenous glucose production was not fully suppressed during the hyperglycemic state under SG and increased with reduced glucose infusion (2.6 ± 1.5 vs. 1.1 ± 1.4 $mg.kg^{-1}.min^{-1}$; LG vs. SG; p = .05). Whole body protein balance derived from leucine and phenylalanine kinetics was slightly negative but not further affected with a decrease in glucose infusion.

Conclusions: The current recommended glucose infusion induces hyperglycemia in post-surgical children. A reduced glucose infusion safely reduced high glucose levels, while children were capable to sustain normoglycemia with increased endogenous glucose production. The reduced glucose infusion did not exacerbate the mild catabolic state in which the patients were.

LIST OF ABBREVIATIONS

α-KIC	α-Ketoisocaproate
APE	Atom percent excess
CRP	C-reactive protein
EGP	Endogenous glucose production
LG	Low glucose infusion
MPE	Mass percent excess
NHPD	Non-hydroxylation phenylalanine disposal
NOLD	Non-oxidative leucine disposal
PICU	Pediatric Intensive Care Unit
Ra	Rate of appearance
Rd	Rate of disappearance
SG	Standard glucose infusion

INTRODUCTION

Plasma glucose levels are the resultant of exogenous glucose supply and endogenous glucose production on the one hand and glucose oxidation or storage as glycogen and triglycerides on the other. Critically ill children are at increased risk to a disturbance in this balance leading to hyper- as well as hypoglycemia [1].

Hyperglycemia is a frequent complication and associated with increased morbidity and mortality in pediatric intensive care units (PICU's) [2]. Notwithstanding the widespread implementation of tight glucose regimens [3], concerns regarding hypoglycemia have been raised [4]. Recently, insulin therapy to achieve normoglycemia has been shown to improve morbidity as well as mortality in critically ill children, but also led to hypoglycemia (≤ 40 $mg.dL^{-1}$ ~ ≤ 2.2 $mmol.L^{-1}$) and severe hypoglycemia (≤ 31 $mg.dL^{-1}$ ~ ≤ 1.7 $mmol.L^{-1}$) in 87 (25%) and 17 (5%) children, respectively[5].

The child's developing brain is more susceptible to hypoglycemia which can result in permanent damage [6-8]. Furthermore, young age is a risk factor for developing hypoglycemia, especially when the child is ill [9, 10]. This has led to a debate questioning the risks of insulin therapy in the pediatric population [11, 12].

An alternative to insulin therapy is to reduce the amount of glucose infusion in post-surgical children admitted to the PICU. This approach, however, also has two potential detrimental side effects; an increased risk for hypoglycemia and an amplification of an already increased protein catabolism. Currently no data exist on the impact of different glucose infusions on glucose kinetics and amino acid metabolism in post-surgical children admitted to the PICU.

We hypothesized that in post-surgical children, reduced glucose infusion will improve plasma glucose levels without affecting glucose production rates or amino acid metabolism. Therefore, our first objective was to determine the impact of standard or low glucose infusions on glucose homeostasis and kinetics. Our second objective was to determine whether a low glucose infusion would affect protein and amino acid catabolism.

METHODS

Patient characteristics

The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. The studies were performed in children after surgical correction for non-syndromal craniosynostosis within 6 hours after admission to the PICU of the Erasmus Medical Center - Sophia Children's Hospital. Written informed consent was obtained from the parents. All were assessed by the Pediatric Logistic Organ Dysfunction (PELOD) score [13], Pediatric Index of Mortality (PIM2) [14, 15] and the Pediatric Risk of Mortality III (PRISM III) score [16], which are validated measures of the severity of multiple

organ dysfunction in PICU's. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded.

Study design

The experimental design, shown in **Figure 4.1**, consisted of a cross-over design, with a 4h period of intravenous low glucose infusion (LG; 2.5 mg.kg^{-1}.min^{-1}) versus a 4h period of standard glucose infusion (SG; 5.0 mg.kg^{-1}.min^{-1}) [17]. Patients were randomized for the order of glucose infusion through a computer generated envelope. Laboratory personnel, nursing staff and investigators were blinded until analyses were finished. Six hours after admission (t=0) to the PICU, an intravenous deuterium oxide infusion (^2H_2O; 4 gr.kg^{-1}) was administered in one hour to prime the body water pool. Two hours later (t=120), after obtaining baseline blood samples, the intravenous glucose infusion as per standard care (4.0–6.0 mg.kg^{-1}.min^{-1}) [17] was stopped and the study glucose infusion (SG or LG) started. Simultaneously, patients received a primed, continuous, 8h intravenous tracer infusion (see below). Four hours after start of the tracer infusion (t=360) the glucose infusion was switched.

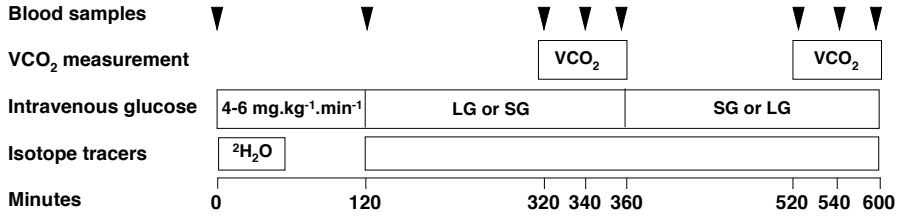


Figure 4.1 – Schematic presentation of the tracer infusion study in eight post-surgical infants receiving either low (LG, 2.5 mg.kg^{-1}.min^{-1}) or standard (SG, 5.0 mg.kg^{-1}.min^{-1}) glucose infusion.

Black triangles indicate time points for plasma collection for laboratory parameters and isotopic enrichment measurements. Square boxes represent carbon dioxide production (VCO_2) measurements.

Tracer Infusion studies

All isotope tracers (Cambridge Isotope Laboratories, Andover, MA, USA) were tested for sterility and pyrogenicity after they were compounded at the investigational pharmacy at Erasmus Medical Center, Rotterdam, the Netherlands.

At t=120, the bicarbonate pool was primed with 2.1 μmol.kg^{-1} $NaH^{13}CO_2$, followed by a 8-hour primed, continuous tracer infusion of [6,6-$^2H_2$]glucose (40 μmol.kg^{-1}; 48 μmol.kg^{-1}.h^{-1}), L-[1-^{13}C]leucine (8 μmol.kg^{-1}; 8 μmol.kg^{-1}.h^{-1}), [ring-$^2H_5$]Phenylalanine (5.4 μmol.kg^{-1}; 4.1 μmol.kg^{-1}.h^{-1}), [3,3-$^2H_2$]Tyrosine (3.6 μmol.kg^{-1}; 3.0 μmol.kg^{-1}.h^{-1}). The [ring-$^2H_5$]Phenylalanine derived tyrosine pool was primed with [ring-$^2H_4$]Tyrosine (2.5 μmol.kg^{-1}).

Measurements and sample analysis

Blood samples were obtained at standard frequent intervals (**Figure 4.1**), centrifuged (2 min 6000 rpg) and frozen at -80°C until samples were analyzed.

Isotopic enrichment of deuterium in plasma body water was determined by isotope ratio mass spectrometry (Delta+XP IRMS Thermo Fisher, Bremen, Germany). Enrichments of glucose labeled with ^{2}H were measured by gas chromatography-mass spectrometry (GC-MS) (GC 6890, MS 5973N; Agilent Technologies, Wilmington, DE) using the penta-acetate derivative as previously described [18, 19].

Leucine kinetics were calculated from plasma alpha-ketoisocaproate (α-KIC) enrichment, which were determined by GC-MS after derivatization to butyldimethyl-silylquinoxalinol derivatives [20]. Plasma isotopic enrichments of [ring-$^{2}H_5$]Phenylalanine, [ring-$^{2}H_4$]Tyrosine and [3,3-$^{2}H_2$]Tyrosine were determined by GC-MS after using the *N*-ethoxycarbonylethylester derivative according to a modified method of Husek[21].

Carbon dioxide production (VCO_2), oxygen (VO_2) consumption and respiratory quotient (RQ), were obtained with a metabolic monitor in canopy mode (Deltatrac™ I MBM-200, Datex Division Instrumentarium Corp., Finland). To determine the enrichment of $^{13}CO_2$ in whole blood, 1.5 mL of perchloric acid 10% was added to 1.5 mL of whole blood in a vacutainer to release the CO_2. The released gas was transferred to a vacuum impermeable glass tube and $^{13}CO_2$ was determined with IRMS [22, 23].

Plasma samples for glucose, insulin, cortisol, triglycerides, and free fatty acids were determined by standard in-house protocols. Plasma glucose levels > 6.1 mmol.L^{-1}(> 110 mg.dL^{-1}) were considered hyperglycemic [24].

Calculations

Whole body kinetics of protein were calculated by conventional isotope dilution equations using a stochastic model during steady state enrichment [25] and glucose kinetics were estimated using the Steele equation [26], based upon the last 40 min of both study periods. At steady state plateau, rate of appearance (Ra) equals the rate of disappearance (Rd) as follows:

$$Ra = Rd = i \times (E_{inf}/E_{pl} - 1) \quad (1)$$

where i is the infusion rate of the labeled tracer, E_{inf} is the tracer enrichment of the infusate and E_{pl} the tracer enrichment in plasma.

Glucose kinetics

Endogenous glucose production (EGP) rate is calculated as follows:

$$EGP = Ra_{Glucose} - GIR \quad (2)$$

where GIR is the total glucose infusion rate in $mg.kg^{-1}.min^{-1}$.

Fractional gluconeogenesis is calculated as previously described [19]. Briefly, the average enrichment of ^2H on each glucose carbon was calculated with the following equation:

$$\text{Average (M+1)d} = (M+1)d_{(m/z169)}/6 \quad (3)$$

where $(M+1)d_{(m/z169)}$ is the M+1 enrichment of deuterium of glucose measured using *m/z* 170/169 and "6" is the number of ^2H labeling sites on the *m/z* 169 fragment of glucose.

Because body water is the precursor pool for deuterium or hydrogen, the extent of deuterium labeling of glucose during the gluconeogenic process when ^2H_2O is infused is a measure of fractional gluconeogenesis. Therefore, with the average deuterium enrichment in *m/z* 170/169 for calculating fractional gluconeogenesis (FracGNG), the equation is

$$\text{FracGNG} = \text{average(M+1)d}/E_{^2H_2O} \quad (4)$$

where $E_{^2H_2O}$ is the deuterium enrichment in body water.

The absolute rate of appearance of plasma glucose from gluconeogenesis (Ra_{GNG}) and glycogenolysis were calculated as follows:

$$\text{Gluconeogenesis} = Ra_{Gluc} \text{ x FracGNG} \quad (5)$$

$$\text{Glycogenolysis} = \text{EGP} - \text{Gluconeogenesis} \quad (6)$$

Amino acid metabolism

Whole body leucine, phenylalanine and tyrosine fluxes were calculated according to *(1)* as previously described [27-29].

Leucine oxidation rates were calculated as follows;

$$\text{LeucineOx} = VCO_2 \text{ x } (E^{13}CO_2/69.18)/[^{13}C]\alpha\text{–KIC} \quad (7)$$

where 69.18 is the $^{13}CO_2$ refraction correction factor for critically ill children [30]. VCO_2 is measured in mL/min and converted to mMol/h by multiplying by 60 min and dividing by 22.4; the number of 1 in 1 mole of an ideal gas at standard temperature and pressure to convert to mL/min. Non-oxidative leucine disposal (NOLD; leucine converted into protein synthesis) was calculated as follows:

$$\text{NOLD} = Ra_{leu} - \text{LeucineOx} \quad (8)$$

Phenylalanine hydroxylation (rate of phenylalanine conversion to tyrosine) was calculated as follows [31, 32];

$$\text{Hydroxylation} = Ra_{tyr} \times (E_{[^2H_4]tyr}/E_{[^2H_5]Phe}) \times (Ra_{Phe}/(i_{Phe}+Ra_{Phe})) \times 2.2 \quad (9)$$

where Ra_{Phe} and Ra_{Tyr} are the phenylalanine and tyrosine fluxes; $E_{[^2H_4]tyr}$ and $E_{[^2H_5]Phe}$ are the plasma enrichments; and i_{Phe} is the infusion rate of labeled phenylalanine and the term (Ra_{Phe}/(i_{Phe}+Ra_{Phe}) corrects for the contribution of the tracer infusion to Ra_{Phe}. The factor 2.2 is to correct for the secondary deuterium-isotope kinetic effect for in vivo hydroxylation in fasted state as described and validated previously [31, 32]. Non-hydroxylation phenylalanine disposal (NHPD; phenylalanine converted into protein synthesis) was calculated as follows:

$$\text{NHPD} = Ra_{Phe} - \text{Hydroxylation} \quad (10)$$

Whole body protein metabolism

Under the assumption that 1 gram of protein contains approximately 621 μmol of leucine [33] and 280 μmol of phenylalanine[34], it is then possible to convert leucine and phenylalanine kinetics ($\mu mol.kg^{-1}.h^{-1}$) into protein kinetics ($g.kg^{-1}.d^{-1}$). Whole body protein turnover was calculated from the model described by Golden and Waterlow [35].

Statistical analysis

A prospective power analysis revealed that 8 patients with complete data, would detect a difference of 20% of plasma glucose levels (80% power, type I error of 5%). The Shapiro-Wilk normality test was used to determine whether data were normally distributed. Comparison between the two different glucose infusions at both infusion rates was made using the paired student's t-test. For non-parametric data the Wilcoxon matched pairs test was used. Data are presented as the mean ± standard deviation unless non-parametric in which case they are presented as median and interquartile range. Statistical significance was considered at $p < 0.05$. Repeated measures ANOVA were used to analyze the effect of glucose infusion on parameters of glucose and protein metabolism over time and between LG and SG. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, La Jolla, CA., USA). This trial was registered in the Dutch trial register (www.trialregister.nl) under number NTR2079.

RESULTS

Patient characteristics

Eight children (9.8±1.9 months) admitted to the PICU after surgical correction for non-syndromal craniosynostosis were included (**Table 4.1**). All patients were hemodynamically

stable without vasoactive drugs and breathing spontaneously with a FiO_2 < 0.6. They received opioids or acetaminophen as pain relief and were not sedated or receiving muscle relaxation. Patients did not receive (par)enteral nutrition other than intravenous glucose as per standard care (4-6 $mg.kg^{-1}.min^{-1}$)[17].

Table 4.1 – Demographic and nutritional data of eight post-surgical children.[a]

Age (months)	9.8 ± 1.9		
Gender (male: female)	6:2		
Weight (kg)	9.5 ± 1.1		
Height (cm)	74.3 ± 3.0		
PELOD[b]	10.1 ± 7.6		
PRISM III[c]	7.4 ± 3.7		
PIM2[d]	14.2 ± 2.8		
	LG[e]	**SG[f]**	**P-value**
Glucose infusion ($mg.kg^{-1}.min^{-1}$)	2.6 ± 0.1	5.2 ± 0.1	< 0.0001
VO_2 ($mL.min^{-1}$)	69 ± 24	67 ± 17	0.79
VCO_2 ($mL.min^{-1}$)	59 ± 11	55 ± 14	0.29
Resting energy expenditure ($kcal.kg^{-1}.d^{-1}$)	49.8 ± 17.6	49.7 ± 14.9	0.98
Respiratory quotient	0.88 ± 0.14	0.83 ± 0.10	0.37
Caloric intake ($kcal.kg^{-1}.day^{-1}$)	12.7 ± 0.2	25.3 ± 0.5	< 0.0001
Caloric intake (%)[g]	24 ± 1	47 ± 2	< 0.0001
Glucose ($mmol.L^{-1}$)	5.9 ± 0.6	7.5 ± 1.7	0.02
Triglycerides ($mmol.L^{-1}$)	0.38 ± 0.25	0.43 ± 0.20	0.54
Free fatty acids ($mmol.L^{-1}$)	0.72 ± 0.20	0.63 ± 0.12	0.34
C-reactive protein ($mg.dL^{-1}$)	24 ± 13	26 ± 16	0.61
Cortisol ($nmol.L^{-1}$)	649 ± 160	681 ± 205	0.77
Insulin ($pmol.L^{-1}$)	64 ± 48	90 ± 51	0.16

[a] All values are mean ± SD.
[b] PELOD, Pediatric Logistic Organ Dysfunction (17).
[c] PRISM III, Pediatric Risk of Mortality III (20).
[d] PIM2, Pediatric index of Mortality (18, 19).
[e] LG, Low glucose.
[f] SG, Standard glucose.
[g] Caloric intake as percentage of requirements according to the Schofield equation (59).

Laboratory values and hormone concentrations

Patients were hyperglycemic during standard glucose infusion (SG), while during LG plasma glucose levels were lower and normoglycemic (**Figure 4.2, Table 4.1**). LG did not cause hypoglycemia, the lowest plasma glucose was 5.1 $mmol.L^{-1}$ (91.8 $mg.dL^{-1}$). Insulin plasma concentrations did not differ significantly (**Table 4.1**).

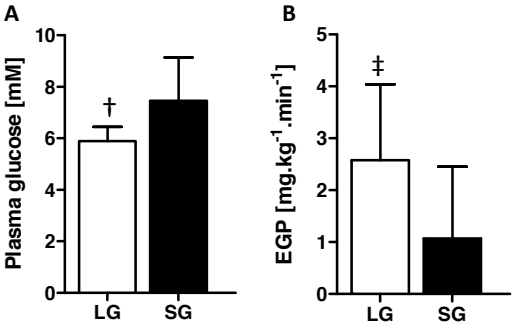


Figure 4.2 – Glucose metabolism during low and standard glucose infusion in eight post-surgical infants.

Panel A. Plasma glucose concentration, mmol.L^{-1}, mean ± SD, † p =0.02. Panel B. Endogenous glucose production mg.kg^{-1}.min^{-1}, mean ± SD, ‡ p =0 .05.

Stable Isotope kinetics

Steady state conditions were achieved during the last 40 minutes of each study period (data not shown).

Glucose kinetics

The deuterium enrichment of body water was 0.59±0.02 and 0.58±0.03 MPE, at LG and SG respectively. Glucose Ra did not differ between glucose protocols (**Table 4.2**). EGP increased during LG (**Figure 4.2, Table 4.2**). Absolute gluconeogenesis and glycogenolysis were not significantly different (p=0.08) and glycogenolysis was not significantly different from zero (**Table 4.2**).

Table 4.2 – Glucose kinetics in eight post-surgical infants during two different glucose infusions.[a]

	LG[d]	SG[e]	P-value
Glucose Ra[b]/Rd[c] mg.kg^{-1}.min^{-1}	5.3 ± 1.5	6.4 ± 1.5	0.14
Endogenous Glucose Production mg.kg^{-1}.min^{-1}	2.6 ± 1.5	1.1 ± 1.4	0.05
Fractional gluconeogenesis % of Ra[b]	43 ± 2	29 ± 7	< 0.01
Absolute Gluconeogenesis mg.kg^{-1}.min^{-1}	2.3 ± 0.6	1.8 ± 0.4	0.08
Glycogenolysis mg.kg^{-1}.min^{-1}	0.3 ± 0.9	−0.7 ± 1.1	0.08

[a] All values are depicted as mean ± SD.
[b] Ra, rate of appearance.
[c] Rd, rate of disappearance.
[d] LG, low glucose.
[e] SG, standard glucose.

Amino acid kinetics

Leucine Ra, oxidation and NOLD did not differ (**Table 4.3**). Phenylalanine and tyrosine Ra did not differ. Phenylalanine hydroxylation was significantly higher at the lower glucose infusion rate (**Table 4.3**). However, NHPD (**Table 4.3**) and the phenylalanine hydroxylation fraction of the total phenylalanine Rd did not differ (12±3% vs. 11±3%; LG vs. SG, p=0.07).

Table 4.3 – Leucine, phenylalanine and tyrosine kinetics in eight post-surgical infants during two different glucose infusions.[a]

	LG[b]	SG[c]	P-value
Leucine Ra[d]	160 ± 15	158 ± 16	0.68
Leucine oxidation	33 (22–82)	29 (22–62)	0.38
NOLD[e]	122 ± 14	124 ± 20	0.76
Phenylalanine Ra	70 ± 6	68 ± 5	0.25
Tyrosine Ra	39 ± 2	37 ± 2	0.06
Phenylalanine hydroxylation	8.4 ± 1.7	7.4 ± 1.6	0.04
NHPD[f]	61 ± 6	61 ± 6	1.0

[a] All values are measured in μmol kg^{-1} h^{-1} and depicted in mean ± SD, except for Leucine oxidation which is depicted in median (range).
[b] LG, Low glucose.
[c] SG; Standard glucose.
[d] Ra; rate of appearance.
[e] NOLD; non-oxidative leucine disposal.
[f] NHPD; non-hydroxylation phenylalanine disposal.

Whole body protein metabolism

Whole body protein metabolism corresponding with leucine kinetics was as follows (**Figure 4.3**). Protein synthesis (4.7±0.5 vs. 4.8±0.8 $g.kg^{-1}.d^{-1}$) and breakdown (5.9±0.6 vs. 5.8±0.6 $g.kg^{-1}.d^{-1}$) did not differ between LG and SG respectively. Protein balance was negative and did not differ (-1.2±0.8 vs. -1.0±0.6 $g.kg^{-1}.d^{-1}$, LG vs. SG). Whole body protein metabolism corresponding with phenylalanine and tyrosine kinetics was as follows (**Figure 4.3**). Protein synthesis (5.3±0.6 vs. 5.2±0.5 $g.kg^{-1}.d^{-1}$) and breakdown (5.6±0.5 vs. 5.4±0.4 $g.kg^{-1}.d^{-1}$) did not differ between LG and SG respectively. Protein balance was negative and showed a statistically significant but not clinical relevant difference (-0.3±0.1 vs. -0.2±0.1 $g.kg^{-1}.d^{-1}$; LG vs. SG, p=0.04).

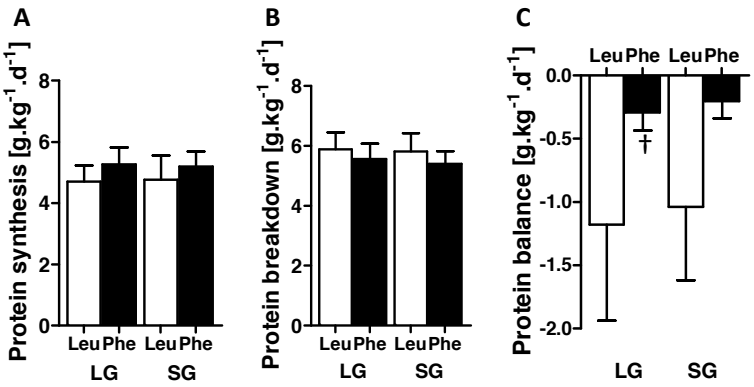


Figure 4.3 – Whole body protein metabolism during low and standard glucose infusion in eight post-surgical infants.

Leu = leucine, Phe = Phenylalanine. Panel A, protein synthesis. Panel B, protein breakdown. Panel C, protein balance; Values are $g.kg^{-1}.d^{-1}$, mean ± SD, †p =0.04; LG vs. SG.

DISCUSSION

Tight glucose control improves morbidity and mortality in critically ill children, although hypoglycemia is a frequent and serious side effect of insulin therapy [5]. In this study we showed that in post-surgical children, normoglycemia could be achieved by a reduced glucose infusion, without occurrence of hypoglycemia, although no conclusion can be drawn outside our study intervention period. The reduced glucose infusion, half of what is considered standard practice for age [17], can be considered an alternative to insulin therapy in the initial phase of glycemic management. Additionally we observed that EGP was not fully suppressed, despite high plasma glucose levels. Moreover, reducing the glucose infusion induced an increase in EGP without a significant increase in either gluconeogenesis or glycogenolysis (both p=0.08). Furthermore, reduced glucose did not notably affect the negative whole body protein balance, measured with leucine and phenylalanine kinetics.

Our study is the first to show the feasibility of reduced glucose infusion in post surgical children to prevent or treat hyperglycemia. We found that during LG plasma glucose levels were lower and normoglycemic, not due to an increased rate of disposal or decreased glucose production, but solely through a reduced glucose infusion rate. Due to the paucity of data in children it is difficult to define lower limits of glucose infusion. Current recommendations are based largely upon data for children extracted from the relation between glucose intake and 1) glucose uptake by the brain, 2) glucose oxidation, 3) endogenous glucose production (EGP), and 4) protein and amino acid catabolism [17]. Although conditions are different in post-surgical children, we used these considerations to determine whether LG was within safe limits in our population. We acknowledge that we did not quantify cerebral glucose uptake. Additionally, exact determination of glucose oxidation with [^{13}C]tracer data was not possible

in our study, because of interference with our [^{13}C]Leucine tracer. Therefore, we cannot provide clear insight in the impact of LG on cerebral glucose uptake and utilization or oxidation. However, glucose oxidation calculated from the oxygen consumption and carbon dioxide production did not change. Of greater importance, hypoglycemia did not occur during low glucose infusion. This suggests that the reduced glucose intake did not negatively impact cerebral glucose uptake or utilization.

EGP rates in our infants, receiving 2.5 $mg.kg^{-1}.min^{-1}$, were lower than those measured in healthy infants, fasted for 8–9h (7.1±0.3 $mg.kg^{-1}.min^{-1}$)[36]. So, although an increased EGP was observed during LG, their production was not at their full potential. During both glucose infusions glycogenolysis was almost completely blocked. Glycogenolysis was probably suppressed by elevated insulin levels and suppressed glucagon levels during glucose infusion [37], although the latter could not be measured in our study due to blood draw limitations. Considering the exogenous glucose our patients received it is not likely that glycogen stores were already depleted. Therefore, it appears safe to conclude that our infants were capable of sustaining normoglycemia, without a direct risk of developing hypoglycemia. However, conclusions cannot be drawn for a longer period beyond the study period of reduced glucose infusion we presented.

Of interest, even during SG, despite supraphysiological glucose and insulin levels, EGP was not fully suppressed. During both glucose infusions, gluconeogenesis constituted almost all of the EGP and it appears that gluconeogenesis in contrast to glycogenolysis was unresponsive to insulin, high glucose concentrations and/or infusion. A clear association exists between increased contribution of gluconeogenesis and insulin resistance [38]. The expression of phosphoenolpyruvate-carboxy-kinase (PEPCK), the rate-limiting enzyme for gluconeogenesis, is increased and less sensitive to insulin during critical illness [39]. Therefore, we hypothesize that the increase in EGP and moreover gluconeogenesis during LG does not indicate that LG was below the threshold, but that it might indicate a certain state of hepatic insulin resistance in these post-surgical infants.

Our study was not able to show which precursors (amino acids, glycerol or lactate) were predominantly used to fuel gluconeogenesis. Although our static measurements of lipid metabolism (FFA and triglycerides) did not differ (**Table 4.1**), these results do not exclude a change in lipolysis, because we didn't confirm this by means of glycerol isotope tracer infusions. Another important gluconeogenic precursor is lactate. However, as our patients were hemodynamically stable with good organ perfusion, lactate levels are not expected to have increased during either intervention. Finally, protein or amino acid catabolism waswere not significantly increased.

Although LG did not affect leucine kinetics and more specifically oxidation, phenylalanine hydroxylation was slightly but statistically significant higher during LG. Increased availability of plasma amino acids, a known stimulant of hydroxylation [40-42], can be ruled out as an explanation. Furthermore, a physiological response to cope with an energy shortage is unlikely

as the leucine oxidation was unaffected. Possibly, phenylalanine catabolism was induced to provide gluconeogenic precursors. Products derived from phenylalanine hydroxylation can be used for gluconeogenesis, while oxidized leucine can merely be used for the ketogenic pathway [43]. This could explain the difference in catabolism between phenylalanine and leucine. These slight differences in amino acid or protein metabolism during low glucose infusion are not likely to have clinical relevance.

Whole body protein balance was as expected slightly negative during both study periods and consistent with those obtained from septic infants and children [33, 34]. In contrast with our data, normoglycemia, independent of plasma insulin levels, improved protein turnover after abdominal surgery in adults [44]. Our different results may be explained by differences in patient populations and the approach used. Furthermore, there are tissue and age specific differences in the metabolic processes [45]. However, consistent with our data, it has been shown in neonates that different glucose infusions do not affect protein turnover [46]. During critical illness a deficiency in energy supply is not solely responsible for protein catabolism [47]. Proteolysis during critical illness is usually caused by activation of the ubiquitin-proteasome proteolytic pathway (UPP) in muscle initiated by activation of caspase 3 [48, 49], and pathophysiological triggers include activation of lysosome- [50] and calpain-dependent pathways [51]. Furthermore, there appears to be a link between muscle wasting and insulin resistance [52-54].

There are some limitations to our study, some of them inherent to studying critically ill children. Our sample size was small and conclusions from our study are restricted to post-surgical infants. Substrate metabolism greatly differs between infants, children and adolescents both for glucose and amino acid metabolism [17, 45]. Furthermore, various diagnoses, such as trauma, burns or sepsis, as well as differences between single and multi-organ failure yield a different response. We also acknowledge that the negative glycogenolysis rates are physiological not possible. This might be explained by a consistent underestimation of the EGP by the tracer model, as part of the diluted tracer pool, due to newly produced glucose, is taken up by the liver again[55]. These limitations, however, do not weaken the conclusions drawn from our study. Finally, although our study does not provide data on reduced glucose infusion for longer than 4h this approach appears to be safe in the initial postoperative phase which duration is individually dependent but usually lasts 6-24h. Confirmatory studies with reduced glucose infusion during this study period are warranted.

CONCLUSION

Reduced glucose infusion, half of what is considered standard practice for age, in the initial post-surgical phase reduced high glucose levels in infants without occurrence of hypoglycemia. Additionally we observed that the endogenous glucose production was not fully suppressed and almost entirely relied on gluconeogenesis, despite high plasma glucose levels

and exogenous glucose infusion. Furthermore, protein and amino acid catabolism was not exacerbated during the reduced glucose infusion. Although further studies on the optimal time period and (long term) clinical implications are warranted, reduced glucose infusion can be considered an alternative to insulin therapy in the initial glycemic postoperative management.

ACKNOWLEDGEMENTS

Our gratitude goes out to the patients and their families at Erasmus MC Sophia Children's hospital for their selfless contribution to this study. We thank Marianne Maliepaard, Hansje Bredero-Boelhouwer, Gardi Minderman-Voortman and Kristien Dorst for their contribution.

REFERENCES

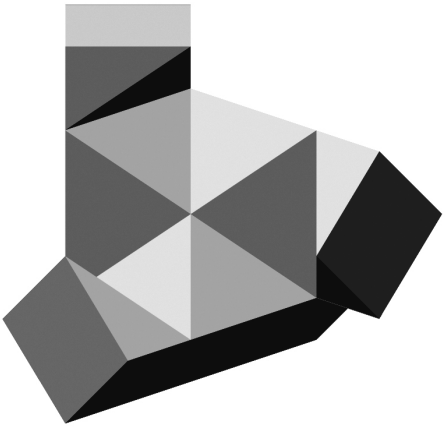
1. Zijlmans WC, van Kempen AA, Serlie MJ, Sauerwein HP. *Glucose metabolism in children: influence of age, fasting, and infectious diseases.* Metabolism. 2009;**58**(9):1356-65.
2. Verbruggen SC, Joosten KF, Castillo L, van Goudoever JB. *Insulin therapy in the pediatric intensive care unit.* Clin Nutr. 2007;**26**(6):677-90.
3. Wilson M, Weinreb J, Hoo GW. *Intensive insulin therapy in critical care: a review of 12 protocols.* Diabetes Care. 2007;**30**(4):1005-11.
4. Wiener RS, Wiener DC, Larson RJ. *Benefits and risks of tight glucose control in critically ill adults: a meta-analysis.* Jama. 2008;**300**(8):933-44.
5. Vlasselaers D, Milants I, Desmet L, Wouters PJ, et al. *Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study.* Lancet. 2009;**373**(9663):547-56.
6. Bober E, Buyukgebiz A. *Hypoglycemia and its effects on the brain in children with type 1 diabetes mellitus.* Pediatr Endocrinol Rev. 2005;**2**(3):378-82.
7. Vannucci RC, Vannucci SJ. *Glucose metabolism in the developing brain.* Semin Perinatol. 2000;**24**(2):107-15.
8. Flykanaka-Gantenbein C. *Hypoglycemia in childhood: long-term effects.* Pediatr Endocrinol Rev. 2004;**1 Suppl 3**:530-6.
9. Solomon T, Felix JM, Samuel M, Dengo GA, et al. *Hypoglycaemia in paediatric admissions in Mozambique.* Lancet. 1994;**343**(8890):149-50.
10. Chaussain JL, Georges P, Calzada L, Job JC. *Glycemic response to 24-hour fast in normal children: III. Influence of age.* J Pediatr. 1977;**91**(5):711-4.
11. Joosten K, Verbruggen SC, Verhoeven JJ. *Glycaemic control in paediatric critical care.* Lancet. 2009;**373**(9673):1423-4; author reply 1424.
12. Preissig C, Rigby M. *Glycaemic control in paediatric critical care.* Lancet. 2009;**373**(9673):1423; author reply 1424.
13. Leteurtre S, Martinot A, Duhamel A, Proulx F, et al. *Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study.* Lancet. 2003;**362**(9379):192-7.
14. Shann F, Pearson G, Slater A, Wilkinson K. *Paediatric index of mortality (PIM): a mortality prediction model for children in intensive care.* Intensive Care Med. 1997;**23**(2):201-7.
15. Slater A, Shann F, Pearson G, Paediatric Index of Mortality Study G. *PIM2: a revised version of the Paediatric Index of Mortality.* Intensive Care Med. 2003;**29**(2):278-85.
16. Pollack MM, Patel KM, Ruttimann UE. *PRISM III: an updated Pediatric Risk of Mortality score.* Crit Care Med. 1996;**24**(5):743-52.
17. Kalhan SC, Kilic I. *Carbohydrate as nutrient in the infant and child: range of acceptable intake.* Eur J Clin Nutr. 1999;**53 Suppl 1**:S94-100.
18. Argoud GM, Schade DS, Eaton RP. *Underestimation of hepatic glucose production by radioactive and stable tracers.* Am J Physiol. 1987;**252**(5 Pt 1):E606-15.
19. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, et al. *Measurement of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide.* J Appl Physiol. 2008;**104**(4):944-51.
20. Matthews DE, Schwarz HP, Yang RD, Motil KJ, et al. *Relationship of plasma leucine and alpha-ketoisocaproate during a L-[1-13C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment.* Metabolism. 1982;**31**(11):1105-12.
21. Husek P. *Amino acid derivatization and analysis in five minutes.* FEBS Lett. 1991;**280**(2):354-6.
22. el-Khoury AE, Sanchez M, Fukagawa NK, Young VR. *Whole body protein synthesis in healthy adult humans: 13CO2 technique vs. plasma precursor approach.* Am J Physiol. 1995;**268**(1 Pt 1):E174-84.
23. Spear ML, Darmaun D, Sager BK, Parsons WR, et al. *Use of [13C]bicarbonate infusion for measurement of CO2 production.* Am J Physiol. 1995;**268**(6 Pt 1):E1123-7.

24. van den Berghe G, Wouters P, Weekers F, Verwaest C, et al. *Intensive insulin therapy in the critically ill patients.* N Engl J Med. 2001;**345**(19):1359-67.
25. Altszuler N, De Bodo RC, Steele R, Wall JS. *Carbohydrate metabolism of hypophysectomized dogs as studied with radioactive glucose.* Am J Physiol. 1956;**187**(1):25-31.
26. Steele R, Wall JS, De Bodo RC, Altszuler N. *Measurement of size and turnover rate of body glucose pool by the isotope dilution method.* Am J Physiol. 1956;**187**(1):15-24.
27. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, et al. *Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine.* Am J Physiol. 1980;**238**(5):E473-9.
28. Clarke JT, Bier DM. *The conversion of phenylalanine to tyrosine in man. Direct measurement by continuous intravenous tracer infusions of L-[ring-2H5]phenylalanine and L-[1-13C] tyrosine in the postabsorptive state.* Metabolism. 1982;**31**(10):999-1005.
29. Thompson GN, Pacy PJ, Merritt H, Ford GC, et al. *Rapid measurement of whole body and forearm protein turnover using a [2H5]phenylalanine model.* Am J Physiol. 1989;**256**(5 Pt 1):E631-9.
30. Sy J, Gourishankar A, Gordon WE, Griffin D, et al. *Bicarbonate kinetics and predicted energy expenditure in critically ill children.* Am J Clin Nutr. 2008;**88**(2):340-7.
31. Marchini JS, Castillo L, Chapman TE, Vogt JA, et al. *Phenylalanine conversion to tyrosine: comparative determination with L-[ring-2H5]phenylalanine and L-[1-13C]phenylalanine as tracers in man.* Metabolism. 1993;**42**(10):1316-22.
32. Marchini JS, Cortiella J, Hiramatsu T, Castillo L, et al. *Phenylalanine and tyrosine kinetics for different patterns and indispensable amino acid intakes in adult humans.* Am J Clin Nutr. 1994;**60**(1):79-86.
33. Argaman Z, Young VR, Noviski N, Castillo-Rosas L, et al. *Arginine and nitric oxide metabolism in critically ill septic pediatric patients.* Crit Care Med. 2003;**31**(2):591-7.
34. Castillo L, Yu YM, Marchini JS, Chapman TE, et al. *Phenylalanine and tyrosine kinetics in critically ill children with sepsis.* Pediatr Res. 1994;**35**(5):580-8.
35. Golden MH, Waterlow JC. *Total protein synthesis in elderly people: a comparison of results with [15N] glycine and [14C]leucine.* Clin Sci Mol Med. 1977;**53**(3):277-88.
36. Bier DM, Leake RD, Haymond MW, Arnold KJ, et al. *Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose.* Diabetes. 1977;**26**(11):1016-23.
37. Grasso S, Fallucca F, Mazzone D, Giangrande L, et al. *Inhibition of glucagon secretion in the human newborn by glucose infusion.* Diabetes. 1983;**32**(6):489-92.
38. Boden G, Chen X, Stein TP. *Gluconeogenesis in moderately and severely hyperglycemic patients with type 2 diabetes mellitus.* Am J Physiol Endocrinol Metab. 2001;**280**(1):E23-30.
39. Van den Berghe G. *How does blood glucose control with insulin save lives in intensive care?* J Clin Invest. 2004;**114**(9):1187-95.
40. Cortiella J, Marchini JS, Branch S, Chapman TE, et al. *Phenylalanine and tyrosine kinetics in relation to altered protein and phenylalanine and tyrosine intakes in healthy young men.* Am J Clin Nutr. 1992;**56**(3):517-25.
41. Young VR, Marchini JS. *Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids, with reference to nutritional adaptation in humans.* Am J Clin Nutr. 1990;**51**(2):270-89.
42. Tessari P, Kiwanuka E, Zanetti M, Barazzoni R. *Postprandial body protein synthesis and amino acid catabolism measured with leucine and phenylalanine-tyrosine tracers.* Am J Physiol Endocrinol Metab. 2003;**284**(5):E1037-42.
43. Garrett RHCMG, *Principles of Biochemistry with a Human Focus.*, ed. Learning. T2002: USA: Brooks/Cole. 578-585.
44. Biolo G, De Cicco M, Lorenzon S, Dal Mas V, et al. *Treating hyperglycemia improves skeletal muscle protein metabolism in cancer patients after major surgery.* Crit Care Med. 2008;**36**(6):1768-75.
45. Verbruggen S, Sy J, Gordon WE, Hsu JW, et al. *Ontogeny of methionine utilization and splanchnic uptake in critically ill children.* Am J Physiol Endocrinol Metab. 2009;(297):E1046–E1055.

46. Hertz DE, Karn CA, Liu YM, Liechty EA, et al. *Intravenous glucose suppresses glucose production but not proteolysis in extremely premature newborns.* J Clin Invest. 1993;**92**(4):1752-8.
47. Jahoor F, Shangraw RE, Miyoshi H, Wallfish H, et al. *Role of insulin and glucose oxidation in mediating the protein catabolism of burns and sepsis.* Am J Physiol. 1989;**257**(3 Pt 1):E323-31.
48. Lecker SH, Solomon V, Mitch WE, Goldberg AL. *Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states.* J Nutr. 1999;**129**(1S Suppl):227S-237S.
49. Voisin L, Breuille D, Combaret L, Pouyet C, et al. *Muscle wasting in a rat model of long-lasting sepsis results from the activation of lysosomal, Ca2+ -activated, and ubiquitin-proteasome proteolytic pathways.* J Clin Invest. 1996;**97**(7):1610-7.
50. Deval C, Mordier S, Obled C, Bechet D, et al. *Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting.* Biochem J. 2001;**360**(Pt 1):143-50.
51. Smith IJ, Lecker SH, Hasselgren PO. *Calpain activity and muscle wasting in sepsis.* Am J Physiol Endocrinol Metab. 2008;**295**(4):E762-71.
52. Wang X, Hu Z, Hu J, Du J, et al. *Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling.* Endocrinology. 2006;**147**(9):4160-8.
53. Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, et al. *A potential role for Akt/FOXO signalling in both protein loss and the impairment of muscle carbohydrate oxidation during sepsis in rodent skeletal muscle.* J Physiol. 2008;**586**(Pt 22):5589-600.
54. Crossland H, Constantin-Teodosiu D, Greenhaff PL, Gardiner SM. *Low-dose dexamethasone prevents endotoxaemia-induced muscle protein loss and impairment of carbohydrate oxidation in rat skeletal muscle.* J Physiol. 2010;**588**(Pt 8):1333-47.
55. Burns SP, Cohen RD. *To: Krebs M, Brehm A, Krssak M et al. (2003) Direct and indirect effects of amino acids on hepatic glucose metabolism in humans. Diabetologia 46:917-925.* Diabetologia. 2004;**47**(1):141; author reply 142-3.

chapter 5

DOES A REDUCED GLUCOSE INTAKE PREVENT HYPERGLYCEMIA IN CHILDREN EARLY AFTER CARDIAC SURGERY? – A RANDOMIZED CONTROLLED CROSSOVER STUDY



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Provisionally accepted Crit Care

ABSTRACT

Introduction: Hyperglycemia in children after cardiac surgery can be treated with intensive insulin therapy, but hypoglycemia is a potential serious side effect. The aim of this study was to investigate the effects of reducing glucose intake below standard intakes to prevent hyperglycemia, on blood glucose concentrations, glucose kinetics and protein catabolism in children after cardiac surgery with cardiopulmonary bypass (CPB).

Methods: Subjects received 4h low glucose (LG; 2.5 $mg.kg^{-1}.min^{-1}$) and 4h standard glucose (SG; 5.0 $mg.kg^{-1}.min^{-1}$) infusion in a randomized blinded crossover setting. Simultaneously, an 8h stable isotope tracer protocol was conducted to determine glucose and leucine kinetics. Data as mean ± SD or median (IQR); comparison by paired samples T-test.

Results: Eleven subjects (age 5.1 (20.2) months) were studied 9.5 ± 1.9 hours post-cardiac surgery. Blood glucose concentrations were lower during LG than SG (LG 7.3 ± 0.7 vs. SG 9.3 ± 1.8 $mmol.L^{-1}$ (131 ± 12 vs.168 ± 32 $mg.dL^{-1}$); $p < 0.01$), although normoglycemia (4.0-6.0 $mmol.L^{-1}$ (72-108 $mg.dL^{-1}$)) was not achieved. No hypoglycemic events occurred. Endogenous glucose production was higher during LG than SG (LG 2.9 ± 0.8 vs. SG 1.5 ± 1.1 $mg.kg^{-1}.min^{-1}$; $p=0.02$), due to increased glycogenolysis (LG 1.0 ± 0.6 vs. SG 0.0 ± 1.0 $mg.kg^{-1}.min^{-1}$; $p < 0.05$). Leucine balance, indicating protein balance, was negative but not affected by glucose intake (LG -54.8 ± 14.6 vs. SG -58.8 ± 16.7 $\mu mol.kg^{-1}.h^{-1}$; $p=0.57$).

Conclusions: Currently recommended glucose intakes aggravated hyperglycemia in children early after cardiac surgery with CPB. Reduced glucose intake decreased blood glucose concentrations without causing hypoglycemia or affecting protein catabolism, but increased glycogenolysis.

LIST OF ABBREVIATIONS

BW	Body weight
CPB	Cardio-pulmonary bypass
EGP	Endogenous glucose production
IV	Intravenous
LG	Low glucose intake
LRP	Leucine release from protein
NOLD	Non-oxidative leucine disposal
PELOD	Pediatric logistic organ dysfunction score
PICU	Pediatric Intensive Care Unit
PIM	Pediatric Index of Mortality score
PRISM	Pediatric Risk of Mortality score
Ra	Rate of appearance
Rd	Rate of disappearance
RACHS-1	Risk adjusted congenital heart surgery score
SG	Standard glucose intake
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption

INTRODUCTION

Critically ill patients often develop hyperglycemia due to an acute stress response after (surgical) trauma and severe illness [1, 2]. Undergoing cardiac surgery with cardiopulmonary bypass (CPB) increases the risk of developing hyperglycemia [3, 4], because of the associated hyperoxia and hypothermia and increased inflammatory response induced by contact of blood to foreign material in the CPB system [5-7]. In addition, intra-operative glucose infusion contributes to hyperglycemia in children undergoing cardiac surgery [8].

Hyperglycemia in critically ill children is described to be associated with increased morbidity and mortality [9-11]. This has led to the widespread use of insulin therapy to achieve normoglycemia in the pediatric intensive care unit (PICU) [12]. A randomized trial in critically ill children, three quarters of whom were cardiac surgery patients, showed that at the research location intensive insulin therapy was associated with a decrease in mortality of 6% to 3% and a decreased morbidity [13]. A major drawback of this therapy was the high incidence of hypoglycemia (25%, 2.2 mmol/L (40 mg.dL^{-1})) [13]. Hypoglycemia has been associated with adverse outcome in the PICU [10] and may adversely affect the developing brain of young children [14-16].

An alternative approach to prevent hyperglycemia and avoid the use of insulin might be reducing intravenous (IV) glucose infusion below current recommendations for glucose intake (~ 5.0 mg.kg^{-1}.min^{-1}) [12, 17, 18]. However, a reduced energy intake could result in increased protein catabolism and subsequently adverse outcome [19]. We hypothesized that currently recommended glucose intake in children after cardiac surgery contributes to the development of hyperglycemia and that reducing glucose intake below these standard intakes would result in normoglycemia, without causing hypoglycemia.

The first aim of this study was to investigate whether reducing IV glucose intake would prevent hyperglycemia in children after cardiac surgery, without causing hypoglycemia. This was determined using a randomized blinded controlled crossover design, providing for both low IV glucose intake (LG 2.5 mg.kg^{-1}.min^{-1}) and standard IV glucose intake (SG 5.0 mg.kg^{-1}.min^{-1}). The second aim was to determine the effects of reduced glucose intake on glucose kinetics and on both leucine kinetics and albumin synthesis as indicators of protein metabolism, using stable isotope tracer methodology.

MATERIALS AND METHODS

Patients and setting

Children admitted to the Intensive Care of Erasmus MC - Sophia Children's Hospital after cardiac surgery for congenital heart disease between June 2010 and October 2010 were consecutively enrolled. Inclusion criteria were: age > 30 days; body weight (BW) < 30 kg; CPB during surgery; arterial and central venous line; and hemodynamic stability (with or without

inotropic support). Exclusion criteria were: chromosomal disorder; pre-existent metabolic or endocrine disorder; liver failure; insulin therapy at start of the study. The medical ethical review board of Erasmus MC, Rotterdam, the Netherlands approved this study. Prior to inclusion into the study we obtained written informed consent from parents or legal representatives of patients.

Cardiac surgery

Anesthetic and peri-operative procedures have been described in detail previously [20]. Maximal arterial oxygen tension was targeted at 20 kPa. On CPB either mild hypothermia of 28-32°C was achieved or deep hypothermia of 18°C nasopharyngeal temperature and 21°C rectal temperature with circulatory arrest (deep hypothermic circulatory arrest). Antegrade cerebral perfusion was established when appropriate. Patients received 30 $mg.kg^{-1}$ methylprednisolone during surgery as standard care. Intra-operatively administered fluids did, among others, contain human albumin but no glucose.

Post-operatively, IV glucose intake was provided at 4.0-6.0 $mg.kg^{-1}.min^{-1}$ and total fluid intake including medications, was restricted in the first 24 hours after surgery to 50 $mL.kg^{-1}.d^{-1}$ if < 10 kg BW and 750 $mL.m^{-2}.d^{-1}$ if 10-30 kg BW. Patients were weaned off the ventilator when possible as standard practice. No corticosteroids were provided in the post-operative course.

Study design and interventions

Eight hours after cardiac surgery, we started the experimental protocol, which lasted for 10 hours. See **Figure 5.1** for the study design. Low glucose intake (LG; 2.5 $mg.kg^{-1}.min^{-1}$) and standard glucose intake (SG; 5.0 $mg.kg^{-1}.min^{-1}$) IV were provided in a crossover manner to diminish the effect of timing after cardiac surgery on metabolic variables. Randomization for the order of glucose intake was performed by means of computer generated sealed envelopes. Indistinguishable syringes with equal volume but different glucose concentrations were prepared in order to keep fluid intake equal throughout the protocol. Laboratory personnel, nursing staff and investigators were blinded until analyses were finished.

In the post-surgical period prior to start of the study, glucose intake was infused as per standard care (4.0-6.0 $mg.kg^{-1}.min^{-1}$). After obtaining baseline blood and breath samples, at t=0 the study glucose intake (LG or SG first) was started. Simultaneously, a primed-continuous 8h intravenous stable isotope tracer infusion (described under "Materials and sample processing") was administered. Four hours after start of the tracer infusion (t=240) the glucose intake was switched to the alternate level. A washout period was not deemed necessary, since glucose turnover is rapid and steady state can be achieved at 80 minutes after start of a glucose infusion [21]; thus carry-over effects were not expected.

Blood glucose concentrations were determined at t=0 and at the end of both interventions (t=240 and t=480) as well as C-reactive protein, pre-albumin, albumin, free fatty acids, triglycerides, insulin and cortisol to describe inflammatory, metabolic, and hormonal

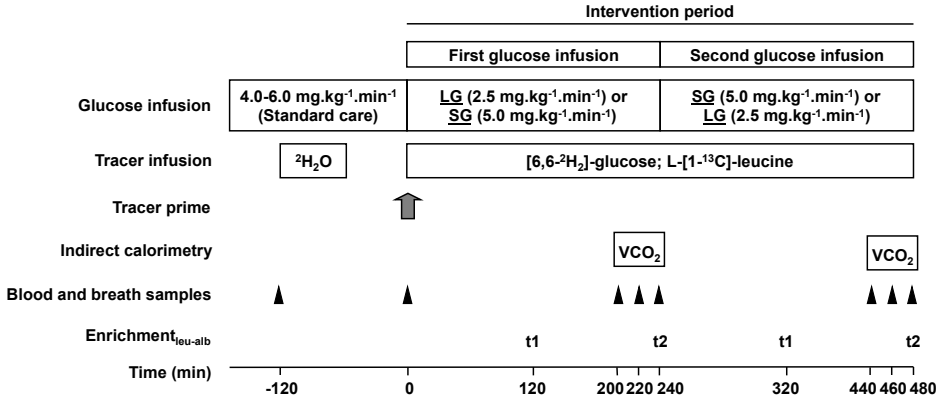


Figure 5.1 – Schematic presentation of the study in children post-cardiac surgery receiving low and standard glucose intake.

In a randomized blinded crossover design subjects received low glucose (LG) and standard glucose (SG) intake, while a primed-continuous stable isotope tracer protocol was conducted. Gray arrow indicates prime of tracers before continuous infusion. Black triangles indicate time points of arterial blood and breath sampling for laboratory parameters and isotopic enrichment measurements of glucose and leucine tracers. VCO_2 = carbon dioxide production measured with indirect calorimetry. $Enrichment_{leu\text{-}alb}$ indicates the enrichment of [1-^{13}C]-leucine incorporated into albumin, "t1" and "t2" represents time points of blood sampling for determination of $Enrichment_{leu\text{-}alb}$ and calculation of fractional albumin synthesis.

characteristics. Blood glucose concentrations < 4.0 mmol.L^{-1} (< 72 mg.dL^{-1}) were considered hypoglycemic; concentrations > 6.0 mmol.L^{-1} (> 108 mg.dL^{-1}) hyperglycemic.

Carbon dioxide production (VCO_2), oxygen consumption (VO_2) and respiratory quotient were determined by indirect calorimetry (Deltatrac^{TM} I MBM-200, Datex Division Instrumentarium Corp., Finland) in the last 40 minutes of each glucose infusion period, either by canopy mode or on the ventilator.

Severity of illness was assessed by the Pediatric Index of Mortality [22], the Pediatric Risk of Mortality score [23] and the Pediatric logistic organ dysfunction score [24]. For all three, higher scores indicate higher severity of disease. Risk Adjustment for Congenital Heart Surgery [25] and Aristotle Comprehensive complexity score [26] were assessed. For both, higher scores indicate increased complexity of cardiac surgery. Furthermore vasopressor score at start of the interventions was calculated as described by Zuppa et al [27]. Estimated energy expenditure was calculated with the Schofield equation [28].

Outcome measures

The primary outcome measure was blood glucose concentrations during LG and SG. Secondary outcome measures were glucose rate of appearance, endogenous glucose production (EGP), rate of gluconeogenesis and glycogenolysis; leucine flux, leucine release from protein, leucine oxidation, non-oxidative leucine disposal and leucine balance; whole body protein

breakdown, whole body protein synthesis and whole body protein balance; albumin synthesis rates and contribution of albumin synthesis to whole body protein synthesis.

Materials and sample processing

Stable isotope tracers (≥ 98% enriched) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The hospital pharmacy of Erasmus MC, Rotterdam, the Netherlands, compounded the tracer solutions and tested them for sterility and pyrogenicity. At t=-120 $^{2}H_2O$ (4 $g.kg^{-1}$) was infused IV over 1 hour to prime the body water pool. At t=0 a bolus of $NaH^{13}CO_3$ (2.1 $\mu mol.kg^{-1}$) was infused to prime the bicarbonate pool followed by primed-continuous administration of [6,6-$^{2}H_2$]glucose (40 $\mu mol.kg^{-1}$; 48 $\mu mol.kg^{-1}.h^{-1}$) and L-[1-^{13}C]leucine (8 $\mu mol.kg^{-1}$; 8 $\mu mol.kg^{-1}.h^{-1}$) to study glucose and leucine metabolism respectively (**Figure 5.1**).

Blood samples were obtained at standard frequent intervals (**Figure 5.1**) from the arterial line, centrifuged (2 min, 2000 g) and plasma was frozen at -80°C until samples were analyzed. Three breath samples of approximately 15 ml expiratory air per time point were taken from the outlet of the ventilator if patients were ventilated [29] or by the direct nasopharyngeal sampling method collecting air from a gastric tube inserted 1-1.5 cm in the nasopharynx [30]. The collected air was transferred into impermeable vacuum glass tubes and stored at room temperature until analysis.

Measurements

Blood glucose concentrations, by the hexokinase method, and plasma albumin concentrations were determined on a Roche Modular Analytics P 800-Module (Roche Diagnostics Nederland, Almere, the Netherlands). Insulin was analyzed in blood with standard human insulin specific radioimmunoassay techniques. C-reactive protein, pre-albumin, free fatty acids, triglycerides and cortisol were determined by standard in-house protocols.

Enrichment of deuterated water in plasma was determined by isotope ratio mass spectrometry (Delta+XP, Thermo Fisher, Bremen, Germany). Glucose M+1 enrichment with ^{2}H derived from $^{2}H_2O$ was analyzed with gas chromatography mass spectrometry (GC 6890, MS 8973, Agilent Technologies, Wilmington, DE, USA) using the penta-acetate derivative in negative chemical ionization mode as previously described [31, 32]. Glucose M+2 enrichment derived from [6,6-$^{2}H_2$]glucose was determined as its aldonitrile penta acetate derivative in electron impact ionization mode using a slightly modified method as previously described [33]. Standard curves were prepared by mixing aqueous solutions of natural and labeled glucose for both enrichment and concentration determination. The mass spectrometric analyses were performed on a mass spectrometer coupled with a gas chromatograph (GC 7890 A, MS 5975 C, Agilent, Amstelveen, the Netherlands). A chemically bonded DB 5ms (J&W Scientific Folsom, CA, USA) capillary column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm was used for the chromatographic separation. The intensities of the fragments 187.2 and 189.2 were selected for measurement of respectively the non-enriched and

the 6,6-$^{2}H_2$ enriched aldonitrile penta-acetate derivative of glucose. All measurements were carried out in selective ion monitoring mode. Leucine kinetics was calculated from plasma alpha-ketoisocaproate (α-KIC) M+1 enrichment that was determined by gas chromatography mass spectrometry after derivatization to butyldimethyl-silylquinoxalinol derivatives [34]. Breath samples were analyzed for enrichment of $^{13}CO_2$ with an infrared isotope analysis technique (Helifan, Fischer Instruments, Leipzig, Germany). ^{13}C enrichment was expressed as atom percentage excess above baseline, for subsequent calculation of leucine oxidation [35]. The enrichment of incorporated leucine in albumin was determined on a gas chromatograph-combustion-isotope ratio mass spectrometer (Delta XP, Thermo Fisher, Bremen, Germany) as described before [36].

Plasma samples were analyzed as triplicates; breath samples were collected in triplicate and analyzed once.

Calculations

Glucose kinetics was estimated using the Steele equation [37], based upon the final 40 min of both glucose infusion periods (steady state); whole body leucine kinetics was calculated by conventional isotope dilution equations using a stochastic model [38]. At steady state plateau, rate of appearance (Ra) equals the rate of disappearance (Rd) as follows:

$$Ra = Rd = i \times (E_{inf}/E_{pl} - 1) \quad \text{(Eq 1)}$$

where i is the infusion rate of the labeled tracer, E_{inf} is the tracer enrichment of the infusate and E_{pl} the tracer enrichment in plasma.

Glucose kinetics

Plasma [6,6-$^{2}H_2$]glucose enrichment (in mole percent excess) and the exogenous glucose infusion rate were used for data calculation. Under steady state conditions, total glucose rate of appearance is equal to the rate of disappearance [37], the latter which reflects glucose utilization. Rates of EGP, glucose clearance, glycogenolysis and gluconeogenesis were calculated as previously described [21, 39, 40].

Endogenous glucose production (EGP) rate was calculated as follows:

$$EGP = Ra_{Glucose} - GIR \quad \text{(Eq 2)}$$

where GIR is the total glucose infusion rate in $mg.kg^{-1}.min^{-1}$.

Fractional gluconeogenesis was calculated as previously described [32]. Briefly, the average enrichment of ^{2}H on each glucose carbon was calculated with the following equation:

$$\text{Average } (M+1)d = (M+1)d_{(m/z\ 169)}/6 \quad \text{(Eq 3)}$$

where $(M+1)d_{(m/z169)}$ is the M+1 enrichment of deuterium of glucose measured using m/z 170/169 and "6" is the number of ^2H labeling sites on the m/z 169 fragment of glucose.

Because body water is the precursor pool for deuterium or hydrogen, the extent of deuterium labeling of glucose during the gluconeogenic process when ^2H_2O is infused

is a measure of fractional gluconeogenesis. Therefore, with the average deuterium enrichment in m/z 170/169 for calculating fractional gluconeogenesis (FracGNG), the equation is

$$\text{FracGNG} = \text{average}(M+1)d/E_{^2H_2O} \quad \text{(Eq 4)}$$

where $E_{^2H_2O}$ is the deuterium enrichment in body water.

The absolute rate of appearance of plasma glucose from gluconeogenesis (Ra_{GNG}) and glycogenolysis were calculated:

$$\text{Gluconeogenesis} = Ra_{Glucose} \text{ x FracGNG} \quad \text{(Eq 5)}$$

$$\text{Glycogenolysis} = \text{EGP} - \text{Gluconeogenesis} \quad \text{(Eq 6)}$$

Glucose clearance, as measure of the disposal of glucose per unit of blood glucose, was calculated with the following equation [21, 40]:

$$\text{Glucose clearance} = Ra_{glucose} / (C_{glucose} \text{ x } 0.01) \quad \text{(Eq 7)}$$

where glucose clearance is expressed in $ml.kg^{-1}.min^{-1}$, $C_{glucose}$ is the glucose concentration in blood in $mg.dL^{-1}$ and 0.01 the factor to convert the concentration to $mg.ml^{-1}$.

Leucine kinetics

Plasma leucine kinetics, which is indicative of whole body protein kinetics, were calculated as follows.

Whole body leucine fluxes (Ra_{Leu}; $\mu mol.kg^{-1}.h^{-1}$) were calculated according to (Eq 1) from [^{13}C]α-ketoisocaproate ([^{13}C]α-KIC) as previously described [34, 41, 42].

Leucine release from protein (LRP), which is indicative of protein breakdown, was calculated as follows:

$$\text{LRP} = Ra_{Leu} - i \quad \text{(Eq 8)}$$

where i represents the tracer infusion rate.

Leucine oxidation rates ($\mu mol.kg^{-1}.h^{-1}$) were calculated with the following equation;

$$\text{LeucineOx} = VCO_2 \text{ x } (E^{13}CO_2/69.18)/[^{13}C]\alpha\text{–KIC} \quad \text{(Eq 9)}$$

where 69.18 is the $^{13}CO_2$ refraction correction factor for critically ill children [35]. VCO_2 is measured in $mL.min^{-1}$ and converted to $mmol.h^{-2}$ by multiplying by 60 min and dividing by 22.4; the number of moles in 1L of an ideal gas at standard temperature and pressure to convert to $mL.min^{-1}$.

Non-oxidative leucine disposal (NOLD; leucine used for protein synthesis, which is indicative of protein synthesis) was calculated:

$$NOLD = Ra_{leu} - LeucineOx \quad \text{(Eq 10)}$$

Leucine balance ($\mu mol.kg^{-1}.h^{-1}$) was calculated as follows:

$$\text{Leucine balance} = LRP - NOLD \quad \text{(Eq 11)}$$

Whole body protein kinetics

Whole body protein turnover was calculated from the model described by Waterlow [43]. In order to convert leucine kinetics into protein kinetics, we assumed that the average content of leucine in human proteins was 621 $\mu mol.g^{-1}$ [44]. Thus leucine kinetics in $\mu mol.kg.^{-1}h.^{-1}$ were divided by 621 $\mu mol.g^{-1}$ and multiplied by 24 h to derive protein kinetics in $g.kg^{-1}.d^{-1}$ (protein synthesis from NOLD, protein breakdown from LRP and protein balance from leucine balance).

Albumin synthesis

By measuring the incorporation of [1-^{13}C]-leucine in albumin we calculated the fractional and absolute synthesis rates of albumin and the contribution of albumin synthesis to the whole body protein synthesis.

Fractional albumin synthesis rate (FSR) represents the renewed fraction of the intravascular albumin pool per time unit ($\%.d^{-1}$) and was calculated by [45] :

$$FSR = (E_{leu\text{-}alb,t2} - E_{leu\text{-}alb,t1})/E_{\alpha\text{-}KIC} \times (24 \times 60) / (t2 - t1) \times 100\% \quad \text{(Eq 12)}$$

where $E_{leu\text{-}alb}$ is the enrichment (mole percent excess) of incorporated leucine in albumin at t1 (t=120 and t=360 for first and second glucose infusion respectively) and t=2

(t=240 and t=480 for first and second glucose infusion respectively) (**Figure 5.1**). $E_{\alpha\text{-}KIC}$ is the

enrichment of the precursor, α-KIC in mole percent excess at these time points in minutes.

The absolute albumin synthesis rate (ASR) ($mg.kg^{-1}.d^{-1}$) was calculated [45]:

$$ASR = FSR \times C_{alb} \times vol_{bl} \times (1\text{-}Ht) \times weight^{-1} \quad \text{(Eq 13)}$$

where C_{alb} is plasma albumin concentration ($g.L^{-1}$), vol_{bl} is the total volume of blood in the body (for these subjects assumed to be 75 $ml.kg^{-1}$), Ht is hematocrit and (1-Ht) the fraction of blood that is plasma.

Furthermore we calculated the contribution (%) of albumin ASR to whole body protein synthesis, by determining the ratio of leucine incorporated into albumin to the total amount of leucine used for protein synthesis [45]:

$$\text{Contribution} = [(\text{ASR} \times 0.104) / (\text{NOLD} \times 131.2 \times 24 \times 0.001)] \times 100\% \quad \text{(Eq 14)}$$

where 0.104 is the mass fraction of leucine residues in albumin, 131.2 is the molecular mass of leucine, 24 is the factor to convert to days, 0.0001 to convert to milligram.

Data analysis

Power analysis showed that inclusion of 8 subjects with complete data would suffice to detect a statistically significant difference of 20% in plasma glucose concentrations between LG and SG (80% power, type I error of 5%). The Shapiro-Wilk normality test was used to determine whether data were normally distributed. Data are presented as mean ± SD unless non-parametric in which case they are presented as median (IQR). Data during the two different glucose infusions were compared by either the paired student's T-test (normal distribution) or the Wilcoxon-matched pairs test with exact significance (non-normal distribution). Differences between subsets of subjects were assessed by the independent samples t-test (normal distribution) or Mann Whitney U test (non-normal distribution). Correlations between baseline characteristics and the primary outcome measure were determined with Spearman's rho correlation coefficient. Statistical significance was defined as $p < 0.05$. Statistical analyses were done with SPSS Statistics version 17.0 (SPSS, IBM Corporation, Armonk, NY, USA).

RESULTS

Patients

We conducted the study protocol in eleven children (8 males, 3 females). In eleven subjects blood glucose concentrations were available during both glucose infusion periods. Due to technical problems glucose kinetics data were collected in 9 of 11 patients. Leucine kinetics data were available in 8 patients due to inability to conduct indirect calorimetry in all patients. Median body weight was 6.8 (7.1) kg. Mean PIM score was 12.6 ± 7.2 % predicted mortality, median PRISM score 7.5 (25.6) % predicted mortality and median PELOD score was 1.3 (1.2) % predicted mortality. **Table 5.1** lists other baseline characteristics.

Table 5.1 – Patient characteristics of 11 children post-cardiac surgery.

	Age (months)	Diagnosis and surgical intervention	RACHS-1 category	Comprehensive Aristotle Complexity Score [1]	CPB time (hh:mm)	Aorta clamp time (hh:mm)	Vasopressor score [2]	Extubation before start intervention period	First glucose infusion (LG or SG)
1	11.7	ASD-II repair	1	3.0	0:37	0:16	0	yes	SG
2	24.4	Sinus venosus defect patch repair	1	3.0	1:23	0:57	0	yes	SG
3	60.0	Sinus venosus defect patch repair	1	3.0	1:11	0:53	0	yes	LG
4	23.3	PCPC for univentricular heart	2	6.8	0:40	0:00	0	yes	LG
5	3.1	VSD repair	2	7.0	1:22	0:47	0	no	SG
6	4.7	VSD repair	2	7.0	1:33	1:08	0	yes	LG
7	2.6	TOF repair with transannular patch	2	8.0	1:14	0:52	0	yes	SG
8	20.6	Redo RVOT procedure after correction of TOF	2	8.5	1:55	0:59	0	yes	LG
9	4.8	CAVSD repair	3	9.0	2:18	1:47	0	yes	LG
10	2.5	Biventricular repair of HLHS with DHCA after hybrid preparation [3]	6	17.0	3:44	1:58	0	no	LG
11	5.2	Biventricular repair of HLHS with DHCA after hybrid preparation [3]	6	17.0	4:32	2:33	7	no	SG
Mean ± SD or median (IQR)	5.1 (20.2)	-	2 (2)	6.0 (4.0)	1:23 (1:07)	1:06 ± 0:44	-	-	-

Normally distributed data (as assessed by Shapiro-Wilk normality test) are presented as mean ± SD, non-normally distributed data as median (IQR); [1] Comprehensive Aristotle complexity score [26]; [2] Vasopressor score [27]; [3] DHCA time (hh:mm) was 0:53 and 1:31 for patient 10 and 11 respectively; antegrade cerebral perfusion time as part of DHCA was 0:32 and 1:21.

ASD-II, ostium secundum atrium septal defect; CAVSD, complete atrial ventricular septal defect; CPB, cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; HLHS, hypoplastic left heart syndrome; LG, low glucose intake (2.5 mg/kg/min); PCPC, partial cavo-pulmonar connection; RACHS-1, Risk adjusted congenital heart surgery score [25]; RVOT, right ventricle outflow tract; SG, standard glucose intake (5.0 mg/kg/min); TOF, Tetralogy of Fallot; VSD, ventricular septal defect.

There were no clinically important nor statistical differences in baseline characteristics between patients randomized to start with LG and those that started with SG (data not shown). All patients received prophylactic antibiotics (cefazolin), diuretics, morphine and/or acetominophen for pain relief. One patient was ventilated with nitric oxide for pulmonary hypertension, but was hemodynamically stable without inotropics. Other drugs administered included norepineprhine (n=1), milrinone (n=2) and IV nitroglycerine (n=2). See **Table 5.1** for vasopressor scores at start of the study protocol.

The first glucose infusion was started a mean 9.5 ± 1.9 hours after cardiac surgery (t=0). During LG glucose intake including glucose tracers was 2.6 ± 0.3 $mg.kg^{-1}.min^{-1}$ and during SG 5.0 ± 0.4 $mg.kg^{-1}.min^{-1}$ ($p < 0.001$; paired samples T-test).

Table 5.2 – Metabolic characteristics of children post-cardiac surgery receiving low and standard glucose intake.

	Before experiment	LG (2.5 $mg.kg^{-1}.min^{-1}$)	SG (5.0 $mg.kg^{-1}.min^{-1}$)	P-value
Glucose intake	3.6 ± 0.7	2.6 ± 0.3	5.0 ± 0.4	< 0.001
Blood glucose ($mmol.L^{-1}$)	9.5 ± 2.0	7.3 ± 0.7	9.3 ± 1.8	0.007
Blood glucose ($mg.dL^{-1}$)	172 ± 37	131 ± 12	168 ± 32	0.007
Estimated Energy expenditure ($kcal.kg^{-1}.d^{-1}$) [7]	54.7 ± 5.8			
Energy intake ($kcal.kg^{-1}.d^{-1}$)		12.1 ± 1.3	23.5 ± 2.1	< 0.001
Measured energy expenditure ($kcal.kg^{-1}.d^{-1}$)		44.9 ± 10.9	46.1 ± 10.7	0.856
VCO_2 ($ml.kg^{-1}.h^{-1}$)		5.6 ± 1.3	5.7 ± 1.2	0.901
VO_2 ($ml.kg^{-1}.h^{-1}$))		6.4 ± 1.7	6.6 ± 1.5	0.813
Respiratory quotient		0.87 (0.21)	0.89 (0.06)	0.719
C-reactive protein ($mg.L^{-1}$)	13 ± 7	32 ± 17	32 ± 16	0.933
Pre-albumin ($g.L^{-1}$)	0.18 (0.04)	0.18 (0.03)	0.17 (0.03)	0.203
Albumin ($g.L^{-1}$)	38 ± 5	38 ± 4	38 ± 5	1.000
Triglycerides ($mmol.L^{-1}$)	0.41 (0.32)	0.41 (0.41)	0.47 (0.35)	0.687
Free fatty acids ($mmol.L^{-1}$)	0.71 ± 0.23	0.66 ± 0.13	0.53 ± 0.12	0.013
Cortisol ($nmol.L^{-1}$)	535 ± 193	229 ± 100	208 ± 42	0.429
Insulin ($pmol.L^{-1}$)	90 (229)	61 (83)	142 (199)	0.064
Insulin/glucose ratio ($pmol.mmol^{-1}$)	0.6 (1.1)	9.0 (13.5)	17.8 (20.8)	0.105

P-values indicate statistical comparison between glucose intakes (LG and SG) only. Normally distributed data (as assessed by Shapiro-Wilk normality test) are presented as mean ± SD and comparison between glucose intakes was done by paired samples T-test. Non-normally distributed data are presented as median (IQR) and comparison between glucose intakes was done by Wilcoxon matched pairs test; [7] estimated with Schofield equation [28]; LG, low glucose intake; SG, standard glucose intake; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

Blood glucose concentrations and laboratory parameters

Blood glucose concentrations were significantly lower during LG than during SG (**Table 5.2**). On average, normoglycemia was not achieved (4.0-6.0 mmol.L^{-1}; 72-108 mg.dL^{-1}) during either of the glucose infusions; No hypoglycemic events occurred, the lowest blood glucose concentration measured was 6.2 mmol.L^{-1} (112 mg.dL^{-1}). **Table 5.2** lists other metabolic characteristics.

Glucose kinetics

Steady state ^2H_2O enrichments were 0.72 ± 0.06 and 0.72 ± 0.07 atom percent excess during the first and second glucose infusion respectively. During SG EGP was not fully suppressed and consisted entirely of gluconeogenesis, while glycogenolysis did not differ from zero (p=0.89; one sample T-test) (**Table 5.3, Figure 5.2**). During LG, glucose rate of appearance tended to be lower, with a significantly higher EGP than during SG, due to increased glycogenolysis, but a similar gluconeogenesis rate as during SG (**Table 5.3, Figure 5.2**).

Leucine kinetics and whole body protein metabolism

VCO_2, VO_2 and respiratory quotient did not differ significantly between both glucose infusions (**Table 5.2**). Respiratory quotient values were within the normal range (0.85-1.00).

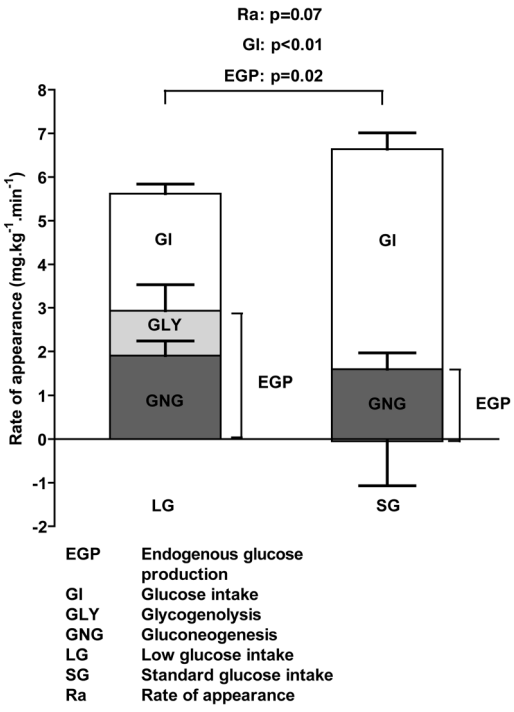


Figure 5.2 – Glucose kinetics in children post-cardiac surgery receiving low and standard glucose intake.

Data as mean ± SD in mg.kg^{-1}.min^{-1} in stacked bars. Comparison between glucose intakes by paired samples T-test. Entire stacked bars represent rate of appearance of glucose, which consists of exogenous glucose intake and endogenous glucose production. The latter is composed of gluconeogenesis and glycogenolysis. Glycogenolysis during SG was not significantly different from zero (p=0.89; one sample T-test).

Table 5.3 – Glucose, leucine and albumin kinetics in children post-cardiac surgery receiving low and standard glucose intake.

	LG (2.5 mg.kg^{-1}.min^{-1})	SG (5.0 mg.kg^{-1}.min^{-1})	P-value
Glucose kinetics (mg.kg^{-1}.min^{-1})			
Glucose Ra	5.6 ± 0.9	6.6 ± 1.1	0.071
Endogenous glucose production	2.3 ± 0.83	1.5 ± 1.1	0.016
Fractional gluconeogenesis as percentage of Ra (%)	34 ± 3	24 ± 5	0.002
Absolute gluconeogenesis	1.91 ± 0.34	1.59 ± 0.37	0.076
Absolute glycogenolysis	1.03 ± 0.59	-0.05 ± 1.02	0.027
Glucose clearance rate (ml.kg^{-1}.min^{-1})	4.19 ± 0.54	4.03 ± 0.64	0.362
Leucine kinetics (μmol.kg^{-1}.h^{-1})			
Leucine Ra	195.2 ± 21.2	209.3 ± 27.3	0.218
Leucine oxidation	63.1 ± 14.6	68.0 ± 15.4	0.573
Leucine release from protein [1]	187.0 ± 20.9	201.1 ± 27.3	0.218
Non-oxidative leucine disposal [2]	132.1 ± 17.7	141.3 ± 35.5	0.496
Leucine balance	-54.8 ± 14.6	-59.8 ± 15.8	0.573
Albumin synthesis			
Fractional albumin synthesis rate (%.d^{-1})	9.2 ± 3.5	9.6 ± 4.0	0.756
Absolute albumin synthesis rate (mg.kg^{-1}.d^{-1})	157.3 (94.6)	139.5 (111.3)	0.742
Contribution to total protein synthesis (%)	4.2 ± 1.3	4.2 ± 1.6	0.976

Normally distributed data (as assessed by Shapiro-Wilk normality test) are presented as mean ± SD and comparison between glucose intakes (LG and SG) was done by paired samples T-test. Non-normally distributed data are presented as median (IQR) and comparison between glucose intakes was done by Wilcoxon matched pairs test;

[1] Indicative of protein breakdown; [2] Indicative of protein synthesis.

LG, low glucose intake; Ra, rate of appearance, SG, standard glucose intake.

Leucine and protein kinetics did not differ significantly between both glucose infusions (**Table 5.3**). Whole body protein kinetics as derived from leucine kinetics were as follows for LG and SG respectively: whole body protein breakdown 7.6 ± 0.8 vs. 8.2 ± 1.1 g.kg^{-1}.d^{-1}, p=0.22, paired samples T-test; whole body protein synthesis 5.4 ± 0.7 vs. 5.7 ± 1.4 g.kg^{-1}.d^{-1}, p=0.46, paired samples T-test. Whole body protein balance was negative during both interventions, but not further aggravated by reduced glucose infusion (LG: -2.2 ± 0.6; SG: -2.4 ± 0.6 g.kg^{-1}.d^{-1}; p=0.57; paired samples T-test).

Patients had normal plasma albumin concentrations (< 35 g.dL^{-1}) (**Table 5.2**). Fractional and absolute albumin synthesis rates did not differ between both glucose infusions (**Table 5.3**). Protein synthesis consisted for 4% of albumin synthesis during both interventions.

Correlations and sub analysis

Age, weight, height, severity of illness scores and complexity of cardiac surgery scores, CPB-time, aorta clamp time or time after surgery of starting the first glucose infusion were not correlated with blood glucose concentrations during LG and SG.

Two subjects underwent deep hypothermic circulatory arrest, which is distinctly different from other cardiac surgical interventions on CPB. Sub analysis without these two patients revealed blood glucose concentrations of LG 7.4 ± 0.7 vs. SG 9.3 ± 1.5 $p < 0.01$ with paired samples T-test. Glucose and leucine kinetics were not affected, apart from slightly changing the significance level of glycogenolysis (LG 1.0 ± 0.6 vs. SG 0.1 ± 1.0; p=0.06; paired samples T-test).

DISCUSSION

Our study showed that currently recommended glucose intakes aggravated hyperglycemia in children admitted to the PICU in the first 24 hours after cardiac surgery with CPB. Furthermore, reduced glucose intake resulted in decreased blood glucose concentrations and not in hypoglycemia or increased protein catabolism. However, in contrast with our hypothesis and our previous study in healthy children undergoing elective craniofacial surgery, normoglycemia (4.0-6.0 $mmol.L^{-1}$ (72-108 $mg.dL^{-1}$)) was not achieved with reduced glucose intake [39]. In addition, it resulted in increased EGP due to increased glycogenolysis.

In the past years the focus on intensive insulin therapy in critically ill children has increased, especially after Vlasselaers et al. showed that in their setting it resulted in decreased morbidity and mortality [13]. However, hypoglycemia of ≤ 2.2 $mmol.L^{-1}$ was observed in a quarter of patients. Hypoglycemia is a serious complication potentially leading to neurological damage at long term [16]. We and others therefore suggested that thresholds for starting insulin therapy should be higher than in the aforementioned trial, at blood glucose concentrations of ~8 $mmol.L^{-1}$ (~140 $mg.dL^{-1}$) [12, 46]. In the present study we showed that when subjects received currently recommended glucose intakes, hyperglycemia was (iatrogenically) aggravated, making them eligible to insulin therapy based on these thresholds. We therefore postulate reduced glucose intake as initial step to prevent hyperglycemia in the early post-operative care. Our study provides mostly a mechanistic view on this approach. Conclusions cannot be drawn for longer periods of reduced glucose intake. Also, it should be noted that our patients were relatively stable and that the intra-operative management in our center includes high dose opioids to suppress the metabolic stress response and hence intra-operative hyperglycemia [20]. Our data therefore ought to be generalized with caution to different and more critically ill populations. However, as reduced glucose intake seems a promising approach, not resulting in either hypoglycemia or aggravated protein catabolism in the early post-operative course after cardiac surgery in relatively stable patients, clinical outcome studies are warranted to provide data to formulate suitable recommendations of (post-operative) glucose intake.

Our study is one of few studies providing data on glucose kinetics and glucose intake in critically ill children. Current recommendations in hospitalized children (varying by BW between 2.8-5.6 $mg.kg^{-1}.min^{-1}$ on day 1 for BW < 30 kg) have been based on the few data available on EGP, glucose oxidation and the consequences of excessive glucose intake during parenteral nutrition [18]. Guidelines recommend to limit glucose intake to 5.0 $mg.kg^{-1}.min^{-1}$ in critically ill children, based on one study indicating that this approximates the maximal glucose oxidation rate of burned patients [18]. Our data suggest that glucose intake needs to be further limited in children in the immediate post-operative course of cardiac surgery. Reducing glucose intake to 2.5 $mg.kg^{-1}.min^{-1}$ however, was not yet effective to achieve normoglycemia in our study and resulted in increased EGP through increased glycogenolysis. This might suggest that LG intake did not meet the metabolic needs of the liver, although enough glucose was available systemically as shown by hyperglycemia. The study was performed at a time that patients likely had substantial glycogen stores. However, with prolonged low glucose intake glycogen stores might eventually deplete, further triggering gluconeogenesis and resulting in increased lipolysis and increased protein catabolism to provide amino acids as gluconeogenic substrate. The fact that we did not find adverse effects of LG on protein metabolism or albumin synthesis rates could have been a result of the relatively short duration of LG (240 minutes), although our findings were consistent with previous studies from our group [36, 39, 47]. Whether prolonged LG, beyond the time that glycogen is available, results in metabolic stress cannot be concluded from our study. However, relatively stable patients in our center often receive oral nutrition and are transferred to a medical ward within 24 hours after PICU admission. In more severely ill children who are likely to receive intravenous glucose intake for longer periods, prolonged LG might result in reduced blood glucose concentrations at the cost of adverse metabolic changes. Whether further reducing glucose intake is more effective to reduce blood glucose concentrations and what the repercussions are on glucose and protein metabolism needs to be investigated.

Our glucose kinetics results differ from those reported in healthy individuals, in whom increased glucose intake results in decreased EGP while blood glucose concentrations remain within normal ranges [21, 48-51]. Our population showed sustained EGP during SG and increased glycogenolysis during LG despite hyperglycemia. A disturbed glucose homeostasis is expected after cardiac surgery with CPB as a result of the metabolic stress response [7]. The response is characterized by release of the counter regulatory hormones glucagon, cortisol and catecholamines on the one hand, leading to increased glucose production [52]. On the other hand, it induces insulin resistance resulting in both impaired suppression of glucose production (central insulin resistance) and impaired insulin-mediated glucose uptake (peripheral insulin resistance) [1, 2, 52]. In our study cortisol concentrations were within the normal range, inotropic support was limited to one patient and all patients received methylprednisolone. The unsuppressed EGP (gluconeogenesis) was therefore most likely the result of insulin resistance. However, craniofacial surgery patients in whom we observed unsuppressed EGP as well, did

achieve normoglycemia when receiving LG [39]. Their insulin resistance was possibly less pronounced, as suggested by lower insulin concentrations, lower insulin/glucose ratios and higher glucose clearance rates as measure for peripheral glucose uptake (n=8, 5.0±1.4 $ml.kg^{-1}.min^{-1}$ on average (unpublished data)) [39].

There are some limitations to this study. First, the study population was heterogeneous and small and comprised relatively stable patients. It does not allow concluding whether the reduction in blood glucose concentrations would differ for different ages, different diagnoses, different types of surgical interventions and different severity of illness. Second, with regard to glucose kinetics, glycogenolysis rates were negative during SG, which is physiologically not possible. These rates were calculated by subtracting two (indirectly) measured values, while the one from which was subtracted (EGP) was possibly underestimated. This underestimation of EGP when using a glucose tracer model as we did, results from dilution of the tracer pool by re-uptake of newly produced glucose in the liver as a consequence of hepatic intralobular functional heterogeneity [53]. Third, we did not measure cerebral glucose uptake as lower limit [17] and glucose oxidation rates as upper limit [18] of glucose intake. Since glucose can diffuse freely across the blood-brain barrier and hypoglycemia was not apparent in our population, we assume that cerebral glucose uptake was not impaired during reduced glucose intake. We refrained from measuring glucose oxidation with [^{13}C]glucose, because our [1-^{13}C]leucine tracer would have interfered with $^{13}CO_2$ measurements for glucose oxidation.

CONCLUSIONS

In conclusion, glucose intake at currently recommended rates in the initial phase of post-operative care at the PICU aggravated hyperglycemia in children < 6 years and < 30 kg BW after cardiac surgery. Reducing glucose intake to 2.5 $mg.kg^{-1}.min^{-1}$ resulted in decreased blood glucose concentrations without causing hypoglycemia or increased protein catabolism, but with increased glycogenolysis. Reduced glucose intake might be feasible as initial step targeting hyperglycemia in the early post-operative course of cardiac surgery in relatively stable children, potentially avoiding insulin use and its complications. We acknowledge that we cannot extrapolate our results to longer durations of glucose infusions or different patient populations. The concept of reduced glucose intake as an alternative to insulin therapy in the (post-operative) care of critically ill children seems promising though. It deserves further investigation during longer time periods and in larger populations of children after cardiac surgery, as well as in other groups of critically ill children.

KEY MESSAGES

- Currently recommended glucose intake (5.0 $mg.kg^{-1}.min^{-1}$) aggravated hyperglycemia (> 6 $mmol.L^{-1}$) in children in the early post-operative phase after cardiac surgery.
- Reducing glucose intake to 2.5 $mg.kg^{-1}.min^{-1}$ reduced blood glucose levels without causing hypoglycemia.
- Reducing glucose intake did not increase protein catabolism.
- The increased endogenous glucose production during reduced glucose intake resulted from increased glycogenolysis, while gluconeogenesis was maintained at the same rate, as compared to standard glucose intake.
- Reducing glucose intake might be used as initial step to prevent hyperglycemia in the early post-operative phase after cardiac surgery in children < 30 kg body weight.

ACKNOWLEDGEMENTS

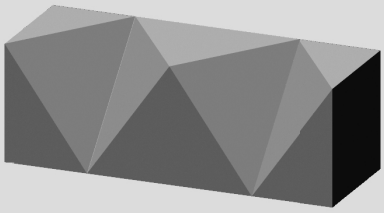
Our gratitude goes out to the patients and their parents for participation in the study. We thank the anesthetists of the Thorax center, Erasmus MC, Rotterdam, the Netherlands for their support in the per-operative setting and for helping to obtain informed consent. We also thank Marianne Maliepaard, Gardi Minderman-Voortman, Kristien Dorst for their contribution, Ko Hagoort for careful editing and Prof. Dr. D. Tibboel for careful review of the manuscript. Furthermore we are grateful to the nursing and medical staff of the Intensive Care of Erasmus MC - Sophia Children's Hospital for their assistance and support in executing the experimental protocol.

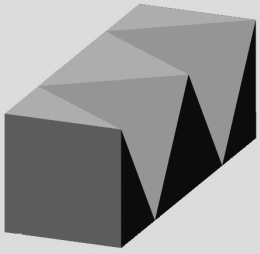
REFERENCES

1. Brealey D, Singer M. *Hyperglycemia in critical illness: a review.* J Diabetes Sci Technol. 2009;**3**(6):1250-60.
2. Mizock BA. *Alterations in fuel metabolism in critical illness: hyperglycaemia.* Best Pract Res Clin Endocrinol Metab. 2001;**15**(4):533-51.
3. Knapik P, Nadziakiewicz P, Urbanska E, Saucha W, et al. *Cardiopulmonary bypass increases postoperative glycemia and insulin consumption after coronary surgery.* Ann Thorac Surg. 2009;**87**(6):1859-65.
4. Mills NL, Beaudet RL, Isom OW, Spencer FC. *Hyperglycemia during cardiopulmonary bypass.* Ann Surg. 1973;**177**(2):203-5.
5. Bandali KS, Belanger MP, Wittnich C. *Does hyperoxia affect glucose regulation and transport in the newborn?* J Thorac Cardiovasc Surg. 2003;**126**(6):1730-5.
6. Lehot JJ, Piriz H, Villard J, Cohen R, et al. *Glucose homeostasis. Comparison between hypothermic and normothermic cardiopulmonary bypass.* Chest. 1992;**102**(1):106-11.
7. Jakob SM, Ensinger H, Takala J. *Metabolic changes after cardiac surgery.* Curr Opin Clin Nutr Metab Care. 2001;**4**(2):149-55.
8. Benzing G, 3rd, Francis PD, Kaplan S, Helmsworth JA, et al. *Glucose and insulin changes in infants and children undergoing hypothermic open-heart surgery.* Am J Cardiol. 1983;**52**(1):133-6.
9. Srinivasan V, Spinella PC, Drott HR, Roth CL, et al. *Association of timing, duration, and intensity of hyperglycemia with intensive care unit mortality in critically ill children.* Pediatr Crit Care Med. 2004;**5**(4):329-36.
10. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, et al. *Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit.* Pediatrics. 2006;**118**(1):173-9.
11. Faustino EV, Apkon M. *Persistent hyperglycemia in critically ill children.* J Pediatr. 2005;**146**(1):30-4.
12. Verbruggen SC, Joosten KF, Castillo L, van Goudoever JB. *Insulin therapy in the pediatric intensive care unit.* Clin Nutr. 2007;**26**(6):677-90.
13. Vlasselaers D, Milants I, Desmet L, Wouters PJ, et al. *Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study.* Lancet. 2009;**373**(9663):547-56.
14. Bober E, Buyukgebiz A. *Hypoglycemia and its effects on the brain in children with type 1 diabetes mellitus.* Pediatr Endocrinol Rev. 2005;**2**(3):378-82.
15. Vannucci RC, Vannucci SJ. *Glucose metabolism in the developing brain.* Semin Perinatol. 2000;**24**(2):107-15.
16. Flykanaka-Gantenbein C. *Hypoglycemia in childhood: long-term effects.* Pediatr Endocrinol Rev. 2004;**1 Suppl 3**:530-6.
17. Kalhan SC, Kilic I. *Carbohydrate as nutrient in the infant and child: range of acceptable intake.* Eur J Clin Nutr. 1999;**53 Suppl 1**:S94-100.
18. Koletzko B, Goulet O, Hunt J, Krohn K, et al. *1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR).* J Pediatr Gastroenterol Nutr. 2005;**41 Suppl 2**:S1-87.
19. Agus MS, Jaksic T. *Nutritional support of the critically ill child.* Curr Opin Pediatr. 2002;**14**(4):470-81.
20. Scohy TV, Golab HD, Egal M, Takkenberg JJ, et al. *Intraoperative glycemic control without insulin infusion during pediatric cardiac surgery for congenital heart disease.* Paediatr Anaesth. 2011;**21**(8):872-9.
21. Wolfe RR, Allsop JR, Burke JF. *Glucose metabolism in man: responses to intravenous glucose infusion.* Metabolism. 1979;**28**(3):210-20.
22. Shann F, Pearson G, Slater A, Wilkinson K. *Paediatric index of mortality (PIM): a mortality prediction model for children in intensive care.* Intensive Care Med. 1997;**23**(2):201-7.
23. Pollack MM, Ruttimann UE, Getson PR. *Pediatric risk of mortality (PRISM) score.* Crit Care Med. 1988;**16**(11):1110-6.

24. Leteurtre S, Martinot A, Duhamel A, Proulx F, et al. *Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study.* Lancet. 2003;**362**(9379):192-7.
25. Jenkins KJ, Gauvreau K, Newburger JW, Spray TL, et al. *Consensus-based method for risk adjustment for surgery for congenital heart disease.* J Thorac Cardiovasc Surg. 2002;**123**(1):110-8.
26. Lacour-Gayet F, Clarke D, Jacobs J, Comas J, et al. *The Aristotle score: a complexity-adjusted method to evaluate surgical results.* Eur J Cardiothorac Surg. 2004;**25**(6):911-24.
27. Zuppa AF, Nadkarni V, Davis L, Adamson PC, et al. *The effect of a thyroid hormone infusion on vasopressor support in critically ill children with cessation of neurologic function.* Crit Care Med. 2004;**32**(11):2318-22.
28. Schofield WN. *Predicting basal metabolic rate, new standards and review of previous work.* Hum Nutr Clin Nutr. 1985;**39 Suppl 1**:5-41.
29. van den Akker CH, te Braake FW, Wattimena DJ, Voortman G, et al. *Effects of early amino acid administration on leucine and glucose kinetics in premature infants.* Pediatr Res. 2006;**59**(5):732-5.
30. van der Schoor SR, de Koning BA, Wattimena DL, Tibboel D, et al. *Validation of the direct nasopharyngeal sampling method for collection of expired air in preterm neonates.* Pediatr Res. 2004;**55**(1):50-4.
31. Argoud GM, Schade DS, Eaton RP. *Underestimation of hepatic glucose production by radioactive and stable tracers.* Am J Physiol. 1987;**252**(5 Pt 1):E606-15.
32. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, et al. *Measurement of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide.* J Appl Physiol. 2008;**104**(4):944-51.
33. Schierbeek H, Moerdijk-Poortvliet TC, van den Akker CH, te Braake FW, et al. *Analysis of [U-13C6] glucose in human plasma using liquid chromatography/isotope ratio mass spectrometry compared with two other mass spectrometry techniques.* Rapid Commun Mass Spectrom. 2009;**23**(23):3824-30.
34. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, et al. *Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine.* Am J Physiol. 1980;**238**(5):E473-9.
35. Sy J, Gourishankar A, Gordon WE, Griffin D, et al. *Bicarbonate kinetics and predicted energy expenditure in critically ill children.* Am J Clin Nutr. 2008;**88**(2):340-7.
36. Verbruggen SC, Schierbeek H, Coss-Bu J, Joosten KF, et al. *Albumin synthesis rates in post-surgical infants and septic adolescents; influence of amino acids, energy, and insulin.* Clin Nutr. 2011;**30**(4):469-77.
37. Steele R, Wall JS, De Bodo RC, Altszuler N. *Measurement of size and turnover rate of body glucose pool by the isotope dilution method.* Am J Physiol. 1956;**187**(1):15-24.
38. Altszuler N, De Bodo RC, Steele R, Wall JS. *Carbohydrate metabolism of hypophysectomized dogs as studied with radioactive glucose.* Am J Physiol. 1956;**187**(1):25-31.
39. Verbruggen SC, de Betue CT, Schierbeek H, Chacko S, et al. *Reducing glucose infusion safely prevents hyperglycemia in post-surgical children.* Clin Nutr. 2011;**30**(6):786-92.
40. Radziuk J, Lickley HL. *The metabolic clearance of glucose: measurement and meaning.* Diabetologia. 1985;**28**(6):315-22.
41. Thompson GN, Pacy PJ, Merritt H, Ford GC, et al. *Rapid measurement of whole body and forearm protein turnover using a [2H5]phenylalanine model.* Am J Physiol. 1989;**256**(5 Pt 1):E631-9.
42. Clarke JT, Bier DM. *The conversion of phenylalanine to tyrosine in man. Direct measurement by continuous intravenous tracer infusions of L-[ring-2H5]phenylalanine and L-[1-13C] tyrosine in the postabsorptive state.* Metabolism. 1982;**31**(10):999-1005.
43. Golden MH, Waterlow JC. *Total protein synthesis in elderly people: a comparison of results with [15N] glycine and [14C]leucine.* Clin Sci Mol Med. 1977;**53**(3):277-88.
44. Argaman Z, Young VR, Noviski N, Castillo-Rosas L, et al. *Arginine and nitric oxide metabolism in critically ill septic pediatric patients.* Crit Care Med. 2003;**31**(2):591-7.

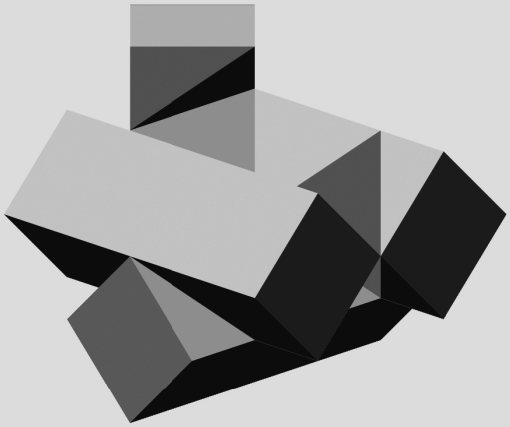
45. van den Akker CH, te Braake FW, Schierbeek H, Rietveld T, et al. *Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life.* Am J Clin Nutr. 2007;**86**(4):1003-8.
46. Preissig C, Rigby M. *Glycaemic control in paediatric critical care.* Lancet. 2009;**373**(9673):1423; author reply 1424.
47. Verbruggen SC, Coss-Bu J, Wu M, Schierbeek H, et al. *Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin-resistant adolescents with tight glucose control.* Crit Care Med. 2011;**39**(11):2518-25.
48. Cowett RM, Oh W, Schwartz R. *Persistent glucose production during glucose infusion in the neonate.* J Clin Invest. 1983;**71**(3):467-75.
49. Van Kempen AA, Romijn JA, Ruiter AF, Ackermans MT, et al. *Adaptation of glucose production and gluconeogenesis to diminishing glucose infusion in preterm infants at varying gestational ages.* Pediatr Res. 2003;**53**(4):628-34.
50. Glamour TS, McCullough AJ, Sauer PJ, Kalhan SC. *Quantification of carbohydrate oxidation by respiratory gas exchange and isotopic tracers.* Am J Physiol. 1995;**268**(4 Pt 1):E789-96.
51. Denne SC, Karn CA, Wang J, Liechty EA. *Effect of intravenous glucose and lipid on proteolysis and glucose production in normal newborns.* Am J Physiol. 1995;**269**(2 Pt 1):E361-7.
52. Van den Berghe G. *How does blood glucose control with insulin save lives in intensive care?* J Clin Invest. 2004;**114**(9):1187-95.
53. Burns SP, Cohen RD. *To: Krebs M, Brehm A, Krssak M et al. (2003) Direct and indirect effects of amino acids on hepatic glucose metabolism in humans. Diabetologia 46:917-925.* Diabetologia. 2004;**47**(1):141; author reply 142-3.





PART 4

INTERVENTIONAL CHALLENGES – PROTEIN AND ENERGY INTAKE

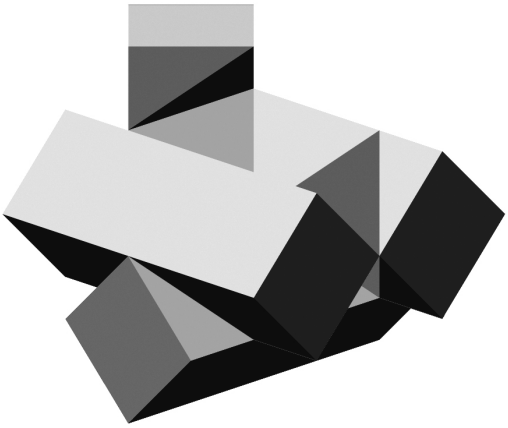


Het leven is wat je gebeurt,
terwijl je andere plannen maakt.

Uit "Laat me slapen" - Acda en de Munnik. Naar John Lennon.

chapter 6

CRITICALLY ILL INFANTS BENEFIT FROM EARLY ADMINISTRATION OF PROTEIN AND ENERGY ENRICHED FORMULA: A RANDOMIZED CONTROLLED TRIAL



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Clin Nutr. 2009;**28**(3):249-55

ABSTRACT

Background & aims: Nutritional support improves outcome in critically ill infants but is impeded by fluid restriction, gastric intolerance and feeding interruptions. Protein and energy-enriched infant formulas may help to achieve nutritional targets earlier during admission and promote anabolism.

Methods: Randomized controlled design. Infants with respiratory failure due to RSV-bronchiolitis received a protein and energy-enriched formula (PE-formula, n=8) or a standard formula (S-formula, n=10) during 5 days after admission. Primary outcome: nutrient delivery, energy and nitrogen balance and plasma amino acid concentrations. Secondary outcome: tolerance and safety.

Results: Nutrient intakes were higher in PE fed infants and met population reference intake (PRI) on day 3–5 whilst in S-fed infants PRI was met on day 5 only. Cumulative nitrogen balance (cNB) and energy balance (cEB) were higher in PE-infants compared to S-infants (cNB: 866 ± 113 vs. 296 ± 71 mg/kg; cEB: 151 ± 31 and 26 ± 17 kcal/kg, both $P < 0.01$). Essential amino acid levels were higher in PE-infants but within reference limits whereas below these limits in S-infants. Both formulas were well tolerated.

Conclusions: 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.

LIST OF ABBREVIATIONS

BCAA	Branched chain amino acids
BUN	Blood urea nitrogen
cEB	Cumulative energy balance
cNB	Cumulative nitrogen balance
CRP	C-reactive protein
Erasmus MC	Erasmus MC-Sophia Children's Hospital, Rotterdam
MREE	Measured resting energy expenditure
MUMC	Maastricht University Medical Center
NB	Nitrogen balance
NEFA	Non-esterified fatty acids
PE	Protein and energy-enriched
PREE	Predicted resting energy expenditure
PRI	Population reference intake
PRISM	Pediatric Risk of Mortality Score
RQ	Respiratory quotient
RSV	Respiratory syncytial virus
S	Standard
TEE	Total energy expenditure
TG	Triglycerides
TUN	Total urinary nitrogen excretion
UUN	Urinary urea nitrogen

INTRODUCTION

Nutritional support is an important aspect of the clinical management of pediatric intensive care patients. Critically ill children receiving better nutritional support show significant improvement in physiologic stability and outcome [1]. There is also evidence for the importance of providing adequate nutritional support in conditions such as prematurity [2] and pediatric burn injury [3]. Malnutrition and nutrient store depletion may be particularly harmful in infants since this is the most critical period of brain growth and endogenous stores of a number of relevant substrates such as glycogen, fat and especially protein are small [4]. Malnutrition in infancy is associated with decreased intelligence and neurological function and even infants undergoing relatively short periods of nutritional deprivation may have poorer learning abilities [5].

Several factors may impede adequate delivery of nutritional support to critically ill infants such as fluid restriction, frequent interruptions of enteral feeding for procedures and relative gastrointestinal intolerance [6]. Therefore enteral nutritional intake frequently differs from the amount prescribed by the physician and protein-energy deficits are often observed.

One of the most important targets of nutritional support in critically ill children is to prevent increased protein breakdown and loss of lean body mass associated with critical illness, whilst at the same time promoting tissue synthesis. It also means preventing under-or overfeeding and ultimately improving clinical outcome to ascertain normal growth and development.

Protein and energy-enriched infant formulas (PE-formulas) may help to achieve nutritional targets in critically ill infants in the early phase of the disease. However, it is not known if these enriched formulas are tolerated by these vulnerable infants or if they could lead to signs of overfeeding, like increased blood urea nitrogen (BUN), hyperglycemia and/or lipogenesis, and thereby further increase the metabolic demands of the acute illness.

The primary goal of the present study was therefore to compare the nutritional effects (nutrient delivery, energy and nitrogen balances, and plasma amino acid profiles) of a protein and energy-enriched infant formula (PE-formula) with a standard infant formula (S-formula) in a double-blind randomized controlled manner, in critically ill infants. Secondary aims were to assess tolerance and safety of these formulas.

SUBJECTS AND METHODS

Subjects and randomization

Infants (n=20) with respiratory failure due to respiratory syncytial virus (RSV) infection (positive RSV immunofluorescence in nasopharyngeal aspirate), admitted between December 2003 and February 2006 in the pediatric ICUs (PICU) of the Maastricht University Medical Center (MUMC) and the Erasmus MC-Sophia Children's Hospital, Rotterdam (Erasmus MC), were recruited if they met the following inclusion criteria: (1) age between 4 weeks and 12 months;

(2) born at term, or born preterm (< 38 weeks of gestation) but > 40 weeks postmenstrual age on enrollment; (3) mechanical ventilation; (4) indwelling arterial and venous catheter; (5) stable hemodynamic condition (normal blood pressure with or without inotropic medication). Exclusion criteria were: (1) breastfeeding; (2) parenteral nutrition besides intravenous dextrose; (3) congenital GI obstructions (stenosis, atresia, etc.); (4) congenital metabolic disease; (5) abnormal liver or kidney function tests or active upper GI bleeding; (6) known chromosomal disorder; (7) diarrhea, vomiting or gastric distension before randomization.

After obtaining informed consent from the parents/caregivers, the infants were randomized within 24-h of admission to receive the PE-formula (Infatrini®)(n=10) or the S-formula (Nutrilon 1®) (both from Nutricia Nederland B.V. Zoetermeer, The Netherlands) (n=10) during 5 days. The compositions of both formulas are summarized in **Table 6.1**. Both study formulas, indistinguishable by smell or consistency, were manufactured, prepared and delivered in identical masked and numbered bottles. Doctors, researchers, nursing staff, parents and caregivers were blinded to the feeds given. Randomization took place separately (parallel) in both centers

Table 6.1 – Composition of the two enteral formulas.

Average content per 100 ml of product	PE-formula	S-formula
Energy (kcal)	100	67
Protein (g) (from cow's milk)	2.6	1.4
En%	10	8
Casein (g)	1.0	0.6
Whey-protein (g)	1.6	0.8
Carbohydrates (g)	10.3	7.5
En%	41	45
Glucose	0.1	–
Lactose	5.3	7.5
Maltose	0.3	–
Polysaccharides	4.6	–
Fat (g) (from vegetable oils)	5.4	3.5
En%	49	47
Saturated	2	1.3
Monounsaturated	2.6	1.7
Polyunsaturated	0.8	0.4
Linoleic acid	0.64	0.35
α-Linolenic acid	0.11	0.06
P:E ratio (g protein/100 kcal)	2.6	2.1
Osmolality (mOsmol/L)	280	285

PE-formula, protein and energy-enriched formula; S-formula, standard infant formula; En%, energy-percentage. P:E ratio, protein:energy ratio.

by permuted block randomization and sequentially numbered, opaque and sealed envelopes. The code of the numbered bottles was guarded by Nutricia and only revealed after completion of the data collection in both centers.

Clinical parameters

Anthropometric measurements were taken at inclusion in the study using calibrated equipment but were not repeated because the severity of illness, with concomitant edema formation in all infants, precluded accurate measurements, whereas the study period was too brief to cause significant alterations in anthropometric values. Severity of illness was assessed by the pediatric risk of mortality score (PRISM) [7]. Clinical parameters recorded during the study period included number of ventilator days and length of stay in the PICU.

Nutritional regimen and tolerance

After randomization the study formulas were started as continuous drip-feeding by nasogastric (MUMC) or nasoduodenal catheter (ErasmusMC) according to institutional guidelines and using a calibrated feeding pump. Infants who already received enteral formula at inclusion in the study were switched to the study formulas. In order to optimize nutritional intake, the target volume of the enteral feed was 130 ml/kg/24 h for all infants. This was estimated to be the maximum achievable volume of fluid available for enteral nutrition in these critically ill ventilated infants. Study feeds were started at 25% of the target volume and increased with steps of 25% of the target volume every 12 h with the aim of reaching the target volume after 36 h. The treating physician, however, could limit the total volume of enteral intake when fluid restriction was deemed necessary on clinical grounds. Total macronutrient intake (enteral and intravenous) was recorded and compared to population reference intakes (PRI) for healthy infants below 6 months of age [8].

Tolerance of both formulas was evaluated by monitoring the volumes of gastric retention every 4 h, the frequency and volumes of vomiting and volumes, frequency, and consistency of stools by the nursing staff. Intolerance was defined as gastric retentions > 50% of the administered volume over the previous 4 h on more than one occasion, occurrence of any gastric distension or vomiting and/or diarrhea defined as > 4 stools per day of watery consistency and leading to substantial fluid loss as either shown by negative fluid balance or hemodynamic consequences (extra fluid requirement, signs of dehydration, tachycardia or hypotension). Nitrogen balance was assessed by measuring urinary urea concentration in urine collected 24 hourly by urinary catheter. Urea was determined on a routine clinical chemistry analyzer (Synchron LX20 Pro, Beckman Coulter Inc., Fullerton, CA, USA). Urinary urea nitrogen excretion (UUN) was converted to total urinary nitrogen excretion (TUN) using the equation, TUN (mg/kg/24 h) = UUN x 1.25, the factor 1.25 was used to correct for non-urea nitrogen. Nitrogen balance (NB) was calculated as total daily protein intake (mg/kg/24h) / 6.25 - TUN,

the factor 6.25 representing the average amount of nitrogen in protein. Cumulative nitrogen balance was calculated by summation of the nitrogen balances of day 1–5.

Energy expenditure and substrate oxidation

Resting energy expenditure was measured (MREE) daily by indirect calorimetry using a previously validated metabolic monitor (Deltatrac II MBM-200, Datex Division Instrumentarium Corp. Finland) [9]. The measurement was only performed in infants fulfilling previously described criteria (tube leakage < 10%, FiO_2 < 60%), to assure accurate measurement. Total energy expenditure (TEE) was then calculated by adding 10% to MREE for patient activity related to nursing care. If assessment of MREE was not possible, resting energy expenditure was predicted (PREE) using the Schofield equation, adapted for age and sex [10] and used instead of MREE. Energy balances were calculated by subtracting TEE from the total daily energy intakes. Cumulative energy balance was calculated by summation of the energy balances of day 1–5. Respiratory quotient (RQ) was defined as VCO_2/VO_2 and lipogenesis was defined as an RQ > 1.0.

Values obtained by indirect calorimetry (VO_2, VCO_2) and UUN on day 5 were used to calculate rates of protein, fat and glucose oxidation during continuous enteral feeding as previously described [11]. Protein, carbohydrate and fat balances were calculated by subtracting the values of nutrient oxidation from the daily nutrient intake.

Biochemical parameters and plasma amino acid concentrations

Plasma concentrations of glucose, insulin, triglycerides (TG), non-esterified fatty acids (NEFA), urea and creatinine were determined in arterial blood collected on day 1 (before start of the study formulas) and on day 5 (at the end of the study period during continuous enteral feeding). Severity of inflammation was assessed by measuring plasma C-reactive protein (CRP) concentrations on days 1 and 5. Amino acid concentrations in plasma were measured on day 5 by high-performance liquid chromatography (HPLC) as described by van Eijk et al. [12] with variation coefficients < 3%. Determination of glucose, TG, NEFA, urea, creatinine and CRP were done on a routine clinical chemistry analyzer. Plasma insulin concentrations were determined in the MUMC and ErasmusMC on an AutoDelfia (PerkinElmer Life and Analytical Sciences, Wellesley, MA, USA) by an Immunofluorimetric monoclonal antibody assay.

Statistical analysis

Power analysis was performed based on results of nitrogen balance studies in critically ill infants [13]. With two-sided significance of 0.05 and sensitivity of 0.80 the number of patients needed in both groups to detect a difference of 50% in nitrogen retention was 8. Data were analyzed using the statistical program SPSS 12.0 for Windows (SPSS Inc, Chicago, IL). Normality of all data was verified by the Kolmogorov–Smirnov test. Variables with a normal distribution

were compared among the groups with the independent samples' t-test. When a variable had no normal distribution the Mann–Whitney U analysis was applied. Results are expressed as mean ± SEM, unless otherwise indicated. A P-value < 0.05 was considered significant.

Ethics

Approval of the Central Committee on Research Involving Human Subject (The Hague, The Netherlands) and the local ethical committees in both centers was obtained. Written informed consent was obtained from all parents/caregivers.

RESULTS

Patient characteristics

Two infants in the PE-group were withdrawn from the study because they were switched by mistake to non-study formula after extubation before day 5 in both cases. Patient characteristics are shown in **Table 6.2**. Gestational age was significantly lower in the PE-group, but no other differences between the groups were found. Also there were no differences noted between patients enrolled in either center (data not shown). Duration of ventilation and length of stay on the pediatric ICU did not differ significantly between the groups.

Table 6.2 – Baseline patient characteristics.

	PE-group (n = 8)	S-group (n = 10)
Medical center (MUMC/Erasmus MC)	4/4	6/4
Gender (M/F)	2/6	3/7
Age (months)	2.7 ± 0.5	3.0 ± 0.6
Body weight (g)	3967 ± 357	4791 ± 371
Crown-heel length (cm)	52.1 ± 1.4	55.2 ± 1.5
Gestational age (weeks)	35.0 ± 1.2*	37.3 ± 0.4
Postmenstrual age (weeks)	46.8 ± 2.9	49.9 ± 3.0
Birth weight (g)	2299 ± 341	2846 ± 71
PRISM score	20.3 ± 1.6	18.6 ± 1.5
CRP on admission (mg/l)	76.7 ± 23.2	64.4 ± 20.1
Mechanical ventilation (days)	7.1 ± 2.2	5.5 ± 0.7
Length of PICU stay (days)	9.0 ± 2.7	6.7 ± 0.7

PE-group, protein and energy-enriched formula fed group; S-group, standard formula fed group; MUMC, Maastricht University Medical Center; Erasmus MC, Erasmus MC - Sophia Children's Hospital, Rotterdam; PRISM, Pediatric Risk of Mortality; CRP, C-reactive protein. Data are presented either as number of subjects or mean ± SEM.

* P < 0.05 (independent samples t-test or Mann–Whitney U).

Nutrient delivery and tolerance

Most infants (six patients in the PE-group and seven in the S-group) received infant formulas before start of the study and were switched to the study formulas after randomization. Study formulas were started 25.3 ± 5.6 and 23.4 ± 5.4 h after admission to the PICU in the PE-and S-group, respectively, and increased every 12 h, according to the study protocol, until the target volume was reached or the treating physician decided to limit further increases of the fluid volume. Volumes of enteral intake did not differ between the groups on any of the study days (22 ± 12, 67 ± 8, 87 ± 7, 101 ±11, 109 ± 7 ml/kg/24 h for the combined group of infants on days 1–5). The same was true for volumes of intravenous fluids (77 ± 15, 46 ± 10, 33 ± 9, 27 ± 10, and 20 ± 9 ml/kg/24 h for the combined group of infants on days 1–5). No patients received (partial) parenteral nutrition besides dextrose. In the majority of patients in both groups (75% and 80% of patients in the PE-group and S-group, respectively) the target volume of enteral intake (130 ml/kg/24 h) was not reached during the study period due to fluid restriction measures by the treating physician.

Energy and protein intakes on days 1–5 in both groups are depicted in **Figure 6.1**. Energy and protein intake were significantly higher on days 3, 4 and 5 in infants in the PE-group compared to the S-group. This was also the case for fat intake on days 3,4 and 5, whilst total carbohydrate intake was higher on days 4 and 5 (fat and carbohydrate intakes on day 5 are presented in **Table 6.3**). Nutritional intake in the PE-infants met PRI for energy (85–95 kcal/

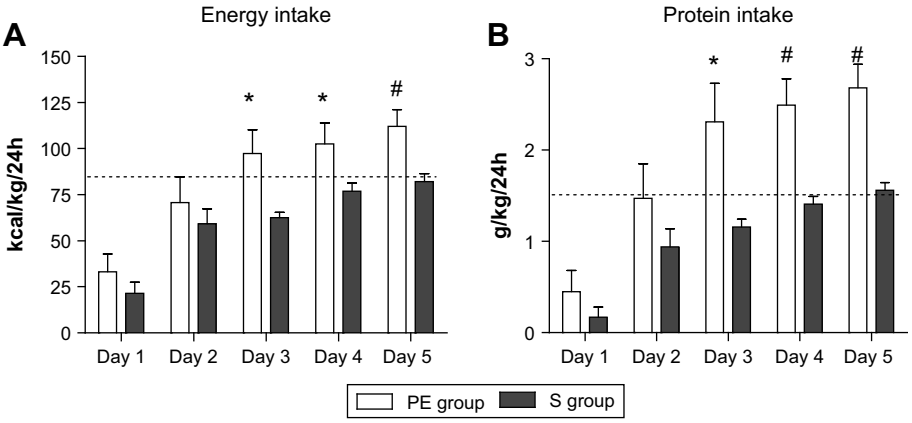


Figure 6.1 – Energy and protein intake on days 1–5.

Data are presented as mean with error bars representing SEM. Dotted lines represent population reference intakes (PRI) for energy, and protein.

PE-group, protein and energy-enriched formula fed group (n = 8); S-group, standard formula fed group (n = 10).

*P < 0.05, #P < 0.01 vs. the S-group (independent samples t-test or Mann-Whitney U).

Table 6.3 – Parameters of substrate oxidation on day 5.

	PE-group	S-group
Energy intake[b] (kcal/kg/day)	112 ± 13#	82 ± 4
MREE[a] (kcal/kg/day)	54 ± 3	50 ± 3
RQ[a]	0.96 ± 0.02#	0.91 ± 0.01
Protein intake[b] (g/kg/day)	2.8 ± 0.3#	1.5 ± 0.1
TUN[b] (protein oxidation) (mg/kg/day)	151 ± 43	117 ± 29
Nitrogen balance[b] (mg/kg/day)	297 ± 41#	123 ± 23
Carbohydrate intake[a] (g/kg/day)	13.1 ± 1.3*	9.9 ± 0.6
Glucose oxidation[a] (g/kg/day)	14.3 ± 1.3*	10.7 ± 1.6
Carbohydrate balance[a] (g/kg/day)	−0.9 ± 1.0	−0.7 ± 1.7
Fat intake[a] (g/kg/day)	5.8 ± 0.4*	3.8 ± 0.2
Fat oxidation[a] (g/kg/day)	0.7 ± 0.3*	1.4 ± 0.2
Fat balance[a] (g/kg/day)	5.1 ± 0.7*	2.4 ± 0.4

PE-group, protein and energy-enriched formula fed group; S-group, standard formula fed group; RQ, respiratory quotient; MREE, measured resting energy expenditure; TUN, total urinary nitrogen excretion; Data are presented as mean ± SEM.

[a] Subgroup of patients (five PE-patients and nine S-patients).

[b] All patients.

* $P < 0.05$.

# $P < 0.01$ versus the S-group (independent samples t-test or Mann–Whitney U).

kg/24 h), protein (1.52 g/kg/24 h), carbohydrates (10 g/kg/24 h) and fat (4.7 g/kg/24 h) already by days 3–5, whilst in S-infants this was only the case on day 5.

Both formulas were well tolerated and gastric distension, vomiting and diarrhea did not occur and feed delivery was not reduced or stopped for reasons of intolerance in any of the patients in both groups during the study period. Gastric retention volumes, however, were significantly higher in the PE-group (9.8 ± 2.7 and 4.7 ± 2.4 ml/24 h in the PE-group and S-group, respectively, $P < 0.01$). Volumes of stools did not differ between the groups (6.4 ± 1.6 and 5.8 ± 1.7 g/24 h in the PE-group and S-group, respectively). Duodenal drip-feed was given in four infants in the PE-group and four in the S-group. There were no significant differences in the amount of feed given between infants receiving nasogastric or nasoduodenal feed and there were no differences in gastric retention volumes or stool volumes (data not shown).

Nitrogen balance and plasma amino acid concentrations

Because of incomplete urinary collections in the majority of patients on the first day of admission, 24 h-nitrogen balances could only be calculated on days 2–5. The daily nitrogen balances on days 2–5 were significantly higher in the PE-infants when compared to the S-infants (147 ± 21, 251 ± 34, 249 ± 48, 219 ± 79 vs. -48 ± 21, 43 ± 58, 60 ± 24 and 146 ± 28 in PE-and S-infants; all $P < 0.05$). The cumulative nitrogen balance calculated over days 2–5 was also significantly

higher (866 ± 113 vs. 297 ± 71 in PE-vs. S-infants, P < 0.01). From the second day onwards all infants receiving PE-formula were in positive nitrogen balance (reflecting anabolism) whereas some infants receiving S-formula had a negative nitrogen balance (reflecting catabolism) until day 4. Significantly higher concentrations of several essential amino acids (histidine, lysine, phenylalanine, methionine and valine) and of the non-essential amino acid ornithine were found in the PE-group compared to the S-group on day 5 (**Table 6.4**). This was also true for

Table 6.4 – Plasma amino acid concentrations on day 5[a].

	PE-group (n = 8)	S-group (n = 10)	Reference values[d] (range)
Methionine[b]	36 ± 2 *	28 ± 2	30–59
Histidine[b]	72 ± 5*	58 ± 4	59–110
Phenylalanine[b]	77 ± 4*	64 ± 3	43–87
Tryptophan[b]	75 ± 5	65 ± 6	36–92
Lysine[b]	238 ± 27*	155 ± 14	38–144
Threonine[b]	244 ± 44	195 ± 20	88–169
Isoleucine[b and c]	36 ± 4	27 ± 2	36–89
Leucine[b and c]	128 ± 6	113 ± 5	48–145
Valine[b and c]	142 ± 9*	104 ± 3	108–214
Ornithine	104 ± 9*	80 ± 8	67–150
Glutamic acid	198 ± 21	191 ± 20	181–379
Asparagine	68 ± 8	67 ± 6	35–78
Serine	154 ± 8	143 ± 9	103–257
Glutamine	646 ± 61	610 ± 30	314–620
Glycine	257 ± 20	250 ± 13	33–180
Citrulline	22 ± 3	22 ± 2	20–125
Arginine	101 ± 13	89 ± 7	40–99
Alanine	332 ± 63	294 ± 20	227–388
Taurine	28 ± 6	27 ± 3	
Tyrosine	70 ± 5	59 ± 5	34–135
Sum of all AA	3026 ± 226	2648 ± 98	
Sum of EAA	1047 ± 80*	817 ± 42	
Sum of BCAA	305 ± 17#	252 ± 9	

PE-group, protein and energy-enriched formula fed group; S-group, standard formula fed group; AA, amino acids; sum of all AA, sum of all measured AA; EAA, essential EAA; BCAA, branched chain AA.

[a] All data in µmol/l and expressed as mean ± SEM.

[b] Essential AA.

[c] BCAA, branched chain AA.

[d] Healthy term breast-fed infants in postnatal week 7 (ref. 14).

* P < 0.05.

# P < 0.01 (independent samples t-test or Mann–Whitney U).

the sum of the branched chain amino acids (BCAA: isoleucine, leucine and valine) and the sum of all essential amino acids (methionine, histidine, phenylalanine, tryptophane, lysine, threonine and BCAA). Plasma concentrations of most amino acids did not exceed age related reference values of healthy breast-fed infants [14] except for concentrations of glycine, lysine and threonine which were elevated in both groups. In the S-group levels of histidine, methionine, valine and isoleucine were below these reference values.

Energy expenditure and substrate oxidation

Indirect calorimetry was performed daily in a subgroup of 14 infants (5 PE-infants and 9 S-infants) fulfilling the entry criteria (tube leakage < 10%, FiO_2 < 60%). Fifty-seven measurements were performed (2.9 ± 0.6 measurements per patient). The results of day 5 in these infants are shown in **Table 6.3**. MREE did not differ significantly between PE-and S-infants on days 1–5. The daily energy balances on days 3–5 (calculated in all infants) were significantly higher in the PE-group when compared to the S-group (40 ± 12, 44 ± 9 and 52 ± 9 vs. 15 ± 3, 27 ± 5 and 30.9 ± 6 in PE-versus S-infants; all $P < 0.05$). The cumulative energy balance calculated over days 1–5 was also significantly higher (51 ± 31 vs. 26 ± 17 in PE-vs. S-infants, $P < 0.01$). RQ on day 4 and 5 was significantly higher in PE-patients than in S-patients. An RQ > 1.0, indicative of overfeeding, was found in two infants in the PE-group but only on a single occasion (one on day 4 and one on day 5) out of a total of 22 measurements.

Substrate oxidation was calculated in the subgroup of patients in whom indirect calorimetry could be performed (**Table 6.3**). Glucose oxidation was significantly higher in infants receiving PE-formula in whom carbohydrate intake was also higher. Carbohydrate balance was not significantly different. Fat oxidation was significantly lower in the PE-group compared to the S-group, in spite of a higher fat intake. This resulted in a significantly higher fat balance in the PE-group.

Biochemical parameters

There were no significant differences in biochemical parameters between the study groups on day 1 and 5 (**Table 6.5**). CRP decreased significantly between day 1 and 5 in both study groups.

DISCUSSION

The present double-blind randomized controlled study compared the nutritional effects of a protein and energy-enriched formula (PE-formula, providing 2.6 g protein and 100 kcal/100 ml) with a standard infant formula (S-formula, providing 1.4 g protein and 67 kcal/100 ml) in similar groups of critically ill infants in the first 5 days of admission to a pediatric ICU. Our results show that the use of a PE-formula in critically ill infants promotes a higher and more adequate nutrient delivery and improves energy and nitrogen balance.

Table 6.5 – Biochemical parameters on day 1[a] and 5[b].

	Day 1		Day 5	
	PE-group (n = 8)	S-group (n = 10)	PE-group (n = 8)	S-group (n = 10)
Glucose (mmol/l)	5.4 ± 0.3	5.5 ± 0.3	5.6 ± 0.2	5.2 ± 0.1
Insulin (mU/l)	19 ± 5	9 ± 3	17 ± 4	12 ± 3
Triglycerides (mmol/l)	0.99 ± 0.14	0.87 ± 0.17	1.36 ± 0.22	1.85 ± 0.35
NEFA (mmol/l)	0.37 ± 0.10	0.25 ± 0.04	0.30 ± 0.08	0.42 ± 0.12
Urea (mmol/l)	2.45 ± 0.48	2.27 ± 0.42	1.88 ± 0.59	1.25 ± 0.20
Creatinine (µmol/l)	27 ± 4	25 ± 3	27 ± 3	27 ± 3
CRP (mg/l)	75 ± 23	75 ± 18	23 ± 7*	28 ± 8*

PE, protein and energy-enriched formula fed group; S, standard formula fed group; NEFA, non-esterified fatty acids; CRP, C-reactive protein. Data are presented as mean ± SEM.

[a] Blood samples taken before start of the study feedings.

[b] Blood samples taken during continuous enteral feeding.

*P < 0.05 day 5 vs. day 1 in the same group (independent samples t-test or Mann–Whitney U).

Gastrointestinal intolerance did not occur in any of the infants in our study and both formulas were well tolerated. This is in agreement with studies showing that gastrointestinal dysfunction is not a major reason for reduced intakes in critically ill infants and children [28]. It is also consistent with the finding of Evans et al. [15] demonstrating that PE-formula is generally well tolerated in infants even when given at full strength from the first day of usage. Further support for the safety of PE-formula in critically ill children was found by the evaluation of biochemical parameters; plasma levels of triglycerides, NEFA, glucose and insulin were all within normal ranges and without significant differences between both groups. The higher protein and energy intake in infants in the PE-group resulted in a significantly higher and positive nitrogen balance suggestive of improved protein accretion and growth in the first days after admission, which is considered the most important target of nutritional support. This is in agreement with the study of Clarke et al. [16] who demonstrated that a high energy, high protein feed can improve length growth in infants with growth faltering with various disease states. This is also in agreement with earlier studies in preterm infants showing that protein balance and growth can be improved by parenteral amino acid supplementation [17], while positive effects of a high protein intake have also been found in severely ill children [3, 18].

The positive effect of increasing nutrient intake on protein metabolism is thought to be primarily mediated by its effect on the availability of essential amino acids. The level of carbohydrate intake alone has no effect on protein metabolism but may have an additive effect by stimulating insulin secretion [19]. Increase of the plasma concentration of essential amino acids and especially the branched chain amino acids has been shown to stimulate (muscle) protein synthesis [19], suppress protein breakdown [20] and improve net protein balance. Indeed in the present study, infants receiving PE-formula had significantly higher plasma

concentrations of many essential amino acids, especially the branched chain amino acids in comparison to the infants receiving S-formula.

High protein intake in infants has been associated with hyperaminoacidemia and increased blood urea levels [21]. However, this did not occur in the infants in the present study. Most plasma essential amino acid concentrations were within normal limits in the PE-group whilst reduced concentrations of several essential amino acids were found in infants in the S-group. Since limitation in the availability of essential amino acids may limit the protein synthetic rate, insufficient intake of essential amino acids in the S-group may have contributed to differences in nitrogen balance found in the present study.

Optimal energy intake in infants recovering from critical illness is not well defined. Measurement of energy expenditure (MREE) with indirect calorimetry for tailoring energy supply is therefore often advocated [22]. However, MREE should be seen as the minimum level of intake for maintenance of body functions and not the optimal energy intake for critically ill infants because it does not take in account the losses of energy via stools (up to 10% in infants) and energy deposition in tissues for growth that may be as high as 50 kcal/kg/24 h [23]. Since the acute metabolic response to severe stress will resolve rapidly (< 48 h) in most infants, normal growth and even catch-up growth may resume early and energy and protein requirements may be equal to PRI. However, a considerable risk of overfeeding also exists, especially due to high carbohydrates' intake, which was the reason for us to evaluate substrate oxidation by indirect calorimetry. Infants receiving PE-formula indeed showed a higher RQ, a higher rate of glucose oxidation and a lower rate of fat oxidation. This is in agreement with earlier studies showing that glucose oxidation increases in a linear manner with increasing carbohydrate intake whilst fat oxidation concomitantly decreases [24]. In two infants receiving PE-formula an RQ > 1.0 was found in a single measurement during the study (2 out of the 22 measurements), which may indicate carbohydrate overfeeding. Total carbohydrate intake (intravenous and enteral) in these two infants was 8.1 and 10.2 mg/kg/min at the time of measurement. Previous studies have shown a maximum glucose oxidation capacity ranging from 5 to 9 mg/kg/min in critically ill children [25, 26] and up to 12.5 mg/kg/min in infants [24]. This large variation may be explained by differences in methodology [25], the severity of the underlying illness [26] and also by individual oxidation capacity of carbohydrates [27]. Although an RQ > 1.0 is often used to detect overfeeding, one should be cautious in using it on a single occasion because other factors may also affect RQ, for instance a period of tachypnea during weaning from the mechanical ventilation and measurement errors [22, 27].

Although the medical teams in both hospitals were strongly encouraged to maximize the delivery of enteral feeding in order to achieve the target volume of enteral intake, the actual volumes of feedings delivered did not match this target in the majority of infants in both groups. This was primarily due to limiting the enteral feeds by the treating physician to compensate for fluids given with drugs and infusions and to a lesser degree also due to interruptions of enteral feeding for procedures. This is a recognized problem in ICUs and has been

reported previously in critically ill children as well as in infants [28]. In spite of the volume limits of the enteral feeds, nutrient intakes in infants receiving the PE-formula matched PRI for protein, fat and carbohydrates as early as the third day of admission while PRI was not met in the S-infants during the 5 days of the study. The importance of our findings is supported by the study of Hulst et al. [29] who prospectively studied energy and protein intake in pediatric ICU patients. In the course of their hospital stay these children showed significant cumulative nutritional deficits compared to PRI associated with declined weight and arm circumference. Since deficits accumulated most rapidly during the first days of admission, they emphasized that special attention should be paid to the adequacy of feeding during the first days of admission. As shown by our data, a protein and energy-enriched formula may be of particular benefit to reach nutritional goals in critically ill infants in this early phase without the risk of overfeeding.

During recovery, when the volume of enteral intake is no longer limited by fluid restriction, a protein and energy-enriched formula may be of benefit too by supplying the necessary nutrients for catch-up growth. However, accelerated catch-up growth (and especially fat growth) should probably be avoided since studies in preterm small for gestational age infants have found that high growth rates in infancy are associated with obesity and increased cardiovascular risk in later life although a better neurocognitive outcome was also found (see for review [30]). Therefore nutritional intake in this phase should be titrated to the estimated individual need of the infant in combination with evaluation of growth.

CONCLUSION

Early enteral administration of PE-formula to critically ill infants is well tolerated and safe, promotes an adequate nutrient delivery early in admission and improves energy and nitrogen balance without adverse metabolic effects. Further research is needed to elucidate the effects of a protein and energy-enriched formula on clinical outcome in critically ill infants.

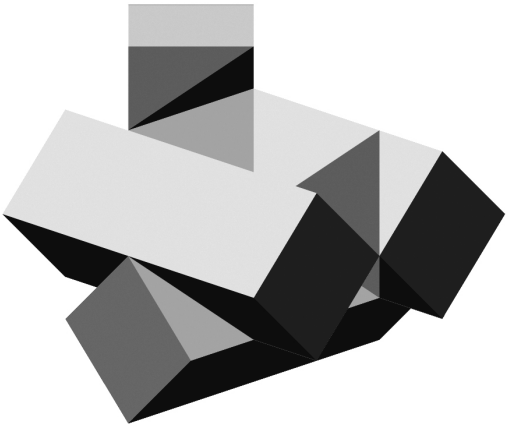
REFERENCES

1. Pollack MM, Ruttimann UE, Wiley JS. *Nutritional depletions in critically ill children: associations with physiologic instability and increased quantity of care.* J Parenter Enteral Nutr 1985;**9**:309–13.
2. Dunn L, Hulman S, Weiner J, Kliegman R. *Beneficial effects of early hypocaloric enteral feeding on neonatal gastrointestinal function: preliminary report of a randomized trial.* J Pediatr 1988;**112**:622–9.
3. Alexander JW, MacMillan BG, Stinnett JD, Ogle CK, et al. *Beneficial effects of aggressive protein feeding in severely burned children.* Ann Surg 1980;**192**:505–17.
4. Cunningham JJ. *Body composition and nutrition support in pediatrics: what to defend and how soon to begin.* Nutr Clin Pract 1995;**10**:177–82.
5. Klein P, Forbes G, Nader P. *Effects of starvation in infancy (pyloric stenosis) on subsequent learning abilities.* J Pediatr 1975;**87**:8–15.
6. Hulst JM, Joosten KF, Tibboel D, van Goudoever JB. *Causes and consequences of inadequate substrate supply to pediatric ICU patients.* Curr Opin Clin Nutr Metab Care 2006;**9**:297–303.
7. Pollack MM, Ruttimann UE, Getson PR. *Pediatric risk of mortality (PRISM) score.* Crit Care Med 1988;**16**:1110–6.
8. *Macronutrients.* In: Dietary reference intakes for macronutrients. Washington: US National Academy of Sciences; 2005.
9. Joosten KF, Verhoeven JJ, Hop WC, Hazelzet JA. *Indirect calorimetry in mechanically ventilated infants and children: accuracy of total daily energy expenditure with 2 hour measurements.* Clin Nutr 1999;**18**:149–52.
10. Schofield WN. *Predicting basal metabolic rate, new standards and review of previous work.* Hum Nutr Clin Nutr 1985;**39**(Suppl. 1):5–41.
11. Consolazio FC, Johnson RE, Pecora LJ. The computation of metabolic balances. In: Physiological measurements of metabolic function in man. New York: McGraw-Hill Book Company; 1963. p. 313–39.
12. van Eijk HM, Rooyakkers DR, Soeters PB, Deutz NE. *Determination of amino acid isotope enrichment using liquid chromatography–mass spectrometry.* Anal Biochem 1999;**271**:8–17.
13. Chaloupecky V, Hucin B, Tlaskal T, Kostelka M, et al. *Nitrogen balance, 3-methylhistidine excretion, and plasma amino acid profile in infants after cardiac operations for congenital heart defects: the effect of early nutritional support.* J Thorac Cardiovasc Surg 1997;**114**:1053–60.
14. Scott PH, Sandham S, Balmer SE, Wharton BA. *Diet-related reference values for plasma amino acids in newborns measured by reversed-phase HPLC.* Clin Chem 1990;**36**:1922–7.
15. Evans S, Twaissi H, Daly A, Davies P, et al. *Should high-energy infant formula be given at full strength from its first day of usage?* J Hum Nutr Diet 2006;**19**:191–7. quiz 199–201.
16. Clarke SE, Evans S, Macdonald A, Davies P, et al. *Randomized comparison of a nutrient-dense formula with an energy-supplemented formula for infants with faltering growth.* J Hum Nutr Diet 2007;**20**:329–39.
17. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, et al. *Amino acid administration to premature infants directly after birth.* J Pediatr 2005;**147**:457–61.
18. Patterson B, Nguyen T, Pierre E, Herndon D, et al. *Urea and protein metabolism in burned children: effect of dietary protein intake.* Metabolism 1997;**46**:573–8.
19. Wolfe RR. *Regulation of muscle protein by amino acids.* J Nutr 2002;**132**:3219S–24S.
20. Poindexter BB, Karn CA, Ahlrichs JA, Wang J, et al. *Amino acids suppress proteolysis independent of insulin throughout the neonatal period.* Am J Physiol 1997;**272**:E592–9.
21. Janas LM, Picciano MF, Hatch TF. *Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentration and various whey/casein ratios.* J Pediatr 1987;**110**:838–48.
22. Joosten KF, Verhoeven JJ, Hazelzet JA. *Energy expenditure and substrate utilization in mechanically ventilated children.* Nutrition 1999;**15**:444–8.
23. Butte NF. *Energy requirements of infants.* Public Health Nutr 2005;**8**:953–67.

24. Jones MO, Pierro A, Hammond P, Nunn A, et al. *Glucose utilization in the surgical newborn infant receiving total parenteral nutrition.* J Pediatr Surg 1993;**28**:1121–5.
25. Sheridan RL, Yu YM, Prelack K, Young VR, Burke JF, et al. *Maximal parenteral glucose oxidation in hypermetabolic young children: a stable isotope study [see comments].* J Parenter Enteral Nutr 1998;**22**:212–6.
26. Coss-Bu JA, Jefferson LS, Walding D, David Y, et al. *Resting energy expenditure in children in a pediatric intensive care unit: comparison of Harris–Benedict and Talbot predictions with indirect calorimetry values.* Am J Clin Nutr 1998;**67**:74–80.
27. Hulst JM, van Goudoever JB, Zimmerman LJ, Hop WC, et al. *Adequate feeding and the usefulness of the respiratory quotient in critically ill children.* Nutrition 2005;**21**:192–8
28. Rogers EJ, Gilbertson HR, Heine RG, Henning R. *Barriers to adequate nutrition in critically ill children.* Nutrition 2003;**19**:865–8.
29. Hulst JM, van Goudoever JB, Zimmermann LJ, Hop WC, et al. *The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population.* Clin Nutr 2004;**23**:1381–9.
30. Yeung MY. *Postnatal growth, neurodevelopment and altered adiposity after preterm birth – from a clinical nutrition perspective.* Acta Paedatr 2006;**95**(8):909-17.

chapter 7

INCREASED PROTEIN-ENERGY INTAKE PROMOTES ANABOLISM IN CRITICALLY ILL INFANTS WITH VIRAL BRONCHIOLITIS: A DOUBLE BLINDED RANDOMISED CONTROLLED TRIAL



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*Arch Dis Child. 2011;**96**(9):817-22*

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ABSTRACT

Objective: Preservation of nutritional status and growth is an important aim in critically ill infants, but difficult to achieve due to the metabolic stress response and inadequate nutritional intake, leading to negative protein balance. This study investigated whether increasing protein and energy intakes can promote anabolism. Primary outcome was whole body protein balance, secondary outcome first pass splanchnic phenylalanine extraction (SPE_{Phe}).

Design: Double blinded randomised controlled trial. Infants (n=18) admitted to the paediatric ICU with respiratory failure due to viral bronchiolitis were randomised to be continuously enterally fed with Protein and Energy enriched (PE-)formula (n=8; 3.1±0.3 g protein/kg/24h, 119±25 kcal/kg/24h) or standard (S-)formula (n=10; 1.7±0.2 g protein/kg/24h, 84±15 kcal/kg/24h; equivalent to recommended intakes for healthy infants < 6 months). Using a combined intravenous-enteral phenylalanine stable isotope protocol on day 5 post-admission, whole body protein metabolism and SPE_{Phe} were determined.

Results: Protein balance was significantly higher with PE-formula than with S-formula (PE-formula, 0.73±0.5 vs. S-formula, 0.02±0.6 g/kg/24h) resulting from significantly increased protein synthesis (PE-formula: 9.6±4.4, S-formula: 5.2±2.3 g/kg/24h), despite significantly increased protein breakdown (PE-formula: 8.9±4.3, S-formula: 5.2±2.6 g/kg/24h). SPE_{Phe} was not statistically different between groups (PE-formula: 39.8±18.3%, S-formula: 52.4±13.6%).

Implications: Increasing protein and energy intakes promotes protein anabolism in critically ill infants in the first days post-admission. Since this is an important target of nutritional support, increased protein and energy intakes should be preferred above standard intakes in these infants.

LIST OF ABBREVIATIONS

BCAA	Branched chain amino acids
CRP	C-reactive protein
EAA	Essential amino acids
ErasmusMC	Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, The Nether lands
MUMC	Maastricht University Medical Center, Maastricht, The Netherlands
PE-formula	Protein and Energy enriched formula
PE-group/ PE-infants	Group of infants receiving protein and energy enriched formula
Phe	Phenylalanine
PICU	Paediatric Intensive Care Unit
PRISM	Pediatric Risk of Mortality
S-formula	Standard infant formula
S-group/ S-infants	Group of infants receiving standard infant formula
SPE	Splanchnic extraction, i.e. the fraction of absorbed amino acids, retained in the splanchnic area during first pass
SPE_{Phe}	Splanchnic extraction of phenylalanine
TUN	Total urinary nitrogen
WbPB	Whole body protein breakdown
WbPBal	Whole body protein balance
WbPM	Whole body protein metabolism
WbPS	Whole body protein synthesis

OBJECTIVE

Preservation of nutritional status and growth is a specific aim in critically ill children, but difficult to achieve. This is due to a metabolic stress response with profound changes in protein metabolism leading to a negative protein balance and loss of lean body mass. Inadequate nutritional intake at the paediatric intensive care unit (PICU), often due to fluid restriction, further leads to protein and energy deficits especially early after admission [1]. Other factors that hinder adequate nutrition are impaired intracellular insulin signaling [2], impaired glucose uptake,[3] and reduced mitochondrial capacity during critical illness [4]. These factors are probably the reason why protein-energy malnutrition is observed in 16-24% of critically ill children [5, 6] and is associated with adverse clinical outcome [7-9].

A common but threatening disease in infants is viral bronchiolitis, which in severe cases leads to respiratory failure with need for ventilatory support and PICU admission. Adequate nutritional support in these critically ill infants is important, with protein anabolism as goal. However, up until now common practice is to use standard infant formulas to provide approximately 1.5 gram protein/kg/day and 100 kcal/kg/day.

Increased protein intake with adequate energy provision promotes anabolism in preterm infants [10-12], in neonates undergoing surgery [13] and in children with burns [14] and cystic fibrosis [15]. Important in relation to these observations is that protein synthesis is a high-energy consuming process [16] and energy deficiency worsens nitrogen balance [17, 18]. Hence, to induce net protein anabolism, it is essential to provide adequate energy intake. We therefore hypothesized that increasing protein and energy intakes will induce net protein anabolism in critically ill infants.

Stable isotope amino acid methods are used to determine net protein balance [19]. During feeding, amino acids appearing in the circulation come from protein breakdown and from the fraction of meal-derived amino acids that are not retained in the splanchnic area. Protein synthesis during feeding can be calculated from the disappearance of essential amino acids like phenylalanine from the circulation, corrected for non-protein synthesis related disposal (e.g. oxidation, hydroxylation). Therefore, to be able to calculate whole body net protein anabolism during feeding, all these factors need to be taken into account [20, 21]. Splanchnic extraction (SPE) of meal-derived amino acids has not been reported before in critically ill children.

The present study was part of a larger study on the nutritional and metabolic effects of increased protein and energy intakes using a Protein and Energy enriched formula (PE-formula) compared with a Standard infant formula (S-formula) [22]. In the present study we studied the efficacy of increased protein and energy intakes to promote protein anabolism and the underlying mechanisms by using a combined intravenous-enteral phenylalanine stable isotope protocol. Primary outcome measure was whole body protein balance at day 5 after admission. SPE of phenylalanine was a secondary outcome measure. 24-Hour nitrogen balance was used as alternative method to assess protein balance. To gain more insight in

the role of separate amino acids in protein kinetics, correlations between plasma amino acid concentrations and protein metabolism were assessed.

DESIGN

Setting and patients

Infants admitted to the PICU of Erasmus Medical Center-Sophia Children's Hospital (Erasmus MC) or Maastricht University Medical Center (MUMC) meeting the inclusion criteria were enrolled: 1) respiratory failure due to viral bronchiolitis; 2) age 4 weeks–12 months; 3) > 40 weeks postmenstrual age; 4) ability to start enteral feeding < 24-hours after admission; 5) expected length of stay > 96-hours; 6) venous and arterial catheter. Exclusion criteria were: 1) gastrointestinal, metabolic or chromosomal disorder; 2) parenteral nutrition other than intravenous dextrose; 3) breast feeding. Inclusion and exclusion criteria were chosen to create a homogenous population of infants. Inclusion criteria 4, 5 and 6 were necessary to enable performance of the study protocol.

The Central Committee on Research Involving Human Subjects (CCMO, The Hague, the Netherlands) and local ethical committees approved this study. Written informed consent was obtained from parents or caregivers.

Anthropometrics and severity of illness (PRISM II) [23] were assessed at inclusion. Duration of mechanical ventilation and length of PICU-stay were registered. To obtain more insight in the metabolic state of the subjects, plasma amino acid concentrations were determined in arterial blood collected in the fed state at start of the stable isotope protocol on day 5 using a fully automated high-performance liquid chromatography as described before [24]. Correlations with whole body protein metabolism were made to identify the role of specific amino acids.

Interventions

Patients were randomised (randomisation and blinding as described before [22]) within 24-hours after admission to receive continuous enteral feeding with Protein and Energy enriched formula (PE-formula; Infatrini®: 2.6 g protein/100ml, 100 kcal/100 ml) or standard formula (S-formula; Nutrilon 1®: 1.4 g protein/100ml, 67 kcal/100ml) both from Nutricia Advanced Medical Nutrition, Zoetermeer, The Netherlands. Compositions are summarized in **Appendix 7.1**. Formulas were administered as described before, starting 25.3±5.6 versus 23.4 ±5.4 hours after PICU-admission in the PE-group and S-group respectively [22]. The ranges in protein and energy intakes on day 5 in the S-group (1.7±0.2 g protein/kg/24h, 84±15 kcal/kg/24h) covered recommended intakes for healthy infants < 6 months (1.14-1.77 g protein/kg/24h, 81-113 kcal/kg/24h, depending on age in months) [16, 25] and were significantly higher in the PE-group (3.1±0.3 g protein/kg/24h, $p < 0.001$; 119±25 kcal/kg/24h, $p < 0.001$),

respectively 175-272% and 105-147% of recommended intakes for protein and energy**.** Intake by volume was not significantly different between groups, 120.6±13.4 ml/kg/24h in the PE-group versus 118.5±13.4,ml/kg/24h in the S-group. With a target volume of 130 ml/kg/day this was the maximum achievable intake for both groups, because of medical reasons (e.g. fluid restriction) as decided by the treating physician. Details on nutritional intake are summarized in **Appendix 7.2.**

Main outcome measures

Whole body protein metabolism and splanchnic phenylalanine extraction

On day 5 whole body protein metabolism (WbPM) and splanchnic phenylalanine extraction (SPE_{Phe}) were assessed by using a stable isotope protocol in the fed state. Several methods can be used to determine protein metabolism. We used the phenylalanine method because of the advantage that only blood samples are needed instead of both blood and breath samples for methods based on leucine isotopes [26]. In order to attain steady state, the infusion rate of enteral nutrition was not changed in the 6 hours prior to the start of the stable isotope protocol and during the stable isotope protocol. The stable isotope protocol was conducted by a research physician or research nurse. Intravenous amino acid tracers were administered 2-hours continuously with calibrated syringe pumps after a priming dose, using the following tracers, priming doses (μmol/kg) and infusion rates (μmol/kg/h) respectively: L-[ring-$^2H_5$] phenylalanine, 4.4 μmol/kg, 4.5 μmol/kg/h; L-[ring-$^2H_2$]tyrosine, 1.9 μmol/kg, 1.5 μmol/kg/h; L-[ring-$^2H_4$]tyrosine, 0.63 μmol/kg. For assessment of SPE_{Phe} L-[1-^{13}C]-phenylalanine was primed-continuously administered via enteral infusion (4.4 μmol/kg, 9.0 μmol/kg/h respectively). Stable isotope tracers (> 98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). Infusates were prepared by the centres' clinical pharmacists. Arterial blood was sampled (500 μl) before isotope infusion to determine background enrichment and at 60, 90, and 120 minutes of infusion to determine isotopic enrichment. Samples were put on ice and centrifuged (3500xg) 10 minutes at 4°C. Plasma was deproteinised with 5% sulfosalicylic acid, frozen in liquid nitrogen and stored at –80°C until analysis. Tracer-to-tracee ratios (TTRs) were analysed using a liquid chromatography-mass spectrometry system as described before [27]. TTRs were corrected for background enrichment and contribution to the measured TTRs of isotopomers with lower masses as described before [28]. Isotopic enrichment reached a steady state after 1-hour infusion, shown by lack of a statistically significant slope of calculated TTRs at 60, 90 and 120 minutes (data not shown). The mean enrichment was used for further calculations as described before [19]. These calculations are explained in detail in **Appendix 7.4**.

Nitrogen balance

24-Hours nitrogen balance on day 5 was assessed as described before, converting urinary urea to total urinary nitrogen (TUN) excretion [22].

Statistical analysis

Power analysis was based on protein metabolism parameters in infants in earlier reports [29]. To detect 20% difference in protein balance between groups with 0.05 two-sided significance and 0.80 sensitivity the number of patients needed per group was 8. Data were analysed intention-to-threat with SPSS statistical software package (version 12.0; SPSS, Chicago, IL). Differences between groups were assessed with Mann-Whitney U analysis. Correlations amongst parameters were tested with Spearman correlation coefficients. Statistical significance was defined as two-tailed $P < 0.05$. Data are presented as mean ± SD.

RESULTS

Patients

20 Infants with respiratory failure due to viral bronchiolitis were included (MUMC: n=10; Erasmus MC: n=10; December 2003-February 2006). 10 Patients were randomised and

Table 7.1 – Patient characteristics of the study population.

	PE group (n=8)			S group (n=10)			P-value
Medical centre (MUMC/Erasmus MC)	4/4			4/6			
Gender (M/F)	2/6			3/7			
Age (months)	2.7	±	1.4	2.9	±	1.8	NS
Weight at inclusion (gram)	3967	±	944	4791	±	1114	NS
Birth weight (gram)	2299	±	903	2841	±	192	NS
Gestational age (weeks)	35.0	±	3.3	37.3	±	1.0	P < 0.05
Postmenstrual age (weeks)	46.8	±	7.6	49.9	±	8.2	NS
Crown-heel length (cm)	56.3	±	5.9	56.6	±	3.6	NS
PRISM score	20.3	±	4.3	18.6	±	4.5	NS
CRP on admission (mg/l)	75	±	65	75	±	51	NS
Mechanical ventilation (days)	7.1	±	6.2	5.0	±	2.2	NS
Length of PICU stay (days)	9.0	±	7.6	6.7	±	2.2	NS

Data are presented as number of subjects or mean ± SD. PE-group, protein and energy enriched formula fed group; S-group, standard infant formula fed group; MUMC, Maastricht University Medical Center; Erasmus MC, Erasmus Medical Center; PRISM, Pediatric Risk of Mortality; CRP, C-reactive protein; PICU, Paediatric intensive care unit.

allocated to receive PE-formula and 10 to receive S-formula. All patients received the allocated formula. Two patients in the PE-group were lost to follow-up, because vascular catheters were removed after extubation before day 5, hence no whole body protein metabolism results could be achieved. Patient characteristics are shown in **Table 7.1.** Gestational age was significantly lower in PE-infants. Other parameters did not differ significantly. There were no significant differences in characteristics between patients enrolled in MUMC and in Erasmus MC (data not shown).

Main outcome measures

Whole body protein metabolism and splanchnic phenylalanine extraction

Rates of phenylalanine kinetics on day 5 are shown in **Table 7.2**. These values are directly derived from the phenylalanine and tyrosine stable isotope tracer results and subsequently used to calculate whole body protein kinetics as represented in **Figure 7.1**. Whole body

Table 7.2 – Whole body and splanchnic phenylalanine kinetics on day 5.

	PE-group (n=8)			S-group (n=10)			P-values
Whole body Phe kinetics							
Wb Ra_{Phe}	124.5	±	50.0	67.9	±	29.9	P < 0.05
Wb Ra_{Tyr}	115.4	±	56.3	57.6	±	9.0	P < 0.05
Wb Phe utilised for PS	112.5	±	50.7	60.4	±	27.2	P < 0.05
Wb $OH_{Phe>Tyr}$	13.5	±	9.0	7.7	±	4.4	NS
Wb Phe from PB	103.9	±	49.8	60.1	±	30.8	P < 0.05
Wb Phe balance	8.5	±	6.5	0.3	±	5.7	P < 0.05
Splanchnic Phe kinetics							
Dietary Phe intake	34.0	±	3.8	16.4	±	2.1	P < 0.01
SPE_{Phe} (%)	39.8	±	18.3	52.4	±	13.6	NS
$ASPE_{Phe}$	13.4	±	6.6	8.7	±	2.8	NS
$PheI_{SPE}$	20.6	±	7.3	7.7	±	2.1	P < 0.01

All data in μmol/kg/hr unless otherwise specified and presented as mean ± SD. PE-group, protein and energy enriched formula fed group; S-group, standard formula fed group; Phe, phenylalanine; Tyr, tyrosine; Wb Ra, whole body rate of appearance; Wb Phe utilised for PS, whole body phenylalanine used for protein synthesis; $WbOH_{Phe>Tyr}$, whole body hydroxylation of phenylalanine to tyrosine; Wb Phe from PB, whole body phenylalanine coming from protein breakdown; Wb Phe balance, whole body phenylalanine balance; SPE_{Phe}, splanchnic phenylalanine extraction; $ASPE_{Phe}$, absolute splanchnic phenylalanine extraction; $PheI_{SPE}$, phenylalanine intake, corrected for splanchnic phenylalanine extraction, thus available for peripheral protein synthesis and oxidation.

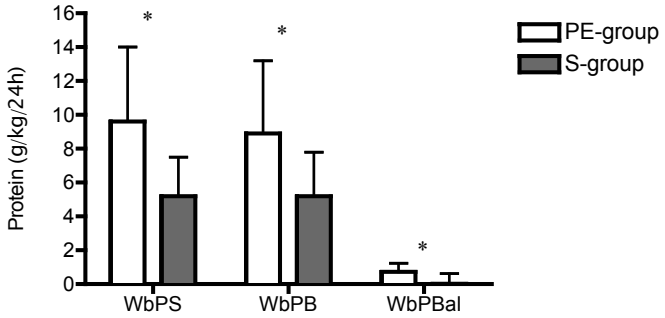


Figure 7.1 – Rates of protein kinetics (g/kg/24h) in both study groups on day 5.

Data presented as mean ± SD. * P < 0.05. PE-group, protein and energy enriched formula fed group; S-group, standard formula fed group; WbPS, whole body protein synthesis; WbPB, whole body protein breakdown; WbPBal, whole body protein balance.
In the PE-group WbPS and WbPB were significantly higher than in the S-group. Consequently, a positive WbPBal was achieved in the PE-group, which was significantly higher than in the S-group.

phenylalanine kinetics were significantly higher in the PE-group than in the S-group, apart from phenylalanine hydroxylation, which was also higher in the PE-group but not significantly. Although splanchnic phenylalanine extraction (%) tended to be higher in the S-group than in the PE-group (p=0.08), absolute splanchnic extraction was highest in the PE-group, however both did not reach significance.

Figure 7.1 depicts rates of whole body protein synthesis (WbPS), whole body protein breakdown (WbPB) and whole body protein balance (WbPBal) in g/kg/24h. It shows that WbPBal on day 5 was positive in the PE-group, whilst in the S-group it did not differ significantly from zero (0.73±0.5 vs. 0.02±0.6 g/kg/24h; p=0.026). The higher WbPBal was achieved by higher WbPS in the PE-group (9.6±4.4 vs. 5.2±2.3 g/kg/24h; p=0.019), despite concomitant higher WbPB (8.9±4.3 vs. 5.2±2.6 g/kg/24h; p=0.046). Negative WbPBal, reflecting catabolism, was found in one subject (13%) in the PE-group, but in four infants in the S-group (40%).

Whole body protein turnover in the PE-group was higher (10.7±4.3 vs. 5.8± 2.6 g/kg/24h; p=0.012) when compared with the S-group. Whole body protein oxidation, calculated from hydroxylation of phenylalanine to tyrosine, was higher with PE-formula than with S-formula, but not significantly (1.2±0.8 vs. 0.7±0.4 g/kg/24h, p=0.25).

Plasma amino acid concentrations on day 5 are shown in **Appendix 7.3**. Concentrations of five essential amino acids (EAA) (methionine, histidine, phenylalanine, lysine, valine) and ornithine were significantly higher in the PE-group. Sums of branched chain amino acids (BCAA) and EAA were also significantly higher. Whole body protein synthesis was positively correlated with concentrations of the EAA histidine (r=0.46, p < 0.05), methionine (r=0.64, p < 0.01), tryptophan (r=0.51, p < 0.05), leucine (r=0.56, p < 0.05), isoleucine (r=0.47, p < 0.05)

and with sums of BCAA ($r=0.51$, $p < 0.05$) and EAA ($r=0.51$, $p < 0.05$). Whole body protein balance was positively correlated with isoleucine ($r=0.52$, $p < 0.05$), valine ($r=0.46$, $p < 0.05$) and the sum of BCAA ($r=0.53$, $p < 0.05$).

Nitrogen balance

24-Hours nitrogen balance on day 5 was significantly higher in PE-infants (274±127 vs. 137±53 mg/kg/24h; $p < 0.05$). Multiplied by 6.25 (average amount of nitrogen in protein) it resulted in protein balances of 1.71 vs. 0.85 g/kg/24h for the PE-group and S-group respectively. Total urinary nitrogen excretion on day 5, as measure of amino acid oxidation, was higher in PE-infants, but not significantly (171±81 vs. 103±54 mg/kg/24h respectively, $p=0.37$).

CONCLUSIONS

The present study is the first to show that protein anabolism, an important target of nutritional support in critically ill infants, can be achieved within the first days after admission to the PICU by increasing enteral protein and energy intakes above dietary reference intakes using a protein-energy enriched formula. This target was not achieved with a standard infant formula. The higher protein balance resulted from stimulation of protein synthesis exceeding the rate of concomitant stimulated protein breakdown. Nitrogen balance data confirmed our phenylalanine results.

Our findings of increased protein synthesis and protein balance are in agreement with several studies in premature and term neonates evaluating effects of amino acid supplementation [10-13, 29-33]. This is also true for protein breakdown that was either increased [33] or not affected by amino acid supplementation [11, 13, 29, 31]. Although Poindexter [30] has also reported suppression of proteolysis, this was in healthy instead of critically ill infants, receiving short-term supplementation. Our finding of both increased protein synthesis and protein breakdown in higher protein and energy intakes is probably due to overall stimulation of protein turnover, shown by the increased whole body protein turnover rate in the PE-group [34].

Increased protein intake promotes protein anabolism, but may lead to increased amino acid oxidation with urea formation as seen in neonates with increasing amino acid supplementation [11, 13, 31], when exceeding needs. In the present study though, neither phenylalanine hydroxylation, nor total urinary nitrogen excretion (both reflecting amino acid oxidation), nor plasma urea concentrations (as described in our previous report) [22] did differ significantly between groups, suggesting that protein intake up to, and probably above, 3.1 g/kg/day does not exceed these infants' needs.

We are aware that using a protein and energy enriched formula makes discern of influences of separate macronutrients on protein metabolism difficult. However studies in adults and children have shown that protein is the major dietary determinant of whole body protein

metabolism as long as energy intake is sufficient [35]. Additionally, supporting this hypothesis, the finding of a positive relationship between plasma essential amino acids and protein synthesis and balance suggests that essential amino acid availability plays a crucial role in increasing protein synthesis and protein balance. It also fits previous observations in healthy adults indicating that (essential) amino acids are the primary stimulus for (muscle) protein synthesis [36].

In these critically ill infants, receiving large amounts of intravenous fluids and medications, 120 ml/kg/day was the maximum achievable nutritional volume intake. Despite these fluid restrictions an anabolic state was obtained within 5 days after admission using a protein-energy enriched formula, thereby limiting the delay in growth and neurodevelopment during critical illness as much as possible. We have previously reported that the PE-formula is safe, well tolerated and improves nitrogen- and energy-balances at day 1 to 5 after admission [22]. This type of formula is thus preferable above standard formulas to achieve adequate nutrition in comparable clinical settings. Since the subjects were a typical sample of infants with respiratory insufficiency due to viral bronchiolitis, we suggest that the results apply to the general population of these critically ill infants.

Our study is also the first to report values of first pass splanchnic phenylalanine extraction in continuously enterally fed critically ill infants. In this population first pass splanchnic phenylalanine extraction did not differ between groups with an average of 46.8%. Comparable values have been described in healthy adults after a meal [21] and in enterally fed piglets [37]. There is discussion about correcting protein intake for SPE in calculations of whole body protein balance, since these retained amino acids are used for constitutive or secreted (glyco-) proteins in the gut,[38, 39] which is then considered part of whole body protein synthesis. We have therefore also calculated the data without correction for SPE (not shown) and found that protein breakdown was 15%-19% lower and protein balance 2.7-3.9 times higher. Only the absolute values are affected by this calculation, the main conclusion of the study is not affected.

There are several limitations of this study. Despite using a randomised design, gestational age was significantly lower in the group receiving protein-energy enriched formula. This might have biased our results of protein metabolism as protein turnover decreases with increased (post-)conceptional age [40]. Furthermore, the proportion of female subjects was relatively high. Protein deposition has been shown to be similar for healthy male and female children prior to adolescence and it is recommended that estimates of protein requirements for healthy children are calculated for both sexes combined [25]. In children with burns (8 years of age in average) though, females had a less negative net muscle protein balance compared to males and females gained lean body mass whereas males lost lean body mass. These differences were possibly due to the observed attenuated hypermetabolic response in females [41]. Assuming that the same differences are true for critically ill infants, this would mean that the achievement of protein anabolism in the first days after admission in our study population could have

been biased by the high proportion of females. However, gender differences in protein kinetics have not been described for critically ill infants. Moreover, our study population of infants with a viral infection is distinctly different from children with burns, who are subject to an extended hypermetabolic stress response with high inflammation [41]. Also, when comparing the female versus the male subjects within the PE- and S-groups of our study, the only notable difference was a non-significant trend towards higher protein turnover, synthesis and breakdown in the females compared to the males within the PE-group, but resulting in similar protein balances in both sexes. Therefore it seems unlikely that our results were affected by gender differences, despite the high proportion of females. Since the female subjects were equally distributed among groups in our study, it neither did influence the comparison of groups. Another limitation is that protein synthesis and protein breakdown were derived by extrapolating phenylalanine metabolism, which in fact only reflects effects on the kinetics of this particular essential amino acid. Other amino acid tracers may have shown different patterns, although the phenylalanine/tyrosine and leucine method are considered to be reference methods to obtain reliable estimates of whole-body protein metabolism in most physiological conditions [26]. The present study was not designed to establish exact protein and energy needs in critically ill infants. Neither was it adequately powered to detect clinical effects. Dose-response studies and studies into clinical effects of improved protein balance in larger groups of critically ill infants are therefore necessary.

In conclusion, protein anabolism in critically ill infants with viral bronchiolitis can be achieved in the first days after admission by increasing protein and energy intakes above reference intakes. Since protein anabolism is an important goal of nutritional support in these infants, increased protein and energy intakes should be preferred above standard intakes.

WHAT IS ALREADY KNOWN ON THIS TOPIC:

- Critical illness in children is associated with increased protein breakdown, negative protein balance and adverse clinical outcome.
- Inadequate nutritional support further leads to protein-energy malnutrition during admission to the paediatric intensive care unit.

WHAT THIS STUDY ADDS:

- Protein anabolism in critically ill infants can be achieved in the first days post-admission by increasing protein and energy intakes above reference intakes.
- The higher protein balance resulted from stimulation of protein synthesis exceeding the rate of concomitant stimulated protein breakdown.
- Increased protein and energy intakes should be preferred above standard intakes in critically ill infants with viral bronchiolitis.

ACKNOWLEDGEMENTS

We would like to thank the participating children and parents. We also thank Marianne Maliepaard for inclusion of patients and data collection. Furthermore our gratitude goes out to nursing and medical staff of the Paediatric Intensive Care Units of Maastricht University Medical Center and Erasmus Medical Center-Sophia Children's Hospital for their support.

REFERENCES

1. Hulst JM, van Goudoever JB, Zimmermann LJ, et al. *The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population.* Clin Nutr. 2004;**23**:1381-9.
2. Sugita H, Kaneki M, Sugita M et al. *Burn injury impairs insulin-stimulated Akt/Pkb activation in skeletal muscle.* Am J Physiol Endocrinol Metab 2005;**288**:E585-91
3. Vanhorebeeke I, Langouche L. *Molecular mechanisms behind clinical benefits of intensive insulin therapy during critical illness: Glucose versus insulin.* Best Pract Res Clin Anaesthesiol. 2009;**23**:449-59.
4. Brealey D, Brand M, Hargreaves I, et al. *Association between mitochondrial dysfunction and severity and outcome of septic shock.* Lancet. 2002;**360**:219-23
5. Hulst J, Joosten K, Zimmermann L, et al. *Malnutrition in critically ill children: from admission to 6 months after discharge.* Clin Nutr. 2004;**23**:223-32.
6. Pollack M. *Nutritional support in the intensive care unit.* In: Suskind R, Suskind L, editors. Textbook of pediatric nutrition. Philadelphia: Saunders, WB 1989:1118-25.
7. Pollack M. *Nutritional support of children in the intensive care unit.* In: Suskind R, Lewinter-Suskind L, editors. Textbook of Pediatric Nutrition. 2nd ed. New York: Raven Press 1993.
8. Pollack MM, Ruttimann UE, Wiley JS. *Nutritional depletions in critically ill children: associations with physiologic instability and increased quantity of care.* JPEN J Parenter Enteral Nutr. 1985;**9**:309-13.
9. Briassoulis G, Zavras N, Hatzis T. *Malnutrition, nutritional indices, and early enteral feeding in critically ill children.* Nutrition. 2001;**17**:548-57.
10. van Lingen RA, van Goudoever JB, Luijendijk IH, et al. *Effects of early amino acid administration during total parenteral nutrition on protein metabolism in pre-term infants.* Clin Sci (Lond). 1992;**82**:199-203
11. Rivera A, Jr., Bell EF, Bier DM. *Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life.* Pediatr Res. 1993;**33**:106-11.
12. te Braake FW, van den Akker CH, Wattimena DJ, et al. *Amino acid administration to premature infants directly after birth.* J Pediatr. 2005;**147**:457-61.
13. Reynolds RM, Bass KD, Thureen PJ. *Achieving positive protein balance in the immediate postoperative period in neonates undergoing abdominal surgery.* J Pediatr. 2008;**152**:63-7.
14. Alexander JW, MacMillan BG, Stinnett JD, et al. *Beneficial effects of aggressive protein feeding in severely burned children.* Ann Surg. 1980;**192**:505-17.
15. Geukers VG, Oudshoorn JH, Taminiau JA, et al. *Short-term protein intake and stimulation of protein synthesis in stunted children with cystic fibrosis.* Am J Clin Nutr. 2005;**81**:605-10.
16. Butte NF. *Energy requirements of infants.* Public Health Nutr 2005;**8**(7A):953-67.
17. Calloway DH, Spector H. *Nitrogen balance as related to caloric and protein intake in active young men.* Am J Clin Nutr. 1954;**2**:405-12.
18. Millward DJ. *Macronutrient intakes as determinants of dietary protein and amino acid adequacy.* J Nutr. 2004;**134**(6Suppl):1588S-96S.
19. Engelen MP, Rutten EP, De Castro CL, et al. *Altered interorgan response to feeding in patients with chronic obstructive pulmonary disease.* Am J Clin Nutr. 2005;**82**: 366 –72.
20. Soeters PB, de Blaauw I, van Acker BA, et al. *In vivo inter-organ protein metabolism of the splanchnic region and muscle during trauma, cancer and enteral nutrition.* Baillieres Clin Endocrinol Metab. 1997;**11**:659-77.
21. Biolo G, Tessari P, Inchiostro S, et al. *Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach.* Am J Physiol. 1992;**262**(4Pt1):E455-63.
22. van Waardenburg DA, de Betue CT, van Goudoever JB, et al. *Critically ill infants benefit from early administration of protein and energy-enriched formula: A randomized controlled trial.* Clin Nutr. 2009;**28**:249–55.
23. Pollack MM, Ruttimann UE, Getson PR. *Pediatric risk of mortality (PRISM) score.* Crit Care Med. 1988;**16**:1110-6.

24. van Eijk HM, Rooyakkers DR, Deutz NE. *Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2-3 microns Spherisorb ODS II column.* J Chromatogr. 1993;**620**:143-8.
25. *Protein and amino acid requirements of infants and children.* In: Protein and amino acid requirements in human nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation. Geneva, Switzerland, 2002 (WHO Technical Report Series, No. 935):161-184.
26. Wagenmakers AJ. *Tracers to investigate protein and amino acid metabolism in human subjects.* Proc Nutr Soc. 1999;**58**:987-1000
27. van Eijk HM, Suylen DP, Dejong CH, et al. *Measurement of amino acid isotope enrichment by liquid chromatography mass spectroscopy after derivatization with 9-fluorenylmethylchloroformate.* J Chromatogr B Analyt Technol Biomed Life Sci. 2007;**856**:48-56.
28. Vogt JA, Chapman TE, Wagner DA, et al. *Determination of the isotope enrichment of one or a mixture of two stable labelled tracers of the same compound using the complete isotopomer distribution of an ion fragment; theory and application to in vivo human tracer studies.* Biol Mass Spectrom. 1993;**22**:600-12.
29. Poindexter BB, Karn CA, Leitch CA, et al. *Amino acids do not suppress proteolysis in premature neonates.* Am J Physiol Endocrinol Metab. 2001;**281**:E472-8.
30. Poindexter BB, Karn CA, Ahlrichs JA, et al. *Amino acids suppress proteolysis independent of insulin throughout the neonatal period.* Am J Physiol. 1997;**272**(4Pt1):E592-9.
31. van den Akker CH, te Braake FW, Wattimena DJ, et al. *Effects of early amino acid administration on leucine and glucose kinetics in premature infants.* Pediatr Res. 2006;**59**:732-5.
32. Denne SC, Rossi EM, Kalhan SC. *Leucine kinetics during feeding in normal newborns.* Pediatr Res. 1991;**30**:23-7.
33. Mitton SG, Garlick PJ. *Changes in protein turnover after the introduction of parenteral nutrition in premature infants: comparison of breast milk and egg protein-based amino acid solutions.* Pediatr Res. 1992;**32**:447-54
34. Wolfe RR, Goodenough RD, Burke JF, et al. *Response of protein and urea kinetics in burn patients to different levels of protein intake.* Ann Surg. 1983;**197**:163-71.
35. Garlick PJ, McNurlan MA, Ballmer PE. I*nfluence of dietary protein intake on whole-body protein turnover in humans. Diabetes Care.* 1991;**14**:1189-98.
36. Volpi E, Mittendorfer B, Rasmussen BB, et al. *The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly.* J Clin Endocrinol Metab. 2000;**85**:4481-90.
37. Van Der Schoor SR, Reeds PJ, Stoll B, et al. *The high metabolic cost of a functional gut.* Gastroenterology. 2002;**123**:1931-40.
38. Van der Schoor SR, Wattimena DL, Huijmans J, et al. *The gut takes nearly all: threonine kinetics in infants.* Am J Clin Nutr. 2007;**86**:1132-8.
39. Van der Schoor SR, Schierbeek H, Bet PM, et al. *Majority of dietary glutamine is utilized in first pass in preterm infants.* Pediatr Res. 2010;67:194-9.
40. Nissim I, Yudkoff M, Pereira G, et al. *Effects of conceptual age and dietary intake on protein metabolism in premature infants.* J Pediatr Gastroenterol Nutr. 1983;**2**:507-16.
41. Jeschke MG, Mlcak RP, Finnerty CC, et al. *Gender differences in pediatric burn patients: does it make a difference?* Ann Surg. 2008;**248**:126-36.

Appendix 7.1 – Macronutrient composition of the infant formulas used in this study.

Average content per 100 ml	PE-formula	S-formula
Energy (kcal)	100	67
Protein (g)	2.6	1.4
En% (protein-energy ratio)	10	8
Casein / whey (g)	1.0 / 1.6	0.6 / 0.8
Amino acids		
L-Alanine (mg)	117	55
L-Arginine (mg)	83	46
L-Aspartic acid / L-Asparagine (mg)	225	120
L-Cystine (mg)	39	22
L-Glutamic acid / L-Glutamine (mg)	556	260
Glycine (mg)	52	27
L-Histidine (mg)	60	35
L-Isoleucine (mg)	159	74
L-Leucine (mg)	278	130
L-Lysine (mg)	257	120
L-Methionine (mg)	68	34
L-Phenylalanine (mg)	112	55
L-Proline (mg)	198	110
L-Serine (mg)	156	69
L-Threonine (mg)	169	73
L-Tryptophan (mg)	39	21
L-Tyrosine (mg)	107	44
L-Valine (mg)	169	82
Carbohydrates (g)	10.3	7.5
En%	41	45
Fat (g)	5.4	3.5
En%	49	47

PE-formula, protein and energy enriched formula; S-formula, standard infant formula; En%, Energy-percentage. Both formulas from Nutricia Advanced Medical Nutrition, Zoetermeer, The Netherlands.

Appendix 7.2 – Rate of nutrient intake (per kg/24h) during the stable isotope infusion protocol on day 5.

	PE-group (n=8)			S-group (n=10)			P-value
Volume (ml)	120.6	±	13.4	118.5	±	13.4	NS
Energy (kcal)	119	±	25	84	±	15	P < 0.001
Energy as % of recommended intakes (81-113 kcal/kg/d)[16]	105-147%			74-104%			
Protein (g)	3.1	±	0.3	1.7	±	0.2	P < 0.001
Protein as % of recommended intakes (1.14-1.77 g/kg/d)[25]	175-272%			96-149%			
Carbohydrates (g)							
Intravenous	1.3	±	1.3	1.7	±	1.7	NS
Enteral	11.4	±	2.8	9.0	±	1.9	P < 0.001
Fat (g)	6.5	±	0.7	4.1	±	0.5	P < 0.001

Data presented as mean ± SD. Intake per kg/24h was calculated from the rate per kg/h during the stable isotope infusion protocol on day 5. PE-group, protein and energy enriched formula fed group; S-group, standard formula fed group.

Appendix 7.3 – Amino acid profile on day 5.

Amino acid (µmol/L)	PE-group (n=8)			S-group (n=10)			P-value	Reference values (µmol/L)
Methionine[1]	36	±	2	28	±	2	< 0.05	30-59
Histidine[1]	72	±	5	58	±	4	< 0.05	59-110
Phenylalanine[1]	77	±	4	64	±	3	< 0.05	43-87
Tryptophane[1]	75	±	5	65	±	6	NS	36-92
Lysine[1]	238	±	27	155	±	14	< 0.05	38-144
Threonine[1]	244	±	44	195	±	20	NS	88-169
Isoleucine[1, 2]	35	±	4	27	±	2	0.091	36-89
Leucine[1,2]	128	±	6	113	±	5	0.075	48-145
Valine[1,2]	142	±	9	113	±	4	< 0.05	108-214
Ornithine	104	±	9	80	±	8	< 0.05	67-150
Glutamine acid	198	±	21	191	±	20	NS	181-379
Asparagine	68	±	8	67	±	6	NS	35-78
Serine	154	±	8	143	±	9	NS	103-257
Glutamine	646	±	61	610	±	30	NS	314-620
Glycine	257	±	20	250	±	13	NS	33-180
Citrulline	22	±	3	22	±	2	NS	20-125
Arginine	101	±	13	89	±	7	NS	40-99
Alanine	332	±	63	294	±	20	NS	227-388
Taurine	28	±	6	27	±	3	NS	
Tyrosine	70	±	5	59	±	5	NS	34-135
Sum of all AA	3026	±	226	2648	±	98	NS	
Essential AA	1047	±	80	817	±	42	< 0.05	
Branched Chain AA	305	±	17	252	±	9	< 0.001	

Data are presented as mean ± SEM. [1] Essential amino acids, [2] Branched chain amino acids. PE-group, protein and energy-enriched formula fed group; S-group, standard formula fed group; AA, amino acids; Sum of all AA, sum of all measured AA.

Data are presented as mean ± SEM.

Reference values derived from Scott PH, et al. Clin Chem. 1990;36:1922–7.

Appendix 7.4 – Calculations of whole body protein metabolism with a combined intravenous-enteral phenylalanine stable isotope protocol.

During steady state, appearance (rate of appearance, Ra) of amino acids into the active metabolic amino acid pool equals disappearance. Appearance in fed subjects comes from protein breakdown (PB) and partially from protein intake (PI). Disappearance (rate of disappearance, Rd) includes amino acids used for protein synthesis (PS) and non-protein synthesis related disposal, e.g. oxidation or hydroxylation. In the case of phenylalanine (Phe) non-protein synthesis related disposal resembles Phe hydroxylation (OH) to tyrosine (Tyr). To be able to calculate protein breakdown during feeding, the amount of amino acids coming from the meal and entering the circulation needs to be known. This is calculated from the fraction of amino acids that is extracted from the meal-derived amino acids in the splanchnic area (splanchnic extraction (SPE)).

Summarized for Phe (µmol/kg/h):

Whole body Ra (WbRa) of Phe = Phe from PB + Phe intake * (1-SPE_{Phe})
= Phe for PS + Phe-OH [a]

WbRa of the traced amino acid is calculated:

1. WbRa = tracer infusion rate / tracer-to-tracee ratio (TTR) in arterial plasma.

SPE and absolute SPE (ASPE) of phenylalanine (%) are calculated [b]:

2. SPE_{Phe} = [1-($WbRa_{[^2H_5]Phe}$ / $WbRa_{[^{13}C]Phe}$)]*100%

where $WbRa_{[^2H_5]Phe}$ and $WbRa_{[^{13}C]Phe}$ represent WbRa of Phe, calculated with intravenous [$^2H_5$]Phe or enteral [^{13}C]Phe.

3. $ASPE_{Phe}$ = Dietary intake of Phe * SPE_{Phe}

Further equations used (µmol/kg/h):

4. Phe hydroxylation to Tyr ($OH_{Phe>Tyr}$) = $WbRa_{[^2H_2]Tyr}$ * ($TTR_{[^2H_4]Tyr}$ / $TTR_{[^2H_5]Phe}$).

5. Phe coming from Whole body Protein Preakdown (Phe from PB) = $WbRa_{[2H5]Phe}$ – (Phe intake * (1-SPE_{Phe}))

6. Phe utilised for Whole body Protein Synthesis (Phe for PS) = $WbRa_{Phe}$ – $OH_{Phe>Tyr}$

7. Whole body Phe balance = Phe for PS – Phe from PB.

Whole body protein kinetics in g/kg/24h (whole body protein breakdown (WbPB), whole body protein synthesis (WbPS) and whole body protein balance (WbPBal)) can be calculated by multiplying Phe kinetics (µmol/kg/h) using the results of calculation 5, 6 and 7 respectively with the average Phe-content in human protein, then multiplying by 24 hours. We used 165.2 g/mol as molecular weight of Phe and 280 µmol/g protein of Phe-content in human protein[c].

References:

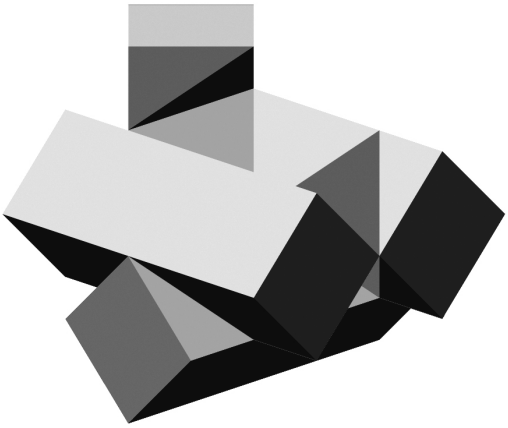
[a] Tessari P, Barazzoni R, Zanetti M, et al. *The role of substrates in the regulation of protein metabolism*. Baillieres Clin Endocrinol Metab. 1996;**10**:511-532.

[b] Matthews DE, Marano MA, Campbell RG. *Splanchnic bed utilization of leucine and phenylalanine in humans. Am J Physiol*. 1993;**264(1 Pt 1)**:E109-118.

[c] Castillo L, Yu YM, Marchini JS, et al. *Phenylalanine and tyrosine kinetics in critically ill children with sepsis*. Pediatr Res. 1994;**35**:580-588.

chapter 8

ACHIEVING NUTRITIONAL GOALS AT DAY 4 AFTER ADMISSION IN CRITICALLY ILL CHILDREN; PREDICTIVE FOR OUTCOME?



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ABSTRACT

Purpose: Adequate nutritional intake is essential during pediatric intensive care admission. We investigated whether achievement of nutritional intake goals at day 4 after admission and route of nutrition were associated with improved outcome.

Methods: Observational study using prospectively acquired data. Patients receiving enteral (EN) and/or parenteral nutrition (PN) were included. Intake goals at day 4 after admission were: energy: resting energy expenditure + 30%; protein: < 1 year 1.8 g/kg/d, ≥ 1 year 1.2 g/kg/d. Clinical outcome measures were length of stay, days on ventilator, number of new infections and duration of antibiotics. Data as median (min-max).

Results: Of 325 subjects (age 0.14 (0.0-18.0) year), 19% were malnourished upon admission. In a subgroup (n=223) the malnourished proportion at discharge (26%) was not significantly different from that upon admission (22%). Fifty-three percent of patients achieved goals; malnourished patients achieved goals more often than non-malnourished (73% vs. 48%, $p < 0.05$). Overall, route of nutrition did not affect goal achievement, but newborns achieved goals more often with PN or EN &PN than EN (70% vs. 69% vs. 23%, $p < 0.05$). Goal achievement, route of nutrition or malnourished status were not associated with changes in clinical outcome.

Conclusions: Malnutrition was highly prevalent upon admission and at discharge. Nutritional goals were achieved in only half of patients; malnourished patients and newborns with PN or EN & PN achieved goals more often. There was no association between achieving goals or route of nutrition and clinical outcome. The use of (supplemental) PN to achieve intake goals should be further investigated.

LIST OF ABBREVIATIONS

BW	Body weight
EI	Energy intake
EN	Enteral nutrition
EN & PN	Combined enteral and parenteral nutrition
PER	Protein-energy ratio
PDMS	Patient data management system
PI	Protein intake
PICU	Pediatric intensive care unit
PN	Parenteral nutrition
REE	Resting energy expenditure
SDS WFA	SD-score for weight-for-age
WHO	World Health Organization

INTRODUCTION

The prevalence of malnutrition at the pediatric intensive care unit (PICU) is up to 24% and has hardly improved over the past decades [1]. Additionally, poor nutritional support results in cumulative energy and protein deficits, which are associated with deteriorated anthropometrics at discharge [2]. Furthermore, malnutrition is associated with increased morbidity and mortality in critically ill children [3]. It follows that adequate nutritional intake is essential in critically ill children. However, energy and protein requirements are not clearly defined and differ among guidelines [4, 5]. Energy requirements are considered to be at or 20-30% above resting energy expenditure (REE) [4]. High protein intakes are associated with improved protein balances in critically ill children [6-9], but exact protein requirements are not known. Importantly, energy intake should be sufficient to prevent utilization of protein as energy source. The World Health Organization (WHO) recommends a protein-energy ratio (PER, energy% (en%)) of 9-11 en% in acutely malnourished children, up to 11-15 en% in chronically malnourished children [10].

Adequate nutritional intake may be difficult to achieve in the PICU though, because of fluid restrictions and interruptions for interventions. Apart from feeding protocols [11-13], protein-energy enriched formulas may help to improve protein and energy balances, as we have previously shown [6, 7]. In our center we use both measures to achieve high energy and protein intakes at day 2 after admission.

In the Netherlands quality of health care is assessed with performance indicators [14]. One of these assesses the proportion of malnourished hospitalized children in general wards and the PICU that achieves intake goals at day 4 after admission. In the current observational study we determined whether patients achieved these intake goals at day 4 after PICU admission and whether achieving goals was associated with improved clinical outcome. Furthermore, we evaluated which route of nutrition was most effective to achieve goals and whether route of nutrition affected clinical outcome.

MATERIAL AND METHODS

Patients and setting

Eligible subjects were patients admitted to the Intensive Care of Erasmus MC - Sophia Children's Hospital, Rotterdam, the Netherlands, in a 20-month period (January 2008 - August 2009) with a minimal length of stay of 4 days and receiving enteral nutrition (EN) and/or parenteral nutrition (PN). Patients with oral food intake other than breastmilk or formula were excluded, because energy and protein intake cannot be accurately calculated. Patients were classified by age as: newborns, 0 - ≤ 28 days; infants, > 28 days - < 1 year; children, ≥ 1 year. Because of the non-invasive character of the study and restriction to prospective dataset analysis, the local medical ethical review board waived the need for medical ethics review and informed consent.

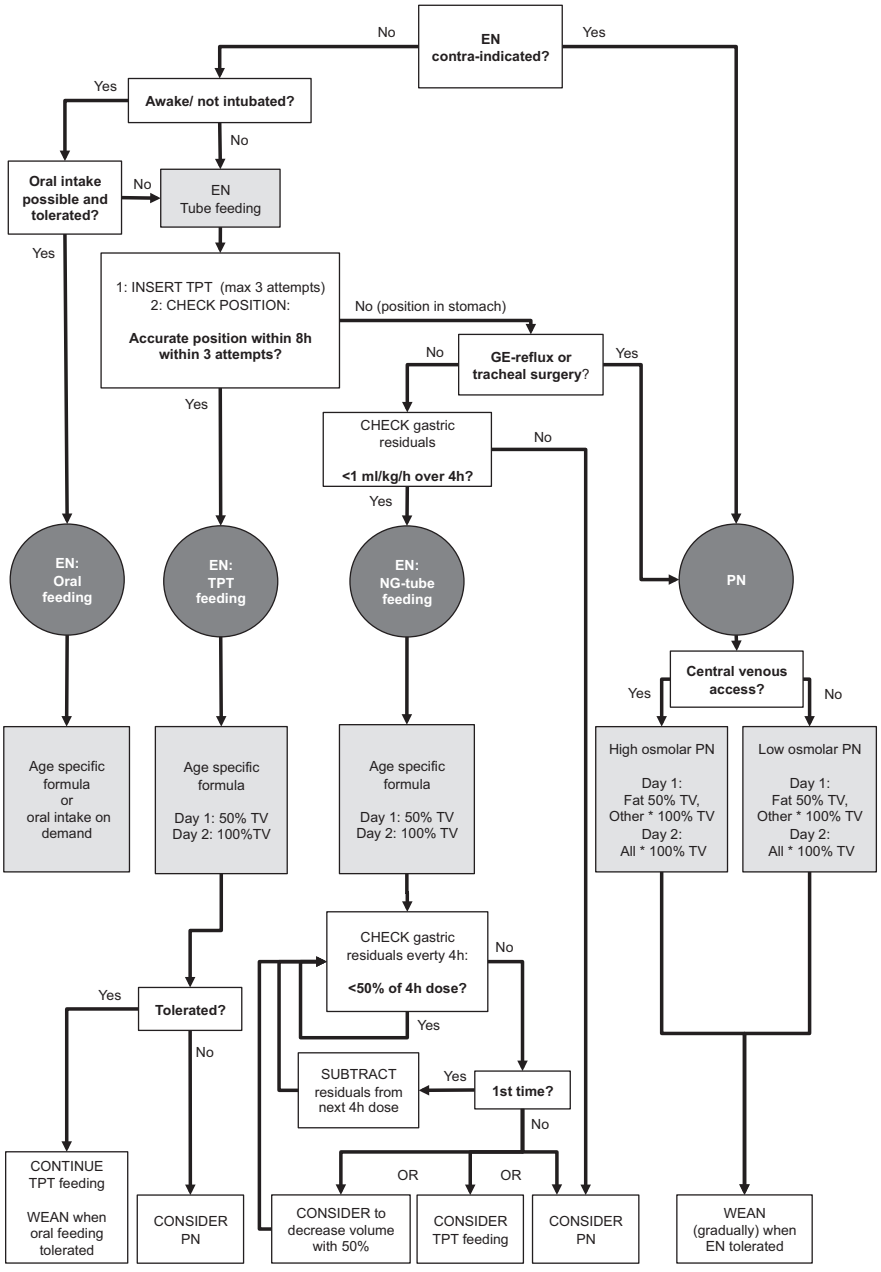


Figure 8.1 – Flow chart to determine the route of nutrition at initiation of nutritional support at the pediatric intensive care unit.

The flow chart represents the procedure to determine the route of nutrition and amount of nutrition at initiation of nutritional support (within 8 hours after admission). * PN components.
EN, enteral nutrition; PN, parenteral nutrition; TPT, trans-pyloric tube; NG-tube, nasogastric tube; TV, target volume.

Nutritional protocol

On admission glucose is provided by maintenance infusion at 4-6 mg/kg/min in children < 30 kg body weight (BW) and 2-4 mg/kg/min ≥ 30 kg BW. Nutrition is introduced within 8 hours after admission; the route of nutrition is determined with an algorithm (**Figure 8.1**). The preferred route is EN via transpyloric tube. Children and ventilated newborns and infants receive age specific enteral formulas (1 kcal/ml; PER of ~10-11 en%); non-ventilated newborns and infants receive fortified breast milk or concentrated standard infant formula. PN is provided when EN is contra-indicated or not tolerated and weaned when EN is tolerated. Our pharmacy provides customized age/weight specific PN compositions, with Intralipid® (Fresenius Kabi, Bad Homburg, Germany) as fat component.

Intake goals

Protein and energy intake goals on day 4 after admission, in line with the above-mentioned performance indicator, were the following (**Table 8.1**). Energy intake: REE + 10% activity factor + 10% illness factor + 10% absorption factor (REE +30%), where REE is calculated with the BW-based Schofield formula [15]. Protein intake: 1.2 g/kg/d for children ≥ 1 year of age. The performance indicator does not set protein goals for children < 1 year of age. For this age group we set the goal at 1.8 g/kg/d, which is the highest recommended protein intake for healthy children in this age group (**Table 8.1**) [16].

Table 8.1 – Nutritional goals.

	Healthy children [a]		**Intake goals [b]**		**Provided by protocol [c]**	
	Protein (g/kg/d)	Energy (kcal/kg/d)	Protein (g/kg/d)	Energy (kcal/kg/d)	Protein (g/kg/d)	Energy (kcal/kg/d)
Newborns [d]	1.8	~95	1.8	~70	≥ 2.0	≥ 90
Infants [e]	1.2	~85	1.8	~70	≥ 2.3 (≥ 1.5 [e])	≥ 90 (≥ 75 [f])
Children [g]						
10-30 kg	0.9	~70	1.2	~55	≥ 2.0	≥ 65
≥ 30 kg	0.9	~55	1.2	~35	≥ 1.7	≥ 45

[a] Dutch Health Council, Dietary reference intakes 2001 [16]; [b] As in performance indicator in Dutch health care [14]. Energy intake defined as resting energy expenditure, as calculated with the Schofield formula + 10% activity + 10% illness factor + 10% absorption factor [15]; [c] Average protein and energy intake according to our nutritional protocol, using preferably 1 kcal/ml age-specific enteral formulas and customized parenteral nutrition (PN) compositions for central venous administration provided by our pharmacy. If using peripheral venous PN ~20% less energy is provided; [d] Newborns: age ≤ 28 days; [e] Infants: age > 28 days – < 1 year; [f] Protein intake when enterally fed and not ventilated; [g] Energy intake when enterally fed and not ventilated; [h] Children: age ≥ 1 year.

Data collection

Data of subjects were retrieved from the electronic Patient Data Management System (PDMS). In PDMS prospectively acquired data are stored on, among others, continuous physiologic parameters, interventions, mechanical ventilation and administration of all medication and nutrition. Additional data, e.g. diagnoses, were collected from medical records.

Nutritional intake

Energy and protein intake at day 4 after admission were compared with intake goals. The proportion of patients achieving intake goals was the primary outcome measure.

Patients were grouped by achieving goals as follows. Goals achieved-group: both energy *and* protein intake goals achieved; Goals not achieved-group; only protein goal or only energy goal or neither achieved. Patients were also grouped by route of nutrition, i.e. EN, PN and EN & PN. Patients who received maintenance infusion besides EN were assigned to the EN-group if ≥ 50% of total energy intake were provided via the enteral route; if < 50% of total energy intake to the EN & PN-group. Also, EN combined with intravenous amino acids and/or intravenous fat was classified as EN & PN.

Anthropometrics

Data on weight upon admission and discharge were collected. SD-scores (SDS) for weight-for-age (WFA) were determined using the software Growth Analyser 3 (Dutch Growth Research Foundation, Rotterdam, the Netherlands), with growth charts for term children > 2 weeks of age [17] and for children < 42 weeks gestational age [17]. SDS WFA of children born < 37 weeks gestational age was corrected for prematurity until 2 years of age. Acute malnutrition was defined as < -2 SDS WFA. Patients were grouped as malnourished or non-malnourished. Difference in SDS WFA between admission and discharge, corrected for length of stay, was a secondary outcome measure.

Clinical outcome

Severity of illness was assessed by Pediatric index of mortality score 2 (PIM2) [18], and Pediatric risk of mortality score III (PRISM III) [19]. For both, higher scores indicate more severe illness. Secondary clinical outcome measures were: number of days until discharged alive, number of days on ventilator after day 4, number of days on antibiotics after day 4, number of new infections after day 4. Mortality was recorded, but not used as outcome measure, because it requires very large sample sizes. New infections were determined as a positive culture; a note of a suspected new infection in PDMS or medical records and/or taking of specimens for culture; start of antibiotics or a switch in antibiotics in combination with a rise in C-reactive protein. New infections were classified as proven by positive culture or suspected. The (suspected) site of infection was classified as bloodstream, airway, wound or other (e.g. abdominal, urinary tract), as deduced from definitions of nosocomial infections by the WHO [20]. We

compared outcome variables between the Goals achieved and the Goals not achieved groups, as well as between the groups classified by route of nutrition and between the malnourished and non-malnourished groups.[20]

Statistical analysis

Data were analyzed using SPSS 17 for Windows, Microsoft (IBM, Armonk, NY, USA). Continuous variables are described as median (minimum-maximum), categorical variables as numbers and proportions. The Mann-Whitney U test served to compare the Goals achieved with the Goals not achieved groups and malnourished with non-malnourished groups. Kruskal-Wallis test was used to compare the route of nutrition groups, with subsequent Mann-Whitney U test with post-hoc Bonferroni correction to compare pairs of groups. Comparison of proportions test served to compare categorical variables between groups. SDS WFA at admission and discharge were compared with Related-samples Wilcoxon Signed Rank test. Correlations were assessed with Spearman's rho. Logistic regression served to determine whether route of nutrition was a determinant for achieving goals or not. Cox-regression analysis and multiple logistic regression in step forward mode and consequently enter mode, to put the factor of interest into the model (Goals achieved vs. Goals not achieved; route of nutrition), were used to identify determinants of these outcomes. In survival analysis, patients who survived were censored at a time point after the last patient died; for discharge alive analysis and days on ventilator, non-survivors were censored at a time point after the last surviving patient was discharged from the PICU.

RESULTS

Patients

Of 2305 admissions during the study period, 325 patients met the inclusion criteria (flow chart in **Appendix 8.1**). Fifty-six percent were male, 19% were acutely malnourished on admission. Baseline characteristics per age group are listed in **Table 8.2.**

Nutritional intake

Figure 8.2 shows protein and energy intake at day 4 after admission**.** Fifty-five percent of patients received EN, 25% EN & PN (21.8 (0.3-93.6) % of total energy intake received via the enteral route) and 20% PN. Fifty-three percent of all patients achieved both protein and energy intake goals at day 4 (Goals achieved-group). These patients had a median younger age, a lower SDS WFA at admission, a higher proportion was malnourished, and a higher proportion underwent surgery (**Table 8.3**). Although overall the proportion of patients achieving goals did not differ by route of nutrition, a significantly smaller proportion of newborns receiving EN achieved goals when compared to the PN or EN & PN groups (23% vs. 69% and 70%, respectively; $p < 0.001$), which was confirmed with multiple logistic regression (EN vs.

Table 8.2 – Baseline characteristics.

	Newborns [a] (n=138)		Infants [b] (n=99)		Children [c] (n=88)		Total (n=325)	
Male – n (%)	74	(54)	62	(63)	48	(55)	184	(57)
Age (at admission, years)	0.00	(0.00-0.08)	0.21	(0.08-0.99)	3.80	(1.06-17.99)	0.14	(0.00-17.99)
SDS WFA	-0.33	(-3.34 – 4.55)	-1.46	(-6.60 – 3.55)	-0.56	(-11.08 – 2.36)	-0.66	(-11.08 – 4.55)
PIM 2 score	-2.74	(-5.96-1.01)	-3.95	(-6.46-0.00)	-3.42	(-6.22-0.07)	-3.40	(-6.46-1.01)
PRISM III score	9	(0-31)	7	(0-31)	8	(0-31)	8	(0-31)
Surgery – n (%) [d]	97	(70)	26	(18)	21	(15)	144	(44)
ECMO – n (%)	26	(19)	7	(7)	4	(5)	37	(11)
Acutely malnourished at admission – no (%) [e]	15	(11)	32	(32)	15	(17)	62	(19)

[a] Newborns: aged ≤ 28 days; [b] Infants: aged > 28 days – 1 year; [c] Children: aged > 1 year;
[d] Including cardiac surgery, thoracic surgery (congenital diaphragmatic hernia), gastro-intestinal surgery, neurosurgery, ear-nose-throat surgery, other (urology, gynaecology); [e] As defined by SD-score weight-for-age < -2SD.
SDS WFA, SD-score weight-for-age.

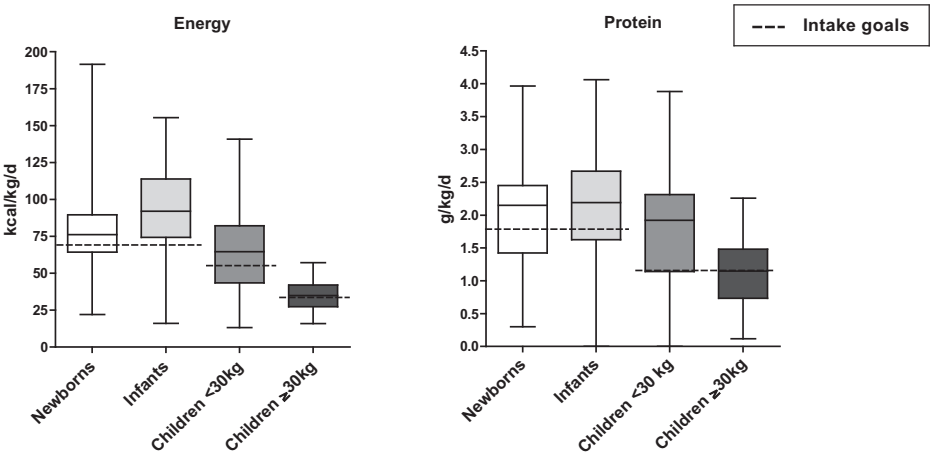


Figure 8.2 – Energy and protein intake at day 4 after admission per age group of critically ill children.

Age specific protein and energy intake goals are shown in dashed lines.

EN & PN p < 0.001; EN vs. PN p=0.002). Newborns in the PN-group were mostly surgical (thoracic and gastro-intestinal surgery) patients, whereas those in the EN-group had mixed diagnoses. For the group as a whole, diagnosis, undergoing surgery and a lower SDS WFA

Table 8.3 – Clinical outcome by groups which achieved or did not achieve nutritional intake goals.

	Goals achieved (n=171)	Goals not achieved (n=154)	P-value
Baseline characteristics			
Male – n (%)	93 (54)	91 (59)	0.393
Age (years)	0.10 (0-18.0)	0.20 (0-17.6)	**0.009**
Malnourished – n (%)	45 (26)	17 (11)	**< 0.001**
Underlying disease – n (%)	134 (78)	112 (73)	0.237
Diagnosis – n (%)			**< 0.001**
Cardiac	16 (9)	31 (20) *	
Respiratory [a]	67 (39) **	40 (26)	
Gastro-intestinal	39 (23) **	13 (9)	
Neurologic	10 (6)	12 (8)	
ENT [b]	7 (4)	18 (12) *	
Severe infection [c]	13 (8)	10 (7)	
Trauma [d]	9 (5)	15 (10)	
Other [e]	10 (6)	15 (10)	
Surgical – n (%)	86 (50)	58 (38)	**0.022**
ECMO – n (%)	21 (12)	16 (10)	0.592
PIM2	-3.29 (-6.46 – 1.01)	-3.52 (-5.96 – 0.62)	0.252
PRISMIII	9 (0-31)	7 (0-27)	0.068
Nutritional intake			
Route of nutrition – n (%)			0.314
EN	88 (52)	92 (60)	
EN & PN	45 (26)	35 (23)	
PN	38 (22)	27 (18)	
Protein-energy ratio (%)	11.2 (4.0-18.3)	8.0 (0.0-31.3)	**< 0.001**
Clinical outcome			
Mortality – n (%)	15 (8.8)	18 (11.7)	0.385
Days until discharged alive	13 (5-349)	10 (5-349)	0.060
Days on ventilator after day 4	2 (0-205)	2 (0-83)	0.338
Infection			
Received antibiotics during admission – n (%)	164 (96)	144 (94)	0.332
Number of days on antibiotics	8 (0-325)	7 (0-85)	**0.031**
New infection during admission – n (%)	80 (47)	68 (44)	0.600

Table 8.3 – Clinical outcome by groups which achieved or did not achieve nutritional intake goals (continued).

	Goals achieved (n=171)	Goals not achieved (n=154)	P-value
Infection site – n (%)			**0.021**
Bloodstream	42 (53)	28 (40)	
Airway	28 (36)	21 (33)	
Wound	8 (10)	6 (10)	
Other (e.g. abdominal, urinary tract)	2 (2)	13 (17) *	

* Proportion in Goals not-achieved-group significantly higher than in Goals achieved-group with comparison of proportions test. ** Proportion in Goals achieved-group significantly higher than in Goals not-achieved group. If neither * or ** mentioned there were significant differences in proportions between groups.

[a] Including postnatal respiratory problems (persistent pulmonary hypertension of the newborn, meconium aspiration), respiratory tract infection and surgery for congenital diaphragmatic hernia, [b] including upper airway obstruction and ENT surgery, [c] Including sepsis, meningitis, organ failure due to infection other than respiratory tract infection, [d] including drowning and neurotrauma; [e] Including craniofacial surgery, urogenital surgery, oncologic and metabolic diagnoses, admission for diagnostic intervention, admission for multiple congenital anomalies and acute life threatening events.

ECMO, extra corporeal membrane oxygenation; ENT, ear nose throat; EN, enteral nutrition; PIM2, Pediatrix Index of Mortality score [18]; PN, parenteral nutrition; PRISMIII, Pediatric Risk of Mortality score [19].

were determinants for achieving goals. For newborns, undergoing surgery was a determinant; for infants, a lower SDS WFA and female gender; for children no determinants were found.

Table 8.4 shows various clinical parameters by route of nutrition. Patients receiving EN were older, had a different distribution of diagnoses, underwent surgery and ECMO less often and had lower severity of illness scores than patients in the PN and EN & PN groups.

Anthropometrics

Weight at discharge was documented in 223 patients. SDS WFA at discharge was -0.84 (-7.28-4.46); 59 patients (26%) were malnourished. Neither was significantly different from baseline in this subgroup (SDS WFA of -0.76 (-7.7-3.55) (p=0.238); 50 malnourished patients at admission (22%)). Overall, malnourished patients on admission showed an improved SDS WFA at discharge (admission: -3.34 (-11.08 - -2.01); discharge: -3.10 (-7.28 – 0.46); p=0.007); 8 out of 50 malnourished patients (16%) were non-malnourished at discharge. Non-malnourished patients at admission showed a decreased SDS WFA at discharge (admission: -0.36 (-1.98 – 4.55); discharge: -0.53 (-4.58 – 4.46); p=0.009); 17 out of 173 non-malnourished patients (10%) were malnourished at discharge. **Figure 8.3** shows the time course of SDS WFA in subgroups of patients being malnourished or non-malnourished at admission and discharge.

Table 8.4 – Clinical outcome by route of nutrition.

	EN (n=180)	EN & PN (n=80)	PN (n=65)	P-value
Baseline characteristics				
Male – n (%)	102 (57)	46 (58)	36 (55)	0.968
Age groups [a]				**< 0.001**
Newborns	40 (22)	52 (65) #	46 (71) #	
Infants	81 (45) †‡	9 (11)	9 (14)	
Children	59 (33) ‡	19 (24)	10 (15)	
Malnourished – n (%)	39 (22)	12 (15)	11 (17)	0.399
Underlying disease – n (%)	126 (70)	61 (76)	59 (91)	**0.004**
Diagnosis – n (%)				**< 0.001**
Cardiac	34 (19) ‡	12 (15) ‡	1 (2)	
Respiratory [b]	57 (32)	24 (30)	26 (40)	
Gastro-intestinal	4 (2)	21 (27) #	27 (42) #	
Neurologic	20 (11) †	2 (3)	0 (0)	
ENT [c]	23 (13) †	2 (3)	0 (0)	
Severe infection [d]	14 (8)	5 (6)	4 (6)	
Trauma [e]	12 (7)	9 (11)	3 (5)	
Other [f]	16 (9)	5 (6)	4 (6)	
Surgical – n (%)	51 (28)	46 (58) #	47 (72) #	**< 0.001**
ECMO – n (%)	10 (6)	14 (18) #	13 (20) #	**0.001**
PIM2	-3.86 (-6.46 – 0)	-2.84 (-5.25 – 1.01) #	-2.60 (-4.94 – 0.62) #	**< 0.001**
PRISMIII	5 (0-31)	13 (0-31) #	11 (1-31) #	**< 0.001**
Nutritional intake				
Goals achieved – n (%)	88 (49)	45 (56)	38 (59)	0.314
Energy intake as % of intake goals (%)	123 (23-246) †‡	105 (31-208)	108 (18-307)	**0.003**
Protein intake as % of intake goals (%)	112 (13-323)	132 (3-220)	131 (0-210)	0.749
Protein-energy ratio (%)	9.3 (3.0-16.0)	11.8 (2.1-31.3) #	12.0 (0-25.3) #	**< 0.001**
Clinical outcome				
Mortality – n (%)	14 (8)	7 (9)	12 (19) #	**0.045**
Days until discharged alive	9 (5-349)	16 (5-349) #	18 (5-349) #	**< 0.001**
Days on ventilator after day 4	2 (0-93)	3 (0-205) #	3 (0-121) #	**0.003**

Table 8.4 – Clinical outcome by route of nutrition (continued).

	EN (n=180)	EN & PN (n=80)	PN (n=65)	P-value
Infection				
Received antibiotics during admission – n (%)	163 (91)	80 (100)	65 (100)	**0.001**
Number of days on antibiotics	7 (0-100)	8 (2-319) #	11 (3-325) #	**< 0.001**
New infection during admission – n (%)	73 (41)	38 (48)	37 (57)	0.076
Proven by positive culture – n (%)	46 (64)	23 (61)	21 (57)	0.766
Infection site – n (%)				**< 0.001**
Bloodstream	25 (34)	21 (55) #	24 (65) #	
Airway	37 (51) †‡	7 (18)	5 (14)	
Wound	4 (6)	4 (11)	6 (16)	
Other (e.g. abdominal or urinary tract)	7 (10)	6 (16)	2 (5)	

Proportion or median in the group at which the sign is indicated is significantly higher than in the EN-group. † Proportion or median in the group at which the sign is indicated is significantly higher than in the EN & PN group. ‡ Proportion or median in the group at which the sign is indicated is significantly higher than in the PN group. If no sign is indicated, there were no significant differences in the proportions or medians between groups.

[a] Newborns: aged ≤ 28 days, infants: aged > 28 days – 1 year; children: aged > 1 year.

[b] Including postnatal respiratory problems (persistent pulmonary hypertension of the newborn, meconium aspiration), respiratory tract infection and surgery for congenital diaphragmatic hernia, [c] including upper airway obstruction and ENT surgery, [d] Including sepsis, meningitis, organ failure due to infection other than respiratory tract infection, [e] including near-drowning and neurotrauma; [f] Including craniofacial surgery, urogenital surgery, oncologic and metabolic diagnoses, admission for diagnostic intervention, admission for multiple congenital anomalies and acute life threatening events. ECMO, extra corporeal membrane oxygenation; ENT, ear nose throat; EN, enteral nutrition; PIM2, Pediatrix Index of Mortality score [18]; PICU-LOS, length-of-stay on pediatric intensive care unit; PN, parenteral nutrition; PRISMIII, Pediatric Risk of Mortality score [19].

Overall, there was no association between the change in SDS WFA between admission and discharge corrected for length of stay, and protein or energy intake at day 4, expressed as percentage of intake goals (r=0.048, p=0.223 and r=-0.038, p=0.570 respectively).

Clinical outcome

Table 8.3 lists clinical outcomes for the Goals achieved-group and the Goals not achieved-group. Number of days on antibiotics and site of infection were significantly different, but multivariate analysis revealed that goal achievement did not significantly affect any of the outcome variables.

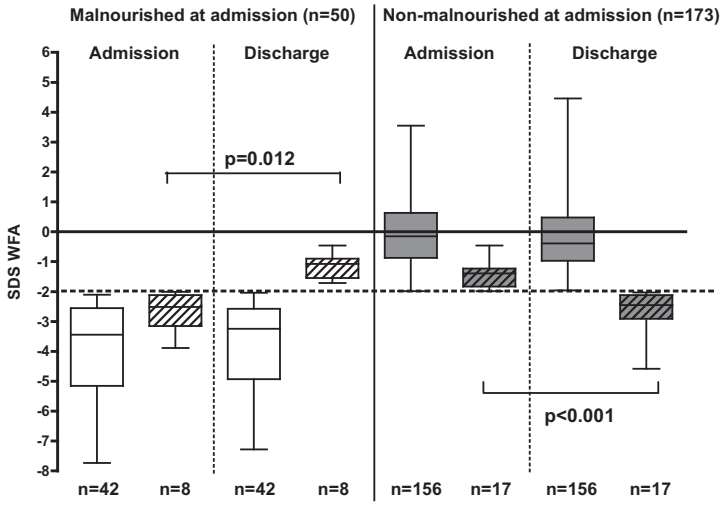


Figure 8.3 – Time course of SD-scores of weight-for-age for malnourished and non-malnourished patients in a subgroup of 223 patients.

In 223 patients weight at discharge was available; 50 were malnourished at admission, 173 were non-malnourished at admission. Eight out of the 50 malnourished patients were non-malnourished at discharge (white-hatched boxplots) and 17 out of 173 non-malnourished patients were malnourished at discharge (gray-hatched boxplots). SDS WFA of these patients and the remaining patients within the malnourished and non-malnourished group are shown separately to indicate the time course in SDS WFA. The horizontal dashed line indicates the cut-off level for acute malnutrition (SDS WFA < -2). In the subgroups that switched to the opposite group at discharge, SDS WFA were significantly different at discharge. In the subgroups that did not switch to the opposite group, the SDS WFA was not significantly different at discharge.
SDS WFA, SD-scores of weight-for-age.

Regarding route of nutrition, univariate analysis (**Table 8.4**) showed that in the EN group the number of days until discharged alive was lower, number of ventilation days was lower, the proportion of patients receiving antibiotics was smaller and duration of antibiotics was shorter than in the other groups. Also, the site of new infections was significantly different between routes of nutrition. Nonetheless, multivariate analysis revealed that route of nutrition did not significantly affect the outcome variables.

Patients in the malnourished group as compared to the non-malnourished group had more often an underlying disease (87% vs. 73% respectively, p=0.02), were less often newborn and more often infant (24% vs. 47% newborns; 52% vs. 26% infants, respectively; $p < 0.001$) and achieved goals more often (**Table 8.3**). With univariate and multivariate analysis, no differences in clinical outcome were found between the malnourished and the non-malnourished group. Also, no differences in clinical outcome were found between patients achieving and not achieving goals within the malnourished group.

DISCUSSION

No more than 53% of our patients achieved both energy and protein intake goals at day 4 after PICU admission. On admission 19% of patients were acutely malnourished, and these achieved goals more often than non-malnourished patients. Route of nutrition did not affect goal achievement for the group as a whole, but PN proved more effective in newborns, which were mostly thoracic and gastro-intestinal surgery patients. After correcting for covariates neither achievement of intake goals nor route of nutrition was associated with changes in number of days until discharged alive, number of days on the ventilator, number of days on antibiotics, number of new infections or site of infection. Within the malnourished group no differences in clinical outcome were found between patients that achieved and those that did not achieve goals.

An important finding in this study is the low proportion of patients achieving intake goals, despite our aggressive nutritional protocol. Other groups, too, concluded that nutritional goals are often not achieved in critically ill children. Reports include median energy intake ranging from 12-70% of goals [21-23]; achievement of energy goals at 26-50% of patient days [13, 24]; and 6-75% of all patients achieving energy goals [11, 21]. Achievement of nutritional goals in critically ill children thus remains difficult. In the present study, not achieving goals resulted from various reasons (not specified above), e.g. fluid restriction and fasting before an intervention, in line with previous reports [22, 25].

Although EN is preferred worldwide, we found that PN was more effective to achieve intake goals in newborns. Since PN is believed to carry more risk of complications and does not maintain gut integrity, we should hesitate to advocate it over EN. The more so, because in recent ASPEN-guidelines for critically ill children EN is the preferred route in patients with a functioning gastro-intestinal tract, if tolerated [5]. The ESPGHAN/ESPEN guidelines even recommend that every effort should be made to avoid PN [4].

Overall, 19% of the patients were malnourished upon admission; in the infant group the prevalence was 32%. These high percentages underline the vulnerable nutritional status of the critically ill child. Remarkably, in the malnourished group goals were achieved more often, although goal achievement did not affect outcome. In 223 patients weight was recorded at discharge; prevalence of malnutrition at discharge in this subgroup increased to 26% (not significantly different from admission). Interestingly, malnourished patients on admission showed an improved SDS WFA at discharge whereas in non-malnourished patients SDS WFA was decreased at discharge. A previous study of our group showed that cumulative protein deficits were associated with a decrease in SDS WFA and skin fold measurements between admission and discharge [2]. In the current study we were not able to demonstrate a similar association, possibly because we only investigated nutritional intake at day 4 as point measurement.

We did not find any effects of achieving intake goals on clinical outcome parameters in our population. An important question is whether these goals were appropriate. We set intake goals as defined in the performance indicator on malnourished, mostly acutely ill, hospitalized children, which are not specific for critically ill children. However, as mentioned in the ASPEN guidelines,

a strong relationship exists between the severity of illness and nutritional status [26]. Therefore severely ill patients are often identified as being malnourished by nutritional assessment tools [26]. Protein requirements for critically ill children are not well defined. A few studies have reported beneficial effects on protein balance at an increased intake up to 3 g/kg/day [6-9, 27, 28]. To date, only two studies in critically ill adults showed that 1.2 g/kg/day should be the target [29-31]. In adults a low energy intake was independently associated with increased ICU-mortality in a retrospective study [32]. Both energy and protein intake are important though, as demonstrated in a prospective adult-ICU study; mortality in those achieving both protein and energy goals was half that of patients achieving only energy goals [33]. Because PICU-mortality is substantial lower than adult-ICU mortality, we did not use mortality as an outcome measure. Our sample size might have been underpowered for other clinical outcome variables, too. Also, the total population and subgroups were very heterogeneous, which is inherent to the PICU population and our study design that was not a randomized trial, but an analysis of observed data.

The question remains whether nutritional intake in PICU patients can be improved. Another approach may be supplementing insufficient EN with PN up to energy goals as recommended in adult-ICU patients. Timing of supplemental PN initiation differs between European and American guidelines for adults [34, 35] and is not recommended as standard in critically ill children [5]. At the time of the present study supplemental PN was not standard in our unit. A recent large randomized controlled trial in critically ill adults (EPANIC study) compared early initiation of supplemental PN with late initiation, to achieve energy goals at day 3 or day 8 after admission respectively. Earlier achievement proved more detrimental than late achievement in terms of longer ICU and hospital stay, higher rate of new infections and a higher proportion of patients requiring mechanical ventilation [36]. We cannot compare our findings with those of the EPANIC study, because study protocols and nutritional policies were different. Early supplemental PN may be less detrimental in critically ill children though. In general children have less energy reserves thus tolerating starvation less well than adults. Additionally, the acute stress response in critically ill children is short and age dependent [37] and EN is tolerated relatively well early after admission. We are currently planning a large randomized controlled trial to study this issue in critically ill children.

In conclusion, this observational study showed that only 53% of critically ill pediatric patients achieved both energy and protein intake goals with an aggressive nutritional protocol. Neither achieving these goals nor route of nutrition, nor malnutrition at admission was associated with changes in clinical outcome. Supplemental PN might be an effective tool to achieve intake goals in critically ill children more often and to improve outcome.

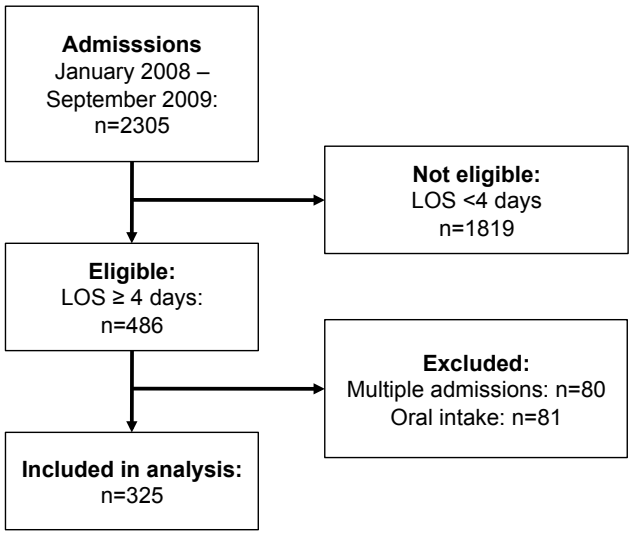
ACKNOWLEDGEMENTS

We would like to thank Neelke Oosterloo for helping to collect data and Ko Hagoort for careful editing.

REFERENCES

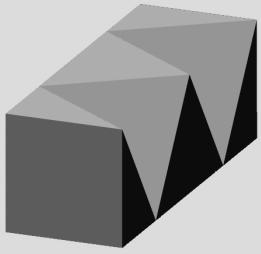
1. Hulst J, Joosten K, Zimmermann L, Hop W, et al. *Malnutrition in critically ill children: from admission to 6 months after discharge.* Clin Nutr. 2004;**23**(2):223-32.
2. Hulst JM, van Goudoever JB, Zimmermann LJ, Hop WC, et al. *The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population.* Clin Nutr. 2004;**23**(6):1381-9.
3. Pollack MM, Ruttimann UE, Wiley JS. *Nutritional depletions in critically ill children: associations with physiologic instability and increased quantity of care.* JPEN J Parenter Enteral Nutr. 1985;**9**(3):309-13.
4. Koletzko B, Goulet O, Hunt J, Krohn K, et al. *1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR).* J Pediatr Gastroenterol Nutr. 2005;**41 Suppl 2**:S1-87.
5. Mehta NM, Compher C. *A.S.P.E.N. Clinical Guidelines: nutrition support of the critically ill child.* JPEN J Parenter Enteral Nutr. 2009;**33**(3):260-76.
6. van Waardenburg DA, de Betue CT, Goudoever JB, Zimmermann LJ, et al. *Critically ill infants benefit from early administration of protein and energy-enriched formula: a randomized controlled trial.* Clin Nutr. 2009;**28**(3):249-55.
7. de Betue CT, van Waardenburg DA, Deutz NE, van Eijk HM, et al. *Increased protein-energy intake promotes anabolism in critically ill infants with viral bronchiolitis: a double-blind randomised controlled trial.* Arch Dis Child. 2011;**96**(9):817-22.
8. Joosten KF, Verhoeven JJ, Hazelzet JA. *Energy expenditure and substrate utilization in mechanically ventilated children.* Nutrition. 1999;**15**(6):444-8.
9. de Klerk G, Hop WC, de Hoog M, Joosten KF. *Serial measurements of energy expenditure in critically ill children: useful in optimizing nutritional therapy?* Intensive Care Med. 2002;**28**(12):1781-5.
10. *Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation*, 2007, WHO: Geneva, Switzerland.
11. Meyer R, Harrison S, Sargent S, Ramnarayan P, et al. *The impact of enteral feeding protocols on nutritional support in critically ill children.* J Hum Nutr Diet. 2009;**22**(5):428-36.
12. Petrillo-Albarano T, Pettignano R, Asfaw M, Easley K. *Use of a feeding protocol to improve nutritional support through early, aggressive, enteral nutrition in the pediatric intensive care unit.* Pediatr Crit Care Med. 2006;**7**(4):340-4.
13. de Neef M, Geukers VG, Dral A, Lindeboom R, et al. *Nutritional goals, prescription and delivery in a pediatric intensive care unit.* Clin Nutr. 2008;**27**(1):65-71.
14. *Basic set quality indicators hospitals 2011 (Basisset kwaliteitsindicatoren ziekenhuizen 2011)*, 2011, Ministry of Health Welfare and Sport; Health Care Inspectorate (IGZ): The Hague, the Netherlands. p. 42-45.
15. Schofield WN. *Predicting basal metabolic rate, new standards and review of previous work.* Hum Nutr Clin Nutr. 1985;**39 Suppl 1**:5-41.
16. *Dietary Reference Intakes: energy, protein and digestible carbohydrates*, 2001, Health Council of the Netherlands: The Hague.
17. Usher R, McLean F. *Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation.* J Pediatr. 1969;**74**(6):901-10.
18. Slater A, Shann F, Pearson G. Paediatric Index of Mortality Study Group. *PIM2: a revised version of the Paediatric Index of Mortality.* Intensive Care Med. 2003;**29**(2):278-85.
19. Pollack MM, Patel KM, Ruttimann UE. *PRISM III: an updated Pediatric Risk of Mortality score.* Crit Care Med. 1996;**24**(5):743-52.
20. *Prevention of hospital-acquired infections: A practical guide.*, 2002, World Health Organization: Geneva, Switzerland.

21. Tume L, Latten L, Darbyshire A. *An evaluation of enteral feeding practices in critically ill children.* Nurs Crit Care. 2010;**15**(6):291-9.
22. Taylor RM, Preedy VR, Baker AJ, Grimble G. *Nutritional support in critically ill children.* Clin Nutr. 2003;**22**(4):365-9.
23. Rogers EJ, Gilbertson HR, Heine RG, Henning R. *Barriers to adequate nutrition in critically ill children.* Nutrition. 2003;**19**(10):865-8.
24. Malakouti A, Sookplung P, Siriussawakul A, Philip S, et al. *Nutrition support and deficiencies in children with severe traumatic brain injury.* Pediatr Crit Care Med. 2012;**13**(1):e18-24.
25. Hulst JM, Joosten KF, Tibboel D, van Goudoever JB. *Causes and consequences of inadequate substrate supply to pediatric ICU patients.* Curr Opin Clin Nutr Metab Care. 2006;**9**(3):297-303.
26. *Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients.* JPEN J Parenter Enteral Nutr. 2002;**26**(1 Suppl):1SA-138SA.
27. Coss-Bu JA, Klish WJ, Walding D, Stein F, et al. *Energy metabolism, nitrogen balance, and substrate utilization in critically ill children.* Am J Clin Nutr. 2001;**74**(5):664-9.
28. Verbruggen SC, Coss-Bu J, Wu M, Schierbeek H, et al. *Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin-resistant adolescents with tight glucose control.* Crit Care Med. 2011;**39**(11):2518-25.
29. Strack van Schijndel RJ, Weijs PJ, Koopmans RH, Sauerwein HP, et al. *Optimal nutrition during the period of mechanical ventilation decreases mortality in critically ill, long-term acute female patients: a prospective observational cohort study.* Crit Care. 2009;**13**(4):R132.
30. Sevette A, Smith RC, Aslani A, Kee AJ, et al. *Does growth hormone allow more efficient nitrogen sparing in postoperative patients requiring parenteral nutrition? A double-blind, placebo-controlled randomised trial.* Clin Nutr. 2005;**24**(6):943-55.
31. Ishibashi N, Plank LD, Sando K, Hill GL. *Optimal protein requirements during the first 2 weeks after the onset of critical illness.* Crit Care Med. 1998;**26**(9):1529-35.
32. Tsai JR, Chang WT, Sheu CC, Wu YJ, et al. *Inadequate energy delivery during early critical illness correlates with increased risk of mortality in patients who survive at least seven days: a retrospective study.* Clin Nutr. 2011;**30**(2):209-14.
33. Weijs PJ, Stapel SN, de Groot SD, Driessen RH, et al. *Optimal protein and energy nutrition decreases mortality in mechanically ventilated, critically ill patients: a prospective observational cohort study.* JPEN J Parenter Enteral Nutr. 2012;**36**(1):60-8.
34. Singer P, Berger MM, Van den Berghe G, Biolo G, et al. *ESPEN Guidelines on Parenteral Nutrition: intensive care.* Clin Nutr. 2009;**28**(4):387-400.
35. McClave SA, Martindale RG, Vanek VW, McCarthy M, et al. *Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.).* JPEN J Parenter Enteral Nutr. 2009;**33**(3):277-316.
36. Casaer MP, Mesotten D, Hermans G, Wouters PJ, et al. *Early versus late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(6):506-17.
37. Bouwmeester NJ, Anand KJ, van Dijk M, Hop WC, et al. *Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized trial comparing the effects of continuous versus intermittent morphine.* Br J Anaesth. 2001;**87**(3):390-9.



Appendix 8.1 – Flow chart of inclusion of subjects.

The flow chart shows the number of patients admitted during the study period and reasons for exclusion from analysis.



PART 5

DISCUSSION AND SUMMARY

After climbing a great hill, one only finds
that there are many more hills to climb.

Nelson Mandela

chapter 9

GENERAL DISCUSSION

LIST OF ABBREVIATIONS

α-KIC	α-Ketoisocaproate
BIS	Bioelectrical impedance spectroscopy
DEXA	Dual energy x-ray absorptiometry
ECMO	Extra-corporeal membrane oxygenation
IVNAA	In vivo neutron activation analysis
LOS	Length-of-stay
mTOR	Mammalian target of rapamycin
N-balance	Nitrogen balance
NO	Nitric oxide
PICU	Pediatric intensive care unit
SDS WFA	SD-score for weight-for-age
SDS WFH	SD-score for weight-for-height
VCO_2	Carbon dioxide production
WHO	World Health Organization

FINDINGS IN THIS THESIS

This thesis focused on protein anabolism in critically ill children, using stable isotope tracer methodology as main method. In **Part 2** pathophysiological aspects of protein metabolism during critical illness in children were studied. **Part 3 and 4** focused on challenges of nutritional support related to protein anabolism in specific phases during critical illness.

The first pathophysiological aspect studied in **part 2** was whether whole body protein metabolism in continuously fed critically ill children shows a circadian rhythm. This is important, as in stable isotope tracer methodology short duration measurements are extrapolated to 24-hours. Hence a circadian rhythm would have implications for the duration of stable isotope tracer protocols. We did not find a circadian rhythm and addressed other important methodological issues. The second pathophysiological aspect of interest was the effect of inflammation and nutrition on arginine metabolism. We found indications that arginine becomes an essential amino acid proportional to the severity of inflammation in critically ill children. Arginine availability in infants was increased *via* intake of a protein-energy enriched formula.

Part 3 focused on the initial post-operative phase, when nutritional support generally consists of intravenous glucose intake. The challenge in this phase is to maintain glucose homeostasis while not aggravating protein catabolism. Indeed, we showed that reducing glucose intake from 5.0 mg/kg/min to 2.5mg/kg/min in infants and young children in the initial phase after elective craniofacial and cardiac surgery, reduced hyperglycemia without increasing protein catabolism.

The next step of nutritional support is to introduce (par)enteral nutrition. The challenge in this phase is to achieve protein anabolism as soon as possible, with the ultimate aim to improve clinical outcome. As such, in **part 4** it appeared that a protein-energy enriched formula was safe and well tolerated and resulted earlier in protein anabolism than a standard infant formula. In addition, we studied the effect of achieving nutritional intake goals with our aggressive nutritional protocol on clinical outcome in 325 critically ill children (0-18 years of age). Half of the patients did not achieve intake goals at day 4 after admission, but achievement of goals did not affect clinical outcome. Neither did the route of nutrition or a malnourished status of individual patients. Possibly, other strategies to achieve nutritional intake goals are needed, such as supplemental parenteral nutrition. The safety and efficacy of this approach in critically ill children needs to be assessed in a large randomized controlled trial.

Contents of the discussion

The above-described aspects were studied using different methods. We have incorporated the used methods in a model of outcome measures to study the effect of protein intake. This model will be introduced in the following section. Also, advantages and disadvantages of these methods will be discussed. Then, the contribution of the data that was generated in this thesis on protein intake and protein balance will be evaluated. We will make recommendations for

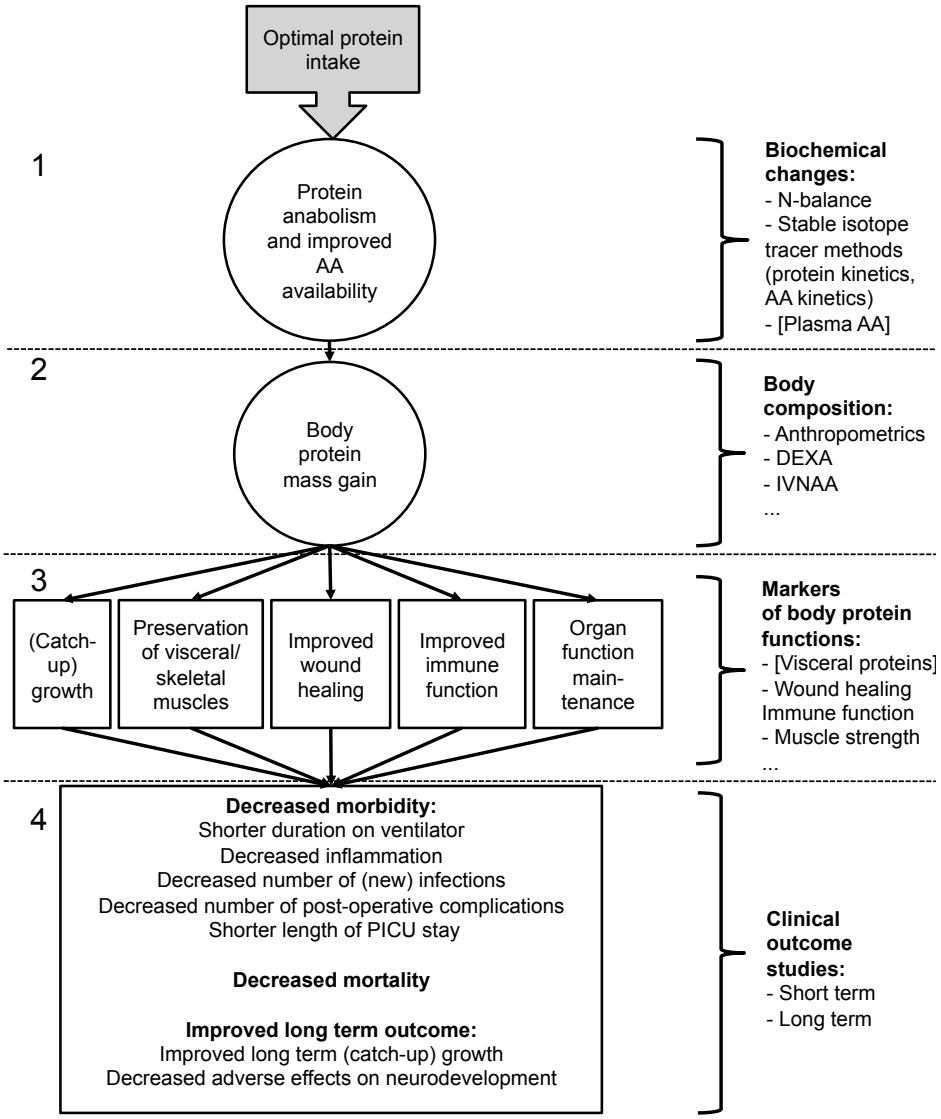


Figure 9.1 – Four levels of outcome measures to assess protein anabolism in critically ill children.

On the left side of the figure, the effect of optimal protein intake on protein anabolism, body composition, body protein functions and subsequently clinical outcome in critically ill children is shown. On the right side suggested methods to assess these effects on the different levels are shown.

AA, amino acid; DEXA, dual energy x-ray absorptiometry; IVNAA, in vivo neutron activation analysis; N-balance, nitrogen balance; [Plasma AA], plasma amino acid concentration.

protein and energy intake in critically ill children. Next, challenges and strategies to improve nutritional intake in the PICU will be addressed. Finally, we make suggestions for subjects of future research.

METHODOLOGICAL CONSIDERATIONS

We propose a model to separate outcome measures, as used to study the effect of protein intake in critically ill children, into four levels as shown in **Figure 9.1.** The first level serves to determine whether a protein anabolic state is achieved at the biochemical level, i.e. if whole body protein synthesis exceeds whole body protein breakdown, resulting in a positive whole body protein balance. If at a whole body level protein anabolism is indeed possible, the amount of structural and functional proteins increases, thus body protein mass is gained (level 2). As a consequence body protein functions may improve (e.g. immune function, wound healing, muscle strength) and linear growth is enabled (level 3). These alterations may eventually lead to improved clinical outcome both on short and on long term (level 4).

In the next section the methods used in this thesis are summarized according to this model.

Level 1: Biochemical changes indicating protein anabolism

Biochemical indices that may indicate whether a protein anabolic state is achieved include nitrogen balance (N-balance) and stable isotope tracer methodology (**Figure 9.2**)[1]. Supporting evidence can be retrieved from plasma amino acid concentrations. These three methods were the main methods used in this thesis and will be discussed below in more detail. Other supporting biochemical markers include plasma concentrations of glucose, fatty acids, triglycerides as well as hormone levels such as insulin and cortisol [2]. These provide a more complete view of metabolism and have been used throughout this thesis in conjunction with the other methods. Plasma urea concentrations are often used as marker of protein tolerance

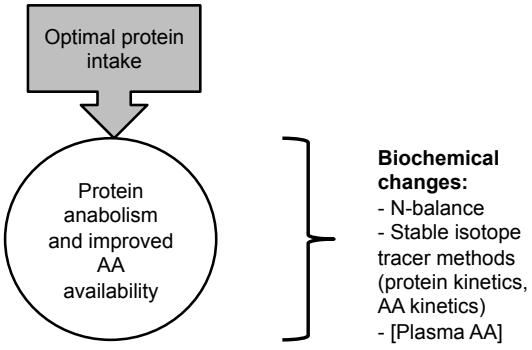


Figure 9.2 – Biochemical changes as outcome measure to study the effect of protein intake.
AA, amino acid; N-balance, nitrogen balance; [Plasma AA], plasma amino acid concentration.

[3-5], because if too much protein is administered, amino acids in excess will be oxidized, and the nitrogen-content will be converted to urea. Accordingly, we used plasma urea concentrations **in chapter 6 and 7** to evaluate whether the protein-energy enriched formula was tolerated well**.**

Plasma amino acid concentrations

Plasma amino acid concentrations have been used in many study populations to identify deficiencies or excesses of individual amino acids under various circumstances and at different protein intakes. E.g. plasma amino acid concentrations were used to evaluate adequacy of parenteral nutrition in premature neonates, infants and children [6-8]. Also, the effects of the acute stress response on plasma amino acids in critically ill adults [9, 10] and after cardiac surgery in children [11, 12] have been studied. The advantage of this method is that information can be achieved on specific amino acids, as part of protein metabolism.

In **chapter 3** we used plasma amino acid concentrations to investigate arginine metabolism in infants receiving either a protein-energy enriched formula or a standard formula. No statistical differences in plasma arginine concentrations were found between the two groups. Strikingly though, arginine kinetics as assessed with stable isotope tracer methodology, meanwhile revealed that the arginine rate of appearance was significantly higher with the protein-energy enriched formula. This means that per time unit more arginine is available in the systemic circulation and is used for its functions, which is supported by the observation of a higher NO synthesis rate in the protein-energy enriched formula fed group. Taking into account plasma amino acid concentrations only, we could not have extrapolated these findings. This illustrates the main drawback of this method to assess protein metabolism: it is a static measurement of dynamic processes. Decreased plasma concentrations can result from decreased influx into the plasma pool (i.e. decreased release from whole body protein breakdown, decreased (or no) dietary intake and decreased *de novo* synthesis in case of non-essential amino acids), increased efflux (i.e. increased utilization for whole body protein synthesis, increased irreversible oxidation or increased synthesis of other small components, such as neurotransmitters and polyamines) or a combination of both [13]. Also, when plasma amino acid concentrations are maintained at the same level, fluxes can be increased or decreased, as described in **chapter 3**. Merely from plasma amino acid concentrations it is not possible to conclude whether a protein anabolic or catabolic state is achieved.

Another disadvantage is that the site and method of sampling, storage and processing of blood influences measurements. Large differences may exist between arterial and venous amino acid concentrations, as a consequence of fluxes across organs [14]. The arterial amino acid profile is considered most representative to amino acid homeostasis [13], but arterial access is not available in all critically ill children and for obvious ethical reasons no reference values of arterial amino acid concentrations in healthy children exist. The processing and storage method after sampling affects concentrations, too. Deproteinization of blood samples

and storage at -70°C prevents decay of certain amino acids [15] and is therefore important. Differences in results between studies may be difficult to interpret if different methods of sample processing are used and/or are not reported in detail. However, it has been suggested that the measurement of plasma amino acid concentrations can be useful as long as the physiologic situation is taken into account [13].

LEVEL 1 BIOCHEMICAL CHANGES

Overall evaluation of plasma amino acid concentrations

- Advantages: provides information on specific amino acids.
- Disadvantages: underlying dynamic processes not addressed; measurements influenced by site, processing and storage of sampling; no reference values of arterial amino acid concentrations.
- Relevance: may provide valuable information on individual amino acids, if used in combination with nitrogen balance or stable isotope tracer methodology.

Nitrogen balance (N-balance)

In **chapter 6** we used N-balance to determine whether and when during admission a protein anabolic state could be achieved when providing a protein-energy enriched formula and a standard formula. We found that with the protein-energy enriched formula all patients were anabolic from day 2 onwards, whereas with the standard formula some patients remained catabolic until day 4.

N-balance has been widely used to determine protein and amino acid requirements [1]. Since protein is the major nitrogen-containing component in the body, loss of nitrogen is regarded as loss of protein. Nitrogen loss in urine can be measured in a non-invasive way, by determining the amount of total urinary nitrogen or urinary urea nitrogen (in case of the latter with a correction factor of 1.25 to account for non-urea nitrogen). Also, nitrogen is lost in feces, which might be measured as well; and from hair, nails, skin desquamation, in sweat and other body secretions, which are more difficult to assess. Often, these "miscellaneous losses" besides urinary nitrogen loss are not accounted for when total nitrogen loss is determined, or a constant value is used. In addition, practical limitations prevent accurate assessment of losses, e.g. urine should be collected preferably during 24-hour, which requires special collection jars and it is very difficult to collect feces accurately. Loss of nitrogen *via* nasogastric tubes, stoma output and in the intestines during intestinal obstruction may further introduce factors of uncertainty.

Subtracting total nitrogen losses from nitrogen intake results in the N-balance; if positive an anabolic state is assumed, if negative it points at a catabolic state. Nitrogen intake can be calculated if the protein content of the ingested food is known, or more accurately, measured from duplicate portions of food. Attention should be paid to food spillage, because if not accounted for, nitrogen intake is overestimated. Overall, nitrogen losses are thought to be underestimated rather than overestimated [1]. Thus, N-balances tend to be too positive, resulting in underestimation of protein and/or amino acid requirements. Indeed, the protein balance results that we obtained with N-balance technique were higher than that obtained with stable isotope techniques in the same patients on day 5 after admission in **chapter 6 and chapter 7.**

When N-balance is used to assess the effect of different protein intakes, an adaptation period to the new protein intake is needed, to allow the urea pool (of which urea nitrogen is derived) to adapt. It is not known precisely what the duration of the adaptation period should be. In healthy males it took 9-12 days to adapt to a switch of 75 g protein/d (~1 g/kg/d) to 225 g protein/d (~3 g/kg/d) with a temporally excessive increase in N-balance until a new equilibrium was achieved at a higher level than with the low intake and *vice versa* when the switch was made from the high protein to the low protein intake [16].

Although N-balance is a non-invasive and relatively simple measurement (despite practical limitations), which allows serial measurement during intensive care admission, it does not give information on the rate of protein synthesis and protein breakdown. A change from a negative to a positive N-balance can result from decreased protein breakdown with unchanged protein synthesis, unchanged protein breakdown with increased protein synthesis or both increased protein breakdown and increased protein synthesis, with the rate of synthesis exceeding the rate of breakdown. In **chapter 7** we performed a stable isotope tracer protocol at day 5 after admission in the same patients as in **chapter 6**. We were able to show that both protein synthesis and breakdown were increased with increased protein-energy intake, which resulted in positive protein balance, which was in agreement with the positive N-balance.

LEVEL 1: BIOCHEMICAL CHANGES

Overall evaluation of nitrogen balance

- Advantages: non-invasive method; serial measurements possible.
- Disadvantages: practical difficulties; overestimation of protein balance; no information on protein breakdown and synthesis rates; long adaptation period.
- Relevance: may be used to compare the effect of different protein intakes between groups or serial measurements in same group of patients.

Stable isotope tracer methodology

Duration of stable isotope tracer protocols

Whereas initially N-balance studies have been used to determine protein and amino acid requirements, stable isotope tracer studies are now considered the reference method [1]. 24-Hour studies are most representative to determine protein requirements, because of resemblance to a normal day [1]. In healthy subjects, study designs comprise both a fed and a fasted state. However, the invasiveness for the subject, the complexity of the study, the burden to the research team and the financial costs of stable isotopes do not allow these studies to be used in large cohorts of patients.

Shorter duration protocols are more feasible, but results obtained during periods of several hours may differ from results obtained during a 24-hour period [17]. In chapter 2 we performed a 24-hour study in critically ill children. This is the first 24-hour study in children, let alone in critically ill children. We found considerable variability in measurements over 24-hour, which could have been caused by several factors. The critically ill children in our study population underwent many medical interventions during the day, e.g. administration of drugs, changes in mechanical ventilation settings and physiotherapy. In addition, as we measured in the fed state, enrichments may have been affected by irregular gastric emptying. Crenn et al studied the differences in plasma enrichments of either intragastric or intraduodenal infusion of stable isotopes and found that enrichments of the intragastric tracer were too variable to be considered reliable as compared with the intraduodenal tracer [18]. However, most patients in our study population received enteral nutrition via transpyloric tube or nasojejunal tube, minimizing the effect of gastric emptying as a confounder.

Apart from the inter-individual variability we observed an upward trend in enrichment, indicating tracer recycling. Since amino acids with tracers are incorporated into body protein, the same proteins may be degraded within 24-hours and the amino acids containing tracers are released into the plasma amino acid pool again. Hence, enrichment increases over time. This is especially possible in critically ill patients, due to high protein turnover and synthesis of proteins with a short half-life (acute phase proteins). In fact, tracer recycling is one of the concerns of stable isotope tracer methodology [19]. In 24-hour stable isotope tracer studies in healthy subjects it has led to designs in which the fasted state was studied at the start of the 24-hour period [17, 19-21]. This ensured that release of amino acids from protein breakdown (during fasting) preceded incorporation of amino acids with tracers during protein synthesis (in the fed state) as much as possible.

In **chapter 3, 4, 5 and 7** we used short duration protocols to assess protein kinetics and glucose kinetics in **chapter 4 and 5**. The study design used in **chapter 3 and 7** was relatively short, namely 2 hours. We obtained relatively steady enrichments, but it cannot be ruled out that a relatively stable measurement around an outlier was done. Outliers were observed in the 24-hour study of **chapter 2** in a substantial proportion of patients and are probably inevitable

in critically ill children for above-mentioned reasons. Probably, it is more sensible to use tracer protocols of 4-6 hours, although in critically ill children the burden to the patient should always be weighed carefully against the benefits of a longer study period. We recommend not to use protocols longer than 10-hours, because of the potentially high amount of tracer recycling.

Selection of the tracer amino acid for protein kinetics

Different tracer methods are available to assess protein metabolism. In **chapter 2, 3, 4, 6 and 7** we used the phenylalanine/tyrosine method. In addition, in **chapter 4 and 5** we used the leucine method, with measurements of leucine oxidation in breath samples. In **chapter 4** we found differences between the results of phenylalanine and leucine kinetics when extrapolated to protein kinetics. This has been described before [22]. Possibly, the conversion rates from amino acid to protein kinetics that we used are not accurate enough. In **chapter 5** we only used the leucine method. Tyrosine is poorly soluble and requires a large volume of fluid to be solved in, which is for instance not feasible in cardiac patients who are fluid restricted in the initial post-operative course. We preferred the phenylalanine/tyrosine method over the leucine method in the other parts of this thesis, since phenylalanine conversion to tyrosine, as measure of irreversible phenylalanine hydroxylation, can be measured within the same blood sample as the phenylalanine enrichment [22]. In contrast, the leucine method requires the use of indirect calorimetry (or other methods to assess VCO_2), breath sampling or extraction of CO_2 from blood samples, in addition to the blood samples that are required for enrichment of α-KIC, which is the intracellular equivalent of leucine.

In the fed state correction of protein intake for splanchnic extraction (retention of amino acids in the splanchnic area during first pass) is required in order to obtain an accurate reflection of the amount of amino acid that is absorbed in the intestine and that enters the blood pool [14]. This is necessary for accurate calculations; otherwise the contribution of amino acids from protein intake to the rate of appearance in plasma is overestimated. Subsequently protein breakdown would be underestimated and protein balance overestimated. Splanchnic extraction can be determined by administrating an enteral version of the tracer amino acid, which has a different stable isotope label than the intravenous tracer amino acid, e.g. ring-[$^2H_5$]-phenylalanine and [^{13}C]-phenyalanine. In **chapter 2** and **chapter 3 and 7** we used this approach to determine whole body protein kinetics in the fed state.

General disadvantages of stable isotope tracer studies.

Although generally accepted as the reference method to study protein kinetics, stable isotope tracer studies have some disadvantages, which make abundant use of this method impossible. The method is invasive, because it requires blood sampling, preferably from an artery. The stable isotope tracers are expensive and there is need for a specialized laboratory for sample analysis with expensive equipment and expert lab technicians. Also, conducting the

experimental protocol requires well-trained personnel. In addition, the calculations from the amino acid enrichments are quite complex. As a consequence, stable isotope tracer studies comprise only small sample sizes. All in all, this results in the fact that stable isotope tracer studies are not possible as bedside tool to evaluate protein intake over the course of time. Nowadays, research groups try to simplify the methods and develop methods to determine isotopic enrichment in urine [23] or in breath samples alone (without additional blood sampling) [24], which may make stable isotope tracer methodology a more feasible method.

LEVEL 1: BIOCHEMICAL CHANGES

Overall evaluation of stable isotope tracer methodology

- Advantages: provides information on protein synthesis and breakdown rates; short duration protocol representative to 24-hour periods; small study populations sufficient to show effect; feasible in all age groups.
- Disadvantages: invasive, expensive, advanced lab equipment and expert personnel required; different tracer amino acids available with inherent practical disadvantages; possibility of tracer recycling during long duration protocols; not feasible as bedside tool.
- Relevance: accepted as reference method to study protein requirements; comparison of different protein intakes well feasible; assessment of kinetics of specific amino acids possible and kinetics on organ level possible.

Overall evaluation of methods to assess biochemical changes

The methods used in this thesis mainly focused on the biochemical changes that occur in protein kinetics after a nutritional intervention and whether anabolism or catabolism is achieved. It should be borne in mind that protein metabolism is very complex, that it consists of dynamic processes and is difficult to access at the actual site of action in vivo (i.e. intracellular). Hence, every method assessing protein metabolism is based on a simplified model with surrogate markers.

It has been subject of debate whether and how long an adaptation period is needed when using N-balance or stable isotope tracer methods, to allow protein metabolism to adapt to the protein intake of interest. Adaptation periods of several days (e.g. 5-7 days) have been standard in healthy subjects [1]. However, in critically ill patients nutritional support is a dynamic process and often food intake is increased over several days. Also, the effects of the acute stress response diminish after a couple of days [25-27]. Therewith metabolism is altered over the days after the start of illness and will not stabilize on a specific rate as provoked by

protein intake alone. Lastly, if an adaptation could be achieved at all, likely devices needed for experimental protocols (e.g. arterial and central venous catheters) are removed as standard of care by that time and/or patients are discharged from the intensive care unit. Therefore, adaptation periods are not feasible in the critically ill population, especially if changes in protein metabolism or energy expenditure over time are assessed.

Level 2: Body composition

The resultant of protein anabolism is body protein accretion. Therefore, ideally to assess the effect of protein intake on protein anabolism is to study the amount of body protein mass gain (**Figure 9.3**). In general, body composition can be approximated by anthropometrics. More in-depth information on the different components of the body, including body protein mass, can be achieved using more advanced methods. Both groups of methods will be adressed below.

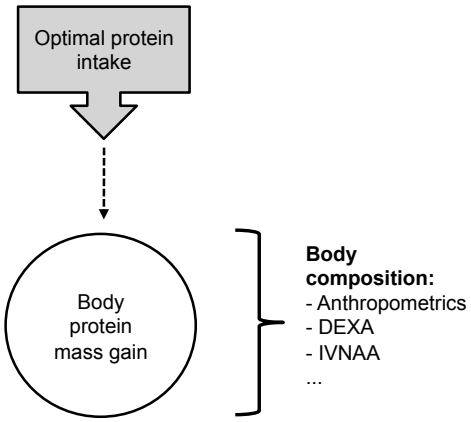


Figure 9.3 – Body composition as outcome measure to study the effect of protein intake.
DEXA, dual energy x-ray absorptiometry; IVNAA, in vivo neutron activation analysis.

Anthropometrics

The simplest and most often used method to study the effect of protein intake on body composition is the measurement of body weight. Other anthropometric methods include length/height, skull circumference, (biceps and triceps) skinfold, mid upper arm circumference and calf circumference measurements, but these measurements require well-trained personnel and longitudinal surveillance of intra-observer variability [28, 29]. In children, to be able to compare body weight with the reference population, SD-scores for age are used. In studies by our group, these SD-scores have been used successfully in a follow-up study on anthropometrics in pediatric intensive care patients [29] and as outcome measure to assess the effect of cumulative energy and protein deficits during PICU admission [28].

In **chapter 8** we determined whether achievement of protein and energy intake goals did affect SD-scores for weight-for-age (SDS WFA) at admission and discharge. We defined acute malnutrition as SDS WFA < -2SD, as used in previous studies [29]. However SDS WFA is not an ideal measure for acute malnutrition, because it does not take the proportionality of the body into account. In children > 1 year of age more accurately, acute malnutrition is defined as SDS weight-for-height (WFH) < -2 SD. This criterion is used by the Dutch STRONGkids nutritional risk screening tool, developed by our group [30] and is recommended by the Dutch Task Force on Malnutrition (www.stuurgroepondervoeding.nl) and in the World Health Organization guidelines [31, 32]. We were not able to assess SDS WFH, because height was sparsely available from the patient data monitoring system. The choice of universal reference values is another limitation, since growth patterns differ among ethnic groups and in children with syndromes or underlying diseases [29].

Concerning the use of anthropometrics as outcome measure to study the effects of protein intake, changes in anthropometrics are not expected quickly and should be used at group level instead of individual patient level. In addition, SDS WFA does not give information on the body composition, more specifically on body protein mass. Changes in SDS WFA can reflect changes in protein mass, fat mass and fluid status (edema) [29]. In addition, weight at admission is often difficult to assess, because of need for e.g. resuscitation, catheters and ventilation [33]. It is our experience that weight at discharge is often not assessed in daily practice. As prognostic value, mid upper arm circumference has been proposed, since this measure is not subject to these disadvantages and is associated with inadequate protein stores and acute malnutrition [29, 33].

LEVEL 2: BODY COMPOSITION

Overall evaluation of anthropometrics

- Advantages: relatively simple to assess; can be used during follow-up; related to cumulative protein and energy deficits.
- Disadvantages: requires well-trained personnel; measurements influenced by edema; effect of protein intake not reflected quickly by changes in anthropometrics; no information on body protein mass.
- Relevance: may be used as initial evaluation of the effect of protein intake on body composition; mid upper arm circumference should be assessed besides weight and height and SD-scores for specific groups of patients and ethnic groups should be used.

Body protein mass

Methods to assess the different components of body composition, hence body protein mass, have been used sparsely in the pediatric critically ill population, although they provide valuable information. In-vivo neutron activation analysis (IVNAA) has been proposed as the golden standard to study the effects of protein intake in adults [34]. This method is based on the principle that with gamma radiation the quantity of individual elements, e.g. nitrogen, in the body can be analyzed and extrapolated to body protein mass [35]. This method has been used in a study in critically ill adults and a study in post-operative adults to assess the effect of protein intake on body composition [36, 37] and current recommendations on protein requirements in critically ill adults are based on these studies. Another method is dual energy x-ray absorptiometry (DEXA) which relies on the principle that x-rays are absorbed with a different intensity by different tissues i.e. bone, lean tissue and fat. Hence, the quantity of lean body mass can be determined [38]. A study in burned children used this method to assess whether an optimal energy intake was effective to decrease lean body mass loss post-burn [39]. These types of study are difficult to perform in critically ill patients, because it requires a specialized facility to provide both critical care and enable metabolic evaluation.

A bedside tool to assess body composition is bioelectrical impedance spectroscopy (BIS). This method relies on the differences in resistance of fluid and fat to a weak electrical current. The measured resistance is extrapolated to the amount of fat mass and fat free mass (fluids, bones and protein). Measurements may be influenced by edema, tubes and catheters and this method has not been validated in critically ill children.

LEVEL 2: BODY COMPOSITION

Overall evaluation of body protein mass

- Advantages: accurate reflection of protein accretion as a result of protein anabolism.
- Disadvantages: requires specialized facilities (IVNAA, DEXA); not validated in critically ill children (BIS).
- Relevance: ideally body protein mass with IVNAA or DEXA should be assessed serially as hard outcome to prove protein anabolism; however most often not feasible due to lack of facilities and interference with critical care.

Level 3: Body protein functions

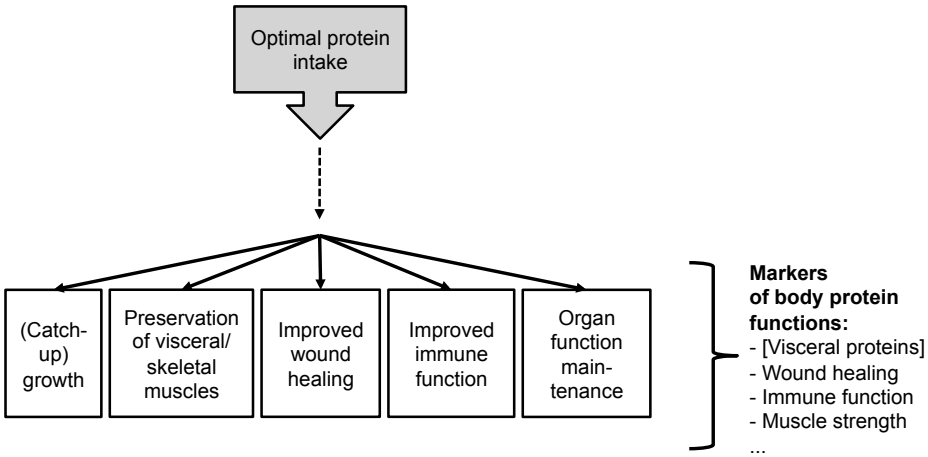


Figure 9.4 – Body protein functions as outcome measure to study the effect of protein intake.

Body protein functions (**Figure 9.4**) are probably the most difficult to assess, since no clear outcome measures are defined and functions of proteins vary widely. Indirect markers of improved body protein function may include markers of wound healing [5], markers of immune function, plasma concentrations of visceral proteins, skeletal muscle strength and visceral muscle strength, such as cardiac function [40]. These methods are most often used in conjunction with other methods, such as stable isotope tracer methodology to assess protein balance, in order to provide additional supporting evidence of the effects of protein intake.

Concerning plasma concentrations of visceral proteins, pre-albumin and retinol-binding protein are considered the most accurate measures to reflect the effect of protein intake, because of their relatively short half-life [41]. Visceral proteins with a longer half-life, such as albumin and transferrin are less suitable to evaluate short term effects of nutritional interventions in critically ill children. Stable isotope tracer techniques may be used to assess synthesis rates of specific proteins, such as skin protein synthesis in burns [5] and albumin synthesis in premutare infants and critically ill children [42, 43], as more dynamic equivalents of visceral protein concentrations. In **chapter 4 and 5** we determined pre-albumin and albumin levels and measured fractional albumin synthesis rates, as indication of visceral proteins, but we found no differences between the groups receiving low glucose intake and standard glucose intake.

It would be interesting to achieve more knowledge on the underlying mechanisms of body protein function that act in-between achieving protein anabolism and gaining body protein mass on the one hand and improving clinical outcome on the other hand.

LEVEL 3: BODY PROTEIN FUNCTIONS

Overall evaluation of indirect markers of body protein function

- Advantages: provide insight in underlying mechanisms of improved outcome.
- Disadvantages: many different functions of protein; indirect markers.
- Relevance: may be used in conjunction with other methods that assess the effect of protein intake to provide more insight into underlying mechanisms of improved clinical outcome.

Level 4: Outcome studies

Outcome studies in critically ill children may focus on short-term outcome and long-term outcome (**Figure 9.5**). Both will be underlined below.

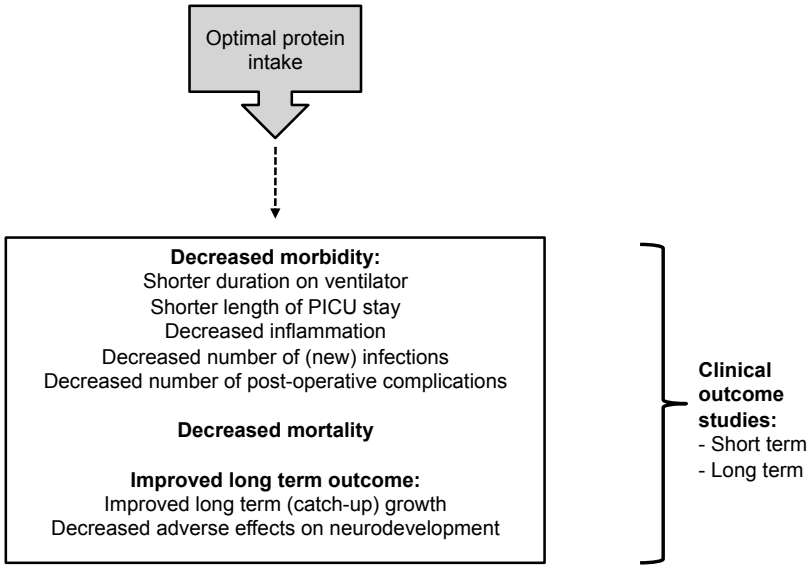


Figure 9.5 – Clinical outcome to study the effect of protein intake.

Short-term outcome

In **chapter 8** we determined whether achievement of protein and energy intake goals was associated with improved clinical outcome. We did not find any effects of achieving intake goals, route of nutrition or malnourished status on clinical outcome. Because the patient population on the PICU is very heterogeneous, a form of stratification is required and therefore sample sizes need to be large. We are one of the few groups that have studied the effect of nutritional intake on clinical outcome in critically ill children. Another large study evaluated nutritional practices in 500 ventilated critically ill children in a multicenter study [44]. Malnutrition was more prevalent than in our study (30%). A higher percentage of energy intake goals received via the enteral route was associated with a decreased 60-day mortality. The use of feeding protocols was associated with a reduced incidence of new infections. To our knowledge no other large clinical outcome studies on the effect of protein intake in critically ill children have been performed, so there is urgent need for this type of studies.

Although studies on nutritional intake in adults have focused on mortality as primary outcome measure [45, 46], in critically ill children it is not a feasible measure, because mortality is low (~5% for the total population) [47] and requires very large sample sizes. Number of new infections seems to be a promising outcome variable, which has been successfully used in a large randomized trial on intensive insulin therapy in children and in another large randomized trial on early versus late parenteral nutrition to complete enteral nutrition in adults [48, 49]. Secondary outcome measures may include the length of PICU-stay (PICU-LOS) and hospital length-of-stay (LOS), number of days on ventilator, duration of antibiotics therapy, the use of inotropics, need for extra-corporeal membrane oxygenation (ECMO), kidney and liver dysfunction (i.e. renal replacement therapy) and the effect on inflammation as assessed by serial C-reactive protein measurements. These have also been used in aforementioned studies [48, 49]. When (intensive) insulin therapy is used, the number of hypoglycemic events should also be assessed. If PICU-LOS and hospital LOS are reduced, it is important to know whether the functional status at discharge is not deteriorated meanwhile, as compared with patients who are discharged later. Therefore, also functional health may be assessed by functional health questionnaires at discharge [48].

LEVEL 4: CLINICAL OUTCOME STUDIES

Overall evaluation of short-term outcome

- Advantages: assesses clinical relevance of achieving protein anabolism.
- Disadvantages: large sample sizes required; stratification needed.
- Relevance: may be used in large randomized controlled trials; results may be used to underline importance of nutritional support/protein intake as part of critical care; number of new infections should be primary outcome measure; secondary outcome measures may include length-of-stay at PICU and hospital, number of days on ventilator and duration of antibiotics therapy.

Long-term outcome

Follow-up studies on the effect of protein intake are lacking in critically ill children. A previous study of Hulst et al. focused on anthropometrics at 6-months follow-up [29]. However, the effect of protein intake during admission was not assessed in relationship to the changes in anthropometrics. Future studies on protein intake should assess long-term growth, e.g. at 3, 6 and 12 months after discharge. Ideally DEXA scans or other methods that can assess body protein mass should be used. In adults, a case report showed that lost body protein mass was replaced by fat only on the long-term [50], despite adequate weight gain. Weight measurement alone is thus not sufficient, although in children linear growth requires protein mass gain and therefore gives indirect information on the protein status. A baseline DEXA scan measurement should then be obtained at PICU discharge to be able to get insight into the growth course, or results may be compared with healthy children. Also, since a history of malnutrition is associated with impaired cognition, behavioral problems and impaired work habits in children [51-54], tests evaluating these aspects may be used at the long-term. In addition functional health can be assessed with questionnaires such as WEEFIM and HUI [55, 56]. The logistic structure of these kind of follow-up studies exists in our center, since patients with congenital anomalies (e.g. esophageal atresia and congenital diaphragmatic hernia) and those treated on ECMO are already participating in long-term growth, functional and cognitive follow-up studies [57, 58].

LEVEL 4: CLINICAL OUTCOME STUDIES

Overall evaluation of long-term outcome

- Advantages: possible to study effect on neurodevelopment.
- Disadvantages: logistic challenges to provide structural follow-up.
- Relevance: data on long term growth and neurodevelopment in critically ill children in relation to protein intake/nutritional support during admission are lacking.

Overall evaluation of outcome measures to study the effect of protein intake

Plasma amino acid concentrations can be used to assess protein metabolism, but provide incomplete information if not combined with kinetics studies. We have now confirmed that for future studies short-duration tracer protocols can be used. However, in the current form stable isotope tracer methods are not as simple enough as to be used on a regular basis. Measurements of tracer enrichments in urine alone would be a step forward.

Urgently, studies are needed on the effect of protein intake on clinical outcome in different subgroups of critically ill children, e.g. brain injury patients, congenital heart disease, septic shock etc. It would be ideal to assess protein metabolic parameters in the same population, to determine whether a catabolic or anabolic state is achieved. It is possible to perform stable isotope studies in a random small sample out of the larger population. Also ideally but possibly not feasible, this could be combined with (serial if possible) assessment of body protein mass, e.g. by DEXA method. Short-term outcome measures may be the number of new infections during PICU-stay, PICU-LOS and hospital-LOS, duration of ventilation, duration of anti-microbial therapy, the need for inotropics and ECMO, inflammation, kidney and liver dysfunction. Indices of body protein fucntions may meanwhile give background information on the pathophysiological mechanisms of improved short-term outcome. Long-term outcome measures may be long-term growth, body protein mass gain, cognitive, behavioral and functional health. Short (and long) term mortality should be reported, but cannot readily be used as outcome measure in critically ill children, because of the low mortality rate in critically ill children.

ACHIEVING PROTEIN ANABOLISM IN CRITICALLY ILL CHILDREN

Protein and energy requirements to achieve protein anabolism

The major aim of the studies in this thesis was to evaluate whether achieving protein anabolism is possible during critical illness in children.

Figure 9.6 summarizes all protein balance data obtained with stable isotope methodology in this thesis in relation to protein and energy intake. Zero protein balance was achieved between 1.0 and 2.1 g/kg/d protein intake. From 2.1 g/kg/d on, all patients except for 1, were in positive protein balance, thus in an anabolic state. When linear regression was performed on the data of the fed patients alone, zero balance was achieved at 1.5 g/kg/d. Our data suggests that 1.5 g protein/kg/d is thus the minimal protein requirement in our study population of critically ill children.

Concerning energy intake, in **chapter 6 and 7** we showed that the protein-energy enriched formula resulted in improved energy balances as determined via indirect calorimetry or from energy expenditure predictions [59], in comparison with the standard infant formula. As shown in **Figure 9.6**, at an energy intake of 57 kcal/kg/d one patient achieved a positive protein balance, but only from an intake of 112 kcal/kg/d all patients were in positive protein balance. Linear regression of all patients showed that zero protein balance was achieved at 85 kcal/kg/d. When only enterally fed patients were included in the analysis, zero protein balance was achieved at 63 kcal/kg/d. For a protein deposition rate of 1.0 g protein/kg/d energy intakes of 100-120 kcal/kg/d were required.

Median age in our patient groups was 0.46 years (min 0.05 years, max 9.9 years; mean 1.24 ± 2.07). In healthy infants average protein deposition for normal growth is 0.6 g protein/kg/d, but for catch-up growth protein deposition may range from 1.0-4.6 g protein/kg/d [1]. The maximum acquired protein deposition rate in our studies was approximately 1.5 g protein/kg/d with intake of ~3.5 g protein/kg/d. Protein deposition of 1.0 g protein/kg/d was achieved at 2.5-3.0 g protein/kg/d and 100-120 kcal/kg/d (protein-energy ratio of 10 energy%). For rapid catch-up growth, even a higher protein intake may be required, but the safety of such high protein intakes should be monitored e.g. by measuring urea plasma concentrations.

Recently, a systematic review of the available literature on protein intake and the effect on N-balance or protein balance was published [60]. Only studies using N-balance were identified, although meanwhile some stable isotope tracer studies have been conducted, among others the study in **chapter 7** and another study from our research group [61]. Data are summarized in **Figure 9.7** in which average values of protein balance per included study are shown plotted against protein and energy intake. The data obtained in this thesis are well in line with the findings of the systematic review, despite the different methods used. Bechard et al. concluded that minimum intakes of 57 kcal/kg/d and 1.5 g protein/kg/d were required to achieve a protein anabolic state (protein-energy ratio of 10.5 energy%).

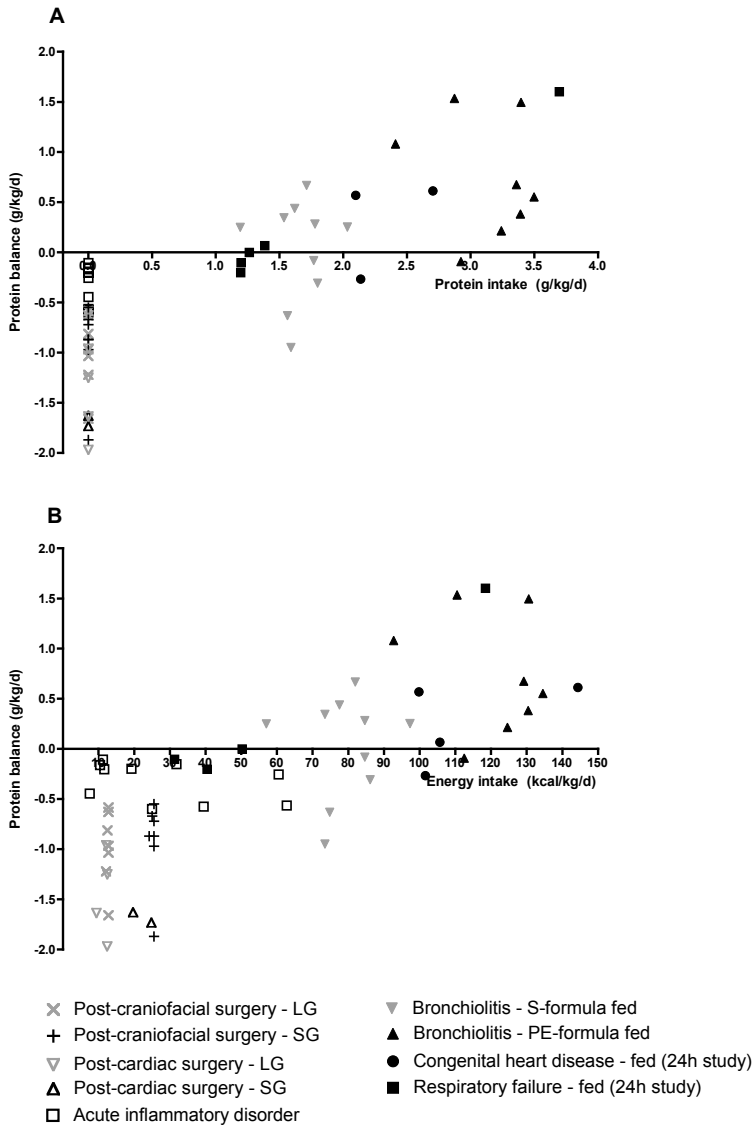


Figure 9.6 – Overview of protein balance data in patients included in this thesis.

Protein balance data are plotted per individual patient against protein intake (A) and energy intake (B). Data were obtained using stable isotope tracer methodology and extrapolated from either phenylalanine/tyrosine or leucine kinetics (leucine tracers used in post-craniofacial surgery and post-cardiac surgery patients). Post-surgical patients are plotted twice, once receiving IV low glucose intake (LG; 2.5 mg glucose/kg/min), once receiving IV standard glucose intake (SG; 5.0 mg glucose/kg/min). Acute inflammatory disorder patients were fasted and received IV glucose at an average of ~5.0 mg/kg/min. The fed patients received enteral nutrition; PE-formula, protein-energy enriched formula; S-formula, standard infant formula. The data of the patients of the 24-hour study of chapter 2 are average protein balance data over the first 6-hour of the stable isotope tracer protocol.

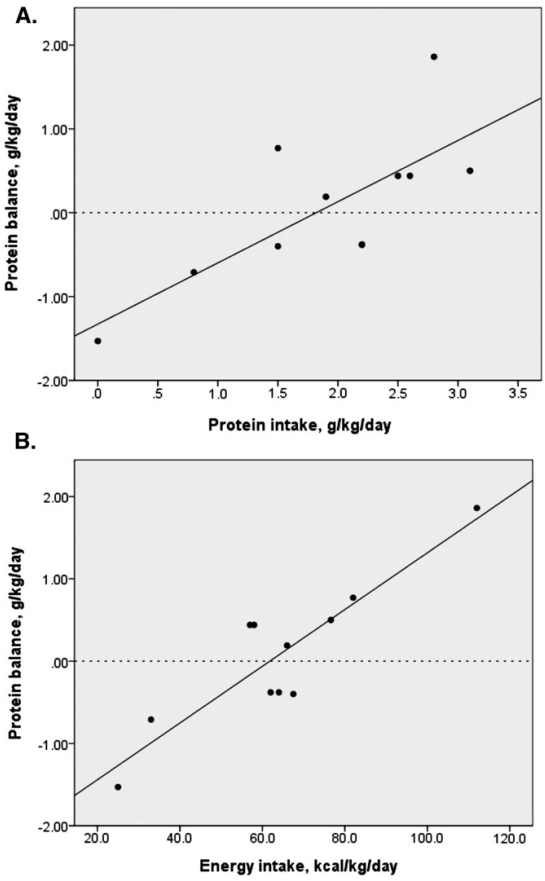


Figure 9.7 – Relationship between protein and energy intake and protein balance (calculated from N-balance) in critically ill children, as extrapolated from available literature. Adapted from Bechard et al. [60]. Each point represents the average protein balance rate per study..

Recommendations

Based on our data and those found by Bechard et al. in critically ill infants we recommend a minimum protein intake of 1.5 g protein/kg/d and ~ 60 kcal/kg/d to achieve zero protein balance, but optimally 2.5-3.0 g protein/kg/d and 100-120 kcal/kg/d should be provided to achieve protein deposition rates of ~1.0 g protein/kg/d. This is in accordance with providing the protein-energy enriched formula, that we used in **chapter 6 and 7** at a volume intake of ~ 100-120 ml/kg/d. For older children, these recommendations are possibly on the high end as older children have lower protein deposition rates during health [1]. On the other hand we and Bechard observed a linear association between protein intake and protein balance, based on data of both infants and older children. Possibly for critically ill children similar protein requirements are applicable as for critically ill infants, driven by critical illness related factors

and not age-dependent factors. In all age groups severity of illness may influence the amount of protein intake that is required to achieve protein anabolism, although in **chapter 3** we were not able to find a correlation between C-reactive protein and protein kinetics.

Furthermore, we recommend the use of formulas with protein-energy ratios of ~10 energy%, such as the protein-energy enriched formula in **chapter 6 and 7**. The protein-energy ratio of the standard infant formula was ~8% and does not allow as fast a protein deposition as formulas with higher protein-energy ratios [1].

It should be noted that the fed patients included in this data analysis were studied at least 5 days after admission. Hence, these recommendations do not apply to the acute phase of critical illness. Ideally, the effect of age, timing after onset of critical illness and severity of inflammation on protein requirements should be studied to be able to make more accurate recommendations under specific circumstances.

Other measures to achieve protein anabolism

The question is whether protein anabolism can be achieved earlier than day 5 after admission. Besides logistic difficulties (i.e. priority to medical care, fluid restriction), the initial phase of the acute stress response probably prevents a protein anabolic state, due to hormonal changes and cytokine expression. In infants after surgery, C-reactive protein levels < 2 mg/dL (defined as resolving stress) as compared with ≥ 2 mg/dL (acute stress) have been associated with reductions in total urinary nitrogen excretion, suggesting return to the anabolic state [62]. In addition, changes in thyroid hormone are associated with changes in C-reactive protein during recovery in critically ill children, suggesting that indeed the recovery of the acute stress response is expressed as a reduction in C-reactive protein levels [63]. Our research group has previously found that the acute stress response is relatively short in critically ill children, lasting 24 - 48 hours [25-27]. This indicates that protein anabolism might be possible early after admission.

In this thesis we investigated only whether increased protein and energy intake was effective to induce protein anabolism. Another approach that is used to boost protein anabolism (muscle protein synthesis) is the administration of leucine or essential amino acids [64-66]. *Via* a mTOR (mammalian target of rapamycin) dependent pathway leucine exerts its anabolic effect [67]. Therefore nutritional supplementation with this amino acid may be feasible to induce protein anabolism to a higher extent than we have achieved in the studies in this thesis.

We did not address the effect of protein composition, which is an important aspect when considering protein requirements though. As explained earlier, a single essential amino acid in the dietary amino acid mixture may be the limiting factor for protein synthesis. Optimal protein synthesis can only be achieved when the amino acid mixture provided as protein in the diet matches the amino acid mixture of proteins that are being synthesized [68]. Acute phase proteins differ in amino acid mixture from skeletal muscle proteins [69]. Thus, it can be hypothesized that during the acute phase of critical illness, when the liver synthesizes acute

phase proteins, the protein composition requirement is different from that in the recovery phase, when wounds are being healed and skeletal muscle loss must be regained. Providing accurate protein compositions might limit protein catabolism and allow protein anabolism earlier, but not much is known on this subject. In healthy subjects different proteins promote anabolism to a different extent, with the milk proteins whey and casein being the most potent [70-72]. Studies evaluating protein composition are therefore necessary in critically ill children to shed more light on this subject.

Specific amino acid requirements

Requirements of specific amino acids during critical illness may be higher than during health. If the endogenous production does not meet the increased needs, a non-essential amino acid becomes conditionally essential [73]. It is therefore important to know more about specific amino acids and provide those that are conditionally essential in higher amounts during circumstances of increased demand.

Arginine

We found strong indications that arginine becomes an essential amino acid proportional to the severity of inflammation in **chapter 3.** Whereas arginine utilization is increased with increasing inflammation, arginine release from protein breakdown is not increased, while citrulline production, hence *de novo* arginine synthesis, is decreased. This was illustrated by decreased arginine and citrulline plasma concentrations, which we have found previously to be inversely related with C-reactive protein levels [74]. Our results were well in line with studies in adults [75-77], but differed somewhat from the only other study in critically ill children [78]. In the latter study arginine and citrulline metabolism in critically ill children was compared with healthy adults and an increased citrulline production was found, hence *de novo* arginine synthesis. Nevertheless, the authors concluded as well that arginine is a conditional essential amino acid in critically ill children. These studies as well as ours imply that exogenous arginine may be needed to improve arginine availability for its functions. However, the amount of arginine required is not known. In children in various disease entities, arginine supplementation has been found to be beneficial and usually is used to improve NO synthesis [79-81]. However, it has been stressed that arginine supplementation may be detrimental in critically ill adults [82], because of possible hypotension due to NO induced vasodilatation. In **chapter 3** we showed that a more physiological approach may be to increase arginine intake as part of increased protein intake at least in children with viral bronchiolitis, by providing a well-balanced protein-energy enriched formula. We were able to increase both arginine appearance and NO synthesis by these means, while the formula was well tolerated and save. Studies on the amount of arginine needed in critically ill children are warranted and should focus on requirements in relation to severity of inflammation.

Citrulline

Citrulline is a non-protein amino acid and the sole precursor of arginine *de novo* synthesis. *De novo* arginine synthesis occurs in the kidneys. Citrulline is made from proline and glutamine in enterocytes [23, 83, 84]. As citrulline production is decreased when functional bowel mass in lost, e.g. in patients with short bowel syndrome, citrulline plasma concentrations have been used as marker of small bowel function [85]. During critical illness in children citrulline plasma concentrations are decreased proportional to the severity of inflammation [74]. In **chapter 3** we showed that also citrulline production, hence *de novo* arginine synthesis, was decreased proportional to the severity of inflammation. To improve arginine availability, citrulline may be supplemented instead of arginine. In children with congenital heart disease oral and intravenous citrulline supplementation has been used to improve NO synthesis to prevent pulmonary hypertension. It appeared that citrulline *via* both routes was well tolerated [86, 87]. In addition to playing an important role in arginine metabolism, it appears that citrulline has muscle protein synthesis stimulating properties, similarly to leucine via the mTOR dependent pathway [88]. In addition, oxidative stress reducing effects and glycemia controlling effects have been ascribed to citrulline [88]. It therefore is an interesting amino acid for future studies in critically ill children.

Glutamine

We did not focus on glutamine requirements in this thesis. We previously showed that plasma glutamine concentrations were decreased proportional to inflammation [74], although we were not able to re-establish this finding in **chapter 3**. The fact that citrulline production was severely decreased proportional to inflammation may indicate that the conversion of glutamine to citrulline in the intestines was impaired, but we cannot conclude this from our results. No differences in glutamine plasma concentrations were found between the protein-energy enriched formula and the standard formula in **chapter 3, 6 and 7**, but neither did we find differences in arginine concentrations, despite different kinetics rates as shown by stable isotope tracer methodology. From the studies in this thesis we cannot make recommendations on glutamine intake.

IMPROVING NUTRITIONAL SUPPORT IN THE PEDIATRIC INTENSIVE CARE UNIT

Challenges to provide adequate nutritional support in critically ill children

As shown in **chapter 8** achievement of intake goals was difficult; half of our patients with a minimal stay of 4 days at the PICU did not achieve both protein and energy intake goals on day 4 after admission. This was despite our aggressive nutritional protocol that aims at achieving nutritional intake higher than these intake goals within 48-hours after admission

(presented in **chapter 8**). Others have also found that a large proportion of their PICU population did not achieve adequate nutritional intake [89-93]. The main factors described to hinder adequate nutritional intake are [33, 94], among others, fluid restriction (reported in 67% of patients), procedural interruptions of feeds (reported in 62% of patients) and gastro-intestinal intolerance (reported in 57% of patients) [92].

Achieving adequate nutritional intake in all patients thus remains a challenge. We aimed to determine in **chapter 8** whether achievement of intake goals improved clinical outcome, however we were not able to demonstrate this. Remarkably we found that malnourished patients achieved goals more often than non-malnourished patients, possibly because of increased attention to their nutritional status.

We provided a new, although not unexpected, insight into protein metabolism in critically ill children in **chapter 2**: no circadian rhythm was present. Circadian rhythms have been increasingly subject of research the past two decades, after clock genes had been discovered, which are part of an intrinsic timekeeping system [95]. Circadian rhythms are involved in the development of a broad range of human diseases, but little attention has been focused on their role in critical illness [96]. A study in critically ill sedated adults showed that 24-hour profiles of blood melatonin, cortisol, heart rate, body temperature and spontaneous motor activity were greatly disturbed [97]. However, it is not known whether these disturbances are adaptive responses or pathologic in themselves. Since circadian desynchrony and genetic disruption of clock genes lead to metabolic pathologies [95], it can be hypothesized that the abolished circadian rhythm in the intensive care unit may contribute to morbidity and mortality. In healthy subjects a diet-based pattern in 24-hour protein metabolism is observed [17, 19-21]. Therefore, although purely speculative, it can be hypothesized that continuous nutritional support contributes to abolishment of circadian rhythms and that in future different approaches of nutritional support have to be sought. This new field of research deserves attention.

Strategies to improve nutritional intake

Several strategies can be used to improve nutritional intake. In **table 9.1** barriers to adequate nutritional intake are shown, with suggested interventions to overcome the problem. Examples of interventions may include instituting nutritional teams and protocols [89], such as our protocol in **chapter 8**, tailored energy intake based on indirect calorimetry measurements [98] and the use of specialized clinical formulas, as used in **chapter 6 and 7**. Within the observational dataset of **chapter 8** we performed a sub-analysis on achievement of intake goals in patients receiving the protein-energy enriched infant formula (n=19) as compared with the standard infant formula (n=30). Patients receiving the protein-energy enriched formula achieved goals more often than those receiving the standard formula (84% vs. 27%, $p < 0.005$ respectively) and had a higher protein intake (median (min-max); 2.6 (1.1-3.9) vs. 1.7 (0.5-3.3) g/kg/d, $p < 0.001$, respectively) and energy intake (median (min-max): 100 (58-151) vs. 80

Table 9.1 – Overview of barriers to adequate nutritional support in critically ill children, underlying problems and recommended approach.

Barrier	Reason	Suggested approach
Interruptions to EN	Intolerance	Apply uniform definition, algorithmic guideline
	Procedures	Review fasting guidelines for procedures Resume feeding of procedure delayed, canceled or complete
	Enteral access issues	Request specialized team for enteral access, radiology collaboration, prompt replacement of displaced enteral tubes
Fluid restriction	Patients with cardiac or renal conditions	Consider concentrated formula Review other fluids Anticipate plan with dietitian
Patient on vasoactive drugs	Concerns for gut ischemia	Prudent to hold EN when actively resuscitating with fluid, hemodynamics worsening or multiple vasoactive drugs required Consider EN if no fluid resuscitation for over 12 hours and on single or stable vasoactive support Monitor closely while advancing feedings
Delayed EN initiation	Failure to prioritize	Educate, develop institution-specific, uniform guidelines for nutrition delivery
General reluctance to address nutrition delivery	Failure to prioritize nutrition support	Create nutrition support team Request dietitian dedicated to the ICU Involve key stakeholders and develop multiprofessional consensus for nutritional therapy goals

Adapted from N. Mehta 2011. EN, enteral nutrition; ICU, intensive care unit.

(29-131) kcal/kg/d, p=0.01; respectively). Thus in clinical practice the use of the protein-energy enriched formula was feasible and beneficial to achieve intake goals.

Another approach to improve nutritional intake, as mentioned above, is to provide supplemental parenteral nutrition when energy and/or protein goals are not met with enteral nutrition alone. Unexpectedly, in adults early initiation of supplemental parenteral nutrition was more detrimental than late initiation (> 7 day after admission) [48]. As early achievement of energy goals has been advocated widely and is part of the European guidelines for critically ill adults [99], the results of the study received much criticism [100-104]. The underlying mechanisms need to be unraveled further, but there seems to be a role for autophagy, which is needed for removal of damaged cell structures and essential for recovery [105]. Autophagy is triggered by fasting and suppressed by nutrient intake, which was indeed shown at least in rabbits receiving early parenteral nutrition after experimental burns [106]. In critically ill patients, an autophagy deficiency phenotype in skeletal muscle and liver biopsies was found as well, of which the severity was correlated with the amount of infused amino acids [107]. In critically ill children supplemental parenteral nutrition is not widely used as standard, nor are there clear recommendations

in guidelines on this subject [108, 109]. Whether similar results as the study in adults will apply to critically ill children is now subject of a randomized controlled trial.

FUTURE PERSPECTIVES

Future studies on protein requirements

The 24-hour study in chapter 2 was the first of three phases of a project to determine protein requirements in critically ill children using stable isotope methodology. In this first phase we aimed to determine whether a circadian rhythm was present in continuously fed critically ill children, hence whether or not measurements at different parts of the day are required to fully appreciate daily metabolism. We hypothesized that this was not the case and that short duration protocols are feasible to determine daily protein balance. Indeed our hypothesis was confirmed. The next step is to introduce 3 different protein intake levels per patient in a 24-hour stable isotope tracer protocol, to determine at which protein intake level zero protein balance is achieved. This would be the minimum protein requirement. In **Figure 9.8** this approach is depicted. The optimal protein requirement is difficult to define, but it should allow optimal (catch-up) growth. Thus, it would be the protein intake at which maximal net protein synthesis is achieved, while not exerting negative effects such as uremia. It is likely that at some point further increasing protein intake would not further stimulate protein balance. In **Figure 9.8** this is indicated as the black dashed line. In the acute phase of illness positive protein balance is possibly never achieved (grey dashed line). The optimal protein requirement would then be the protein intake at which protein balance approaches zero as close as possible. In the third phase of the study, if possible, the 24-hour protocol would be reduced to shorter duration protocols, such that the study would be more feasible in larger cohorts of critically ill children. Then, it will be the aim to study different subgroups of critically ill children, e.g. with different rates of inflammation and different diagnoses, as well as different age groups and at different phases during critical illness. Now that we have shown that shorter duration protocols are representative to 24-hour measurements the second phase of the study can be initiated. The final step would be to investigate the effects of positive protein balance on long term anthropometrics, body composition, clinical outcome and neurodevelopment.

Future studies on glucose intake

Now we have provided a mechanistic view of the effects of reduced glucose intake on glucose and protein metabolism, however we do not know yet whether improved blood glucose levels, as achieved with our approach, are associated with improved clinical outcome. Therefore, a large randomized controlled trial is needed to study the safety, adverse effects and clinical outcome of reduced versus standard glucose intake. Outcome parameters could be the occurrence of hypoglycemia, the occurrence of hyperglycemia, the need for insulin therapy as

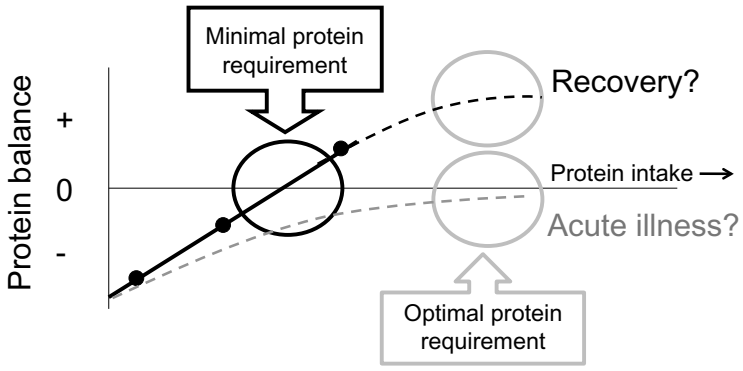


Figure 9.8 – Model of protein balance at different protein intake levels.

With increasing protein intake, protein balance is increased. The minimal protein requirement is the protein intake at which protein balance equals zero. Optimal protein requirement is difficult to define, but should allow optimal growth. In the acute phase of illness positive protein balance may not be achieved.

"escape" method when normoglycemia cannot be maintained, the number of new infections, the effect on inflammation as shown by C-reactive protein, long term neurocognitive outcome and mortality. Currently our research group is planning a large randomized controlled trial to study this.

Future studies on timing and route of nutrition

As described above, supplemental parenteral nutrition may be an interesting approach to improve achievement of intake goals, but first evidence is needed to show that this approach is not detrimental in critically ill children. Currently, our research group is planning to conduct a large randomized controlled trial in critically ill children to study early initiation of supplemental parenteral nutrition versus late initiation.

CLINICAL IMPLICATIONS OF FINDINGS IN THIS THESIS

The clinical implications of this thesis are the following:

- No circadian rhythm is present in protein metabolism in critically ill children, which potentially has detrimental effects. More knowledge is required on this subject, but possibly more attention should be paid to the day-night rhythm in the intensive care unit.
- Short-duration tracer protocols of 6-hours may be used as proxy for 24-hour periods.
- Arginine becomes an essential amino acid during critical illness in children proportional to the rate of inflammation. Arginine supplementation might be needed to restore plasma arginine concentrations. Using a protein-energy enriched formula arginine availability can be improved. This well-balanced nutrition might be a more physiological tool to achieve these goals than merely arginine supplementation
- Plasma arginine concentrations in the fed state might not be representative for arginine availability.
- Reduced glucose intake improves blood glucose levels, without causing hypoglycemia or increased protein catabolism in critically ill children in the early post-operative course after craniofacial surgery and cardiothoracic surgery. Although a large clinical trial is warranted to study the effects on clinical outcome, this approach seems promising as first step to target hyperglycemia.
- Provision of a protein-energy enriched formula is safe, well tolerated and improves nitrogen- and energy balances and promotes protein anabolism early after admission and is therefore recommended over a standard infant formula.
- Intake goals are difficult to achieve in all critically ill children. The use of supplemental parenteral nutrition as tool to achieve goals more often needs to be studied, as clinical outcome may be impaired. Also, a nutritional expert team is an essential part of daily care to guide nutritional support and provide tailored nutritional intake for individual requirements.

REFERENCES

1. *Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation*, 2007, WHO: Geneva, Switzerland.
2. Hulst JM, van Goudoever JB, Zimmermann LJ, Tibboel D, et al. *The role of initial monitoring of routine biochemical nutritional markers in critically ill children.* J Nutr Biochem. 2006;**17**(1):57-62.
3. Reynolds RM, Bass KD, Thureen PJ. *Achieving positive protein balance in the immediate postoperative period in neonates undergoing abdominal surgery.* J Pediatr. 2008;**152**(1):63-7.
4. Rivera A, Jr., Bell EF, Bier DM. *Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life.* Pediatr Res. 1993;**33**(2):106-11.
5. Patterson BW, Nguyen T, Pierre E, Herndon DN, et al. *Urea and protein metabolism in burned children: effect of dietary protein intake.* Metabolism. 1997;**46**(5):573-8.
6. Poindexter BB, Ehrenkranz RA, Stoll BJ, Koch MA, et al. *Effect of parenteral glutamine supplementation on plasma amino acid concentrations in extremely low-birth-weight infants.* Am J Clin Nutr. 2003;**77**(3):737-43.
7. Heird WC, Hay W, Helms RA, Storm MC, et al. *Pediatric parenteral amino acid mixture in low birth weight infants.* Pediatrics. 1988;**81**(1):41-50.
8. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, et al. *Amino acid administration to premature infants directly after birth.* J Pediatr. 2005;**147**(4):457-61.
9. Druml W, Heinzel G, Kleinberger G. *Amino acid kinetics in patients with sepsis.* Am J Clin Nutr. 2001;**73**(5):908-13.
10. Vente JP, von Meyenfeldt MF, van Eijk HM, van Berlo CL, et al. *Plasma-amino acid profiles in sepsis and stress.* Ann Surg. 1989;**209**(1):57-62.
11. Barr FE, Beverley H, VanHook K. *Effect of cardiopulmonary bypass on urea cycle intermediates and nitric oxide levels after congenital heart surgery.* J Pediatr. 2003;**142**:26-30.
12. Chaloupecky V, Hucin B, Tlaskal T, Kostelka M, et al. *Nitrogen balance, 3-methylhistidine excretion, and plasma amino acid profile in infants after cardiac operations for congenital heart defects: the effect of early nutritional support.* J Thorac Cardiovasc Surg. 1997;**114**(6):1053-60.
13. Cynober LA. *Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance.* Nutrition. 2002;**18**(9):761-6.
14. Soeters PB, de Blaauw I, van Acker BA, von Meyenfeldt MF, et al. *In vivo inter-organ protein metabolism of the splanchnic region and muscle during trauma, cancer and enteral nutrition.* Baillieres Clin Endocrinol Metab. 1997;**11**(4):659-77.
15. Van Eijk HM, Dejong CH, Deutz NE, Soeters PB. *Influence of storage conditions on normal plasma amino-acid concentrations.* Clin Nutr. 1994;**13**(6):374-80.
16. Oddoye EA, Margen S. *Nitrogen balance studies in humans: long-term effect of high nitrogen intake on nitrogen accretion.* J Nutr. 1979;**109**(3):363-77.
17. Sanchez M, el-Khoury AE, Castillo L, Chapman TE, et al. *Phenylalanine and tyrosine kinetics in young men throughout a continuous 24-h period, at a low phenylalanine intake.* Am J Clin Nutr. 1995;**61**(3):555-70.
18. Crenn P, Thuillier F, Rakatoambinina B, Rongier M, et al. *Duodenal vs. gastric administration of labeled leucine for the study of splanchnic metabolism in humans.* J Appl Physiol. 2000;**89**(2):573-80.
19. el-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, et al. *Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-13C]leucine and [15N-15N]urea.* Am J Clin Nutr. 1994;**59**(5):1000-11.
20. el-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, et al. *The 24-h pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults.* Am J Clin Nutr. 1994;**59**(5):1012-20.

21. Sanchez M, El-Khoury AE, Castillo L, Chapman TE, et al. *Twenty-four-hour intravenous and oral tracer studies with L-[1-13C]phenylalanine and L-[3,3-2H2]tyrosine at a tyrosine-free, generous phenylalanine intake in adults.* Am J Clin Nutr. 1996;**63**(4):532-45.
22. Wagenmakers AJ. *Tracers to investigate protein and amino acid metabolism in human subjects.* Proc Nutr Soc. 1999;**58**(4):987-1000.
23. Tomlinson C, Rafii M, Sgro M, Ball RO, et al. *Arginine is synthesized from proline, not glutamate, in enterally fed human preterm neonates.* Pediatr Res. 2011;**69**(1):46-50.
24. Huang L, Hogewind-Schoonenboom JE, de Groof F, Twisk JW, et al. *Lysine requirement of the enterally fed term infant in the first month of life.* Am J Clin Nutr. 2011;**94**(6):1496-503.
25. de Groof F, Joosten KF, Janssen JA, de Kleijn ED, et al. *Acute stress response in children with meningococcal sepsis: important differences in the growth hormone/insulin-like growth factor I axis between nonsurvivors and survivors.* J Clin Endocrinol Metab. 2002;**87**(7):3118-24.
26. Joosten KF, de Kleijn ED, Westerterp M, de Hoog M, et al. *Endocrine and metabolic responses in children with meningoccocal sepsis: striking differences between survivors and nonsurvivors.* J Clin Endocrinol Metab. 2000;**85**(10):3746-53.
27. Verhoeven JJ, Hokken-Koelega AC, den Brinker M, Hop WC, et al. *Disturbance of glucose homeostasis after pediatric cardiac surgery.* Pediatr Cardiol. 2011;**32**(2):131-8.
28. Hulst JM, van Goudoever JB, Zimmermann LJ, Hop WC, et al. *The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population.* Clin Nutr. 2004;**23**(6):1381-9.
29. Hulst J, Joosten K, Zimmermann L, Hop W, et al. *Malnutrition in critically ill children: from admission to 6 months after discharge.* Clin Nutr. 2004;**23**(2):223-32.
30. Hulst JM, Zwart H, Hop WC, Joosten KF. *Dutch national survey to test the STRONGkids nutritional risk screening tool in hospitalized children.* Clin Nutr. 2010;**29**(1):106-11.
31. *Management of severe malnutrition: a manual for physicians and other senior health workers*, 1999, World Health Organization: Geneva.
32. Joosten KF, Hulst JM. *Prevalence of malnutrition in pediatric hospital patients.* Curr Opin Pediatr. 2008;**20**(5):590-6.
33. Hulst JM, Joosten KF, Tibboel D, van Goudoever JB. *Causes and consequences of inadequate substrate supply to pediatric ICU patients.* Curr Opin Clin Nutr Metab Care. 2006;**9**(3):297-303.
34. Sauerwein HP,Strack van Schijndel RJ. *Perspective: How to evaluate studies on peri-operative nutrition? Considerations about the definition of optimal nutrition for patients and its key role in the comparison of the results of studies on nutritional intervention.* Clin Nutr. 2007;**26**(1):154-8.
35. Pollock CA, Ibels LS, Allen BJ, Ayass W, et al. *Total body nitrogen as a prognostic marker in maintenance dialysis.* J Am Soc Nephrol. 1995;**6**(1):82-8.
36. Ishibashi N, Plank LD, Sando K, Hill GL. *Optimal protein requirements during the first 2 weeks after the onset of critical illness.* Crit Care Med. 1998;**26**(9):1529-35.
37. Sevette A, Smith RC, Aslani A, Kee AJ, et al. *Does growth hormone allow more efficient nitrogen sparing in postoperative patients requiring parenteral nutrition? A double-blind, placebo-controlled randomised trial.* Clin Nutr. 2005;**24**(6):943-55.
38. Ellis KJ. *Human body composition: in vivo methods.* Physiol Rev. 2000;**80**(2):649-80.
39. Hart DW, Wolf SE, Herndon DN, Chinkes DL, et al. *Energy expenditure and caloric balance after burn: increased feeding leads to fat rather than lean mass accretion.* Ann Surg. 2002;**235**(1):152-61.
40. Faddan NH, Sayh KI, Shams H, Badrawy H. *Myocardial dysfunction in malnourished children.* Ann Pediatr Cardiol. 2010;**3**(2):113-8.
41. Chwals WJ, Fernandez ME, Charles BJ, Schroeder LA, et al. *Serum visceral protein levels reflect protein-calorie repletion in neonates recovering from major surgery.* J Pediatr Surg. 1992;**27**(3):317-20; discussion 320-1.

42. van den Akker CH, te Braake FW, Schierbeek H, Rietveld T, et al. *Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life.* Am J Clin Nutr. 2007;**86**(4):1003-8.
43. Verbruggen SC, Schierbeek H, Coss-Bu J, Joosten KF, et al. *Albumin synthesis rates in post-surgical infants and septic adolescents; influence of amino acids, energy, and insulin.* Clin Nutr. 2011;**30**(4):469-77.
44. Mehta NM, Bechard LJ, Cahill N, Wang M, et al. *Nutritional practices and their relationship to clinical outcomes in critically ill children-An international multicenter cohort study.* Crit Care Med. 2012.
45. Tsai JR, Chang WT, Sheu CC, Wu YJ, et al. *Inadequate energy delivery during early critical illness correlates with increased risk of mortality in patients who survive at least seven days: a retrospective study.* Clin Nutr. 2011;**30**(2):209-14.
46. Weijs PJ, Stapel SN, de Groot SD, Driessen RH, et al. *Optimal protein and energy nutrition decreases mortality in mechanically ventilated, critically ill patients: a prospective observational cohort study.* JPEN J Parenter Enteral Nutr. 2012;**36**(1):60-8.
47. Naghib S, van der Starre C, Gischler SJ, Joosten KF, et al. *Mortality in very long-stay pediatric intensive care unit patients and incidence of withdrawal of treatment.* Intensive Care Med. 2010;**36**(1):131-6.
48. Casaer MP, Mesotten D, Hermans G, Wouters PJ, et al. *Early versus late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(6):506-17.
49. Vlasselaers D, Milants I, Desmet L, Wouters PJ, et al. *Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study.* Lancet. 2009;**373**(9663):547-56.
50. Reid CL, Murgatroyd PR, Wright A, Menon DK. *Quantification of lean and fat tissue repletion following critical illness: a case report.* Crit Care. 2008;**12**(3):R79.
51. Benton D. *The influence of children's diet on their cognition and behavior.* Eur J Nutr. 2008;**47 Suppl 3**:25-37.
52. Benton D. *The influence of dietary status on the cognitive performance of children.* Mol Nutr Food Res. 2010;**54**(4):457-70.
53. Berkman DS, Lescano AG, Gilman RH, Lopez SL, et al. *Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study.* Lancet. 2002;**359**(9306):564-71.
54. Dykman RA, Casey PH, Ackerman PT, McPherson WB. *Behavioral and cognitive status in school-aged children with a history of failure to thrive during early childhood.* Clin Pediatr (Phila). 2001;**40**(2):63-70.
55. Niemeijer AS, Reinders-Messelink HA, Disseldorp LM, Nieuwenhuis MK. *Feasibility, Reliability, and Agreement of the WeeFIM Instrument in Dutch Children With Burns.* Phys Ther. 2012.
56. Buysse CM, Raat H, Hazelzet JA, Hulst JM, et al. *Long-term health status in childhood survivors of meningococcal septic shock.* Arch Pediatr Adolesc Med. 2008;**162**(11):1036-41.
57. Hanekamp MN, Mazer P, van der Cammen-van Zijp MH, van Kessel-Feddema BJ, et al. *[Neurological defects and developmental disorders following neonatal extracorporeal membrane oxygenation: results of a follow-up study after 5 years].* Ned Tijdschr Geneeskd. 2008;**152**(4):207-12.
58. Gischler SJ, Mazer P, Duivenvoorden HJ, van Dijk M, et al. *Interdisciplinary structural follow-up of surgical newborns: a prospective evaluation.* J Pediatr Surg. 2009;**44**(7):1382-9.
59. Schofield WN. *Predicting basal metabolic rate, new standards and review of previous work.* Hum Nutr Clin Nutr. 1985;**39 Suppl 1**:5-41.
60. Bechard LJ, Parrott JS, Mehta NM. *Systematic Review of Energy and Protein Intake on Protein Balance in Critically Ill Children.* J Pediatr. 2012.
61. Verbruggen SC, Coss-Bu J, Wu M, Schierbeek H, et al. *Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin-resistant adolescents with tight glucose control.* Crit Care Med. 2011;**39**(11):2518-25.
62. Letton RW, Chwals WJ, Jamie A, Charles B. *Early postoperative alterations in infant energy use increase the risk of overfeeding.* J Pediatr Surg. 1995;**30**(7):988-92; discussion 992-3.

63. Hulst JM, van Goudoever JB, Visser TJ, Tibboel D, et al. *Hormone levels in children during the first week of ICU-admission: is there an effect of adequate feeding?* Clin Nutr. 2006;**25**(1):154-62.
64. van Loon LJ. *Leucine as a pharmaconutrient in health and disease.* Curr Opin Clin Nutr Metab Care. 2012;**15**(1):71-7.
65. Pasiakos SM, McClung HL, McClung JP, Margolis LM, et al. *Leucine-enriched essential amino acid supplementation during moderate steady state exercise enhances postexercise muscle protein synthesis.* Am J Clin Nutr. 2011;**94**(3):809-18.
66. Coker RH, Wolfe RR. *Bedrest and sarcopenia.* Curr Opin Clin Nutr Metab Care. 2012;**15**(1):7-11.
67. Li F, Yin Y, Tan B, Kong X, et al. *Leucine nutrition in animals and humans: mTOR signaling and beyond.* Amino Acids. 2011;**41**(5):1185-93.
68. *Dietary Reference Intakes: energy, protein and digestible carbohydrates*, 2001, Health Council of the Netherlands: The Hague.
69. Reeds PJ, Fjeld CR, Jahoor F. *Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states?* J Nutr. 1994;**124**(6):906-10.
70. Soop M, Nehra V, Henderson GC, Boirie Y, et al. *Co-ingestion of whey protein and casein in a mixed meal - demonstration of a more sustained anabolic effect of casein.* Am J Physiol Endocrinol Metab. 2012.
71. Phillips SM, Tang JE, Moore DR. *The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons.* J Am Coll Nutr. 2009;**28**(4):343-54.
72. Engelen MP, Rutten EP, De Castro CL, Wouters EF, et al. *Casein protein results in higher prandial and exercise induced whole body protein anabolism than whey protein in Chronic Obstructive Pulmonary Disease.* Metabolism. 2012.
73. Newsholme E, Leech A, *Functional biochemistry in health and disease.*2008: Wiley - Blackwell.
74. van Waardenburg DA, de Betue CT, Luiking YC, Engel M, et al. *Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation.* Am J Clin Nutr. 2007;**86**(5):1438-44.
75. Luiking YC, Poeze M, Ramsay G, Deutz NE. *Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production.* Am J Clin Nutr. 2009;**89**(1):142-52.
76. Kao CC, Bandi V, Guntupalli KK, Wu M, et al. *Arginine, citrulline and nitric oxide metabolism in sepsis.* Clin Sci (Lond). 2009;**117**(1):23-30.
77. Villalpando S, Gopal J, Balasubramanyam A, Bandi VP, et al. *In vivo arginine production and intravascular nitric oxide synthesis in hypotensive sepsis.* Am J Clin Nutr. 2006;**84**(1):197-203.
78. Argaman Z, Young VR, Noviski N, Castillo-Rosas L, et al. *Arginine and nitric oxide metabolism in critically ill septic pediatric patients.* Crit Care Med. 2003;**31**(2):591-7.
79. Morris CR, Kuypers FA, Larkin S, Sweeters N, et al. *Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease.* Br J Haematol. 2000;**111**(2):498-500.
80. Morris CR, Morris SM, Jr., Hagar W, Van Warmerdam J, et al. *Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease?* Am J Respir Crit Care Med. 2003;**168**(1):63-9.
81. Amin HJ, Zamora SA, McMillan DD, Fick GH, et al. *Arginine supplementation prevents necrotizing enterocolitis in the premature infant.* J Pediatr. 2002;**140**(4):425-31.
82. Heyland DK, Novak F, Drover JW, Jain M, et al. *Should immunonutrition become routine in critically ill patients? A systematic review of the evidence.* JAMA. 2001;**286**(8):944-53.
83. Tomlinson C, Rafii M, Ball RO, Pencharz P. *Arginine synthesis from enteral glutamine in healthy adults in the fed state.* Am J Physiol Endocrinol Metab. 2011;**301**(2):E267-73.
84. Tomlinson C, Rafii M, Ball RO, Pencharz PB. *Arginine can be synthesized from enteral proline in healthy adult humans.* J Nutr. 2011;**141**(8):1432-6.
85. Piton G, Manzon C, Cypriani B, Carbonnel F, et al. *Acute intestinal failure in critically ill patients: is plasma citrulline the right marker?* Intensive Care Med. 2011;**37**(6):911-7.
86. Smith HA, Canter JA, Christian KG, Drinkwater DC, et al. *Nitric oxide precursors and congenital heart surgery: a randomized controlled trial of oral citrulline.* J Thorac Cardiovasc Surg. 2006;**132**(1):58-65.

87. Barr FE, Tirona RG, Taylor MB, Rice G, et al. *Pharmacokinetics and safety of intravenously administered citrulline in children undergoing congenital heart surgery: potential therapy for postoperative pulmonary hypertension.* J Thorac Cardiovasc Surg. 2007;**134**(2):319-26.
88. Cynober L, Moinard C, De Bandt JP. *The 2009 ESPEN Sir David Cuthbertson. Citrulline: a new major signaling molecule or just another player in the pharmaconutrition game?* Clin Nutr. 2010;**29**(5):545-51.
89. Meyer R, Harrison S, Sargent S, Ramnarayan P, et al. *The impact of enteral feeding protocols on nutritional support in critically ill children.* J Hum Nutr Diet. 2009;**22**(5):428-36.
90. de Neef M, Geukers VG, Dral A, Lindeboom R, et al. *Nutritional goals, prescription and delivery in a pediatric intensive care unit.* Clin Nutr. 2008;**27**(1):65-71.
91. Tume L, Latten L, Darbyshire A. *An evaluation of enteral feeding practices in critically ill children.* Nurs Crit Care. 2010;**15**(6):291-9.
92. Rogers EJ, Gilbertson HR, Heine RG, Henning R. *Barriers to adequate nutrition in critically ill children.* Nutrition. 2003;**19**(10):865-8.
93. Taylor RM, Preedy VR, Baker AJ, Grimble G. *Nutritional support in critically ill children.* Clin Nutr. 2003;**22**(4):365-9.
94. Mehta NM, McAleer D, Hamilton S, Naples E, et al. *Challenges to optimal enteral nutrition in a multidisciplinary pediatric intensive care unit.* JPEN J Parenter Enteral Nutr. 2010;**34**(1):38-45.
95. Bass J,Takahashi JS. *Circadian integration of metabolism and energetics.* Science. 2010;**330**(6009):1349-54.
96. Chan MC, Spieth PM, Quinn K, Parotto M, et al. *Circadian rhythms: from basic mechanisms to the intensive care unit.* Crit Care Med. 2012;**40**(1):246-53.
97. Paul T, Lemmer B. *Disturbance of circadian rhythms in analgosedated intensive care unit patients with and without craniocerebral injury.* Chronobiol Int. 2007;**24**(1):45-61.
98. Mehta NM, Bechard LJ, Leavitt K,Duggan C. *Cumulative energy imbalance in the pediatric intensive care unit: role of targeted indirect calorimetry.* JPEN J Parenter Enteral Nutr. 2009;**33**(3):336-44.
99. Singer P, Berger MM, Van den Berghe G, Biolo G, et al. *ESPEN Guidelines on Parenteral Nutrition: intensive care.* Clin Nutr. 2009;**28**(4):387-400.
100. Singer P,Pichard C. *Parenteral nutrition is not the false route in ICU.* Clin Nutr. 2012;**31**(2):153-5.
101. Bistrian BR. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1840; author reply 1841-2.
102. Felbinger TW, Weigand MA,Mayer K. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1839; author reply 1841-2.
103. Marik PE. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1840-1; author reply 1841-2.
104. O'Leary MJ,Ferrie S. *Early or late parenteral nutrition in critically ill adults.* N Engl J Med. 2011;**365**(19):1839-40; author reply 1841-2.
105. Van den Berghe G. *Intensive insulin therapy in the ICU-reconciling the evidence.* Nat Rev Endocrinol. 2012;**8**(6):374-8.
106. Derde S, Vanhorebeek I, Guiza F, Derese I, et al. *Early parenteral nutrition evokes a phenotype of autophagy deficiency in liver and skeletal muscle of critically ill rabbits.* Endocrinology. 2012;**153**(5):2267-76.
107. Vanhorebeek I, Gunst J, Derde S, Derese I, et al. *Insufficient activation of autophagy allows cellular damage to accumulate in critically ill patients.* J Clin Endocrinol Metab. 2011;**96**(4):E633-45.
108. Koletzko B, Goulet O, Hunt J, Krohn K, et al. *1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR).* J Pediatr Gastroenterol Nutr. 2005;**41 Suppl 2**:S1-87.
109. Mehta NM,Compher C. *A.S.P.E.N. Clinical Guidelines: nutrition support of the critically ill child.* JPEN J Parenter Enteral Nutr. 2009;**33**(3):260-76.

SUMMARY

Critical illness in children is characterized by a metabolic stress response, resulting in a protein and caloric catabolic state leading to loss of lean body mass. Since loss of lean body mass is associated with increased morbidity and mortality and possibly delays in neurodevelopment, protein catabolism should be limited as much as possible during critical illness in children and protein anabolism should be aimed for. Adequate nutritional support is essential to achieve these goals. However, protein requirements of critically ill children have not been investigated in great detail, except for prematurely born infants. In addition, hyperglycemia results from the metabolic stress response and is associated with increased morbidity and mortality as well. As such, glucose homeostasis is an additional goal to achieve in critically ill children.

In **chapter 1** we describe the metabolic changes during critical illness, the importance and difficulties of nutritional support and the currently available data on protein requirements in critically ill children. In addition, we outline different methods to study protein metabolism. We conclude with the aims of this thesis, which were twofold. First, we studied certain pathophysiological aspects of protein metabolism. Second, interventions were sought to limit protein catabolism in the phase when food intake is not yet established, while aiming for glucose homeostasis. In the phase when food intake is constituted, we aimed to identify interventions to promote protein anabolism as soon as possible.

PATHOPHYSIOLOGICAL ASPECTS

The reference method to study protein requirements is 24-hour stable isotope tracer methodology, which has been used in healthy adult volunteers. However, in children such studies have not been conducted. In critically ill children usually short term stable isotope studies are used, but it is unknown whether these are representative to a 24-hour period. Neither is it known whether a circadian rhythm is present in protein metabolism in critically ill children. In **chapter 2** we investigated 24-hour protein metabolism in relatively stable pediatric ICU patients with stable isotope tracer methodology. We did not find an innate pattern or circadian rhythm, but we found indications of substantial tracer recycling over 24-hours, which was not unexpected, but may influence kinetics data. We therefore recommend not extending stable isotope tracer protocols for more than 10 hours in critically ill children. Intra-individual variation was high, but probably largely inevitable in critically ill children. Average kinetics over the first 6-hours were not significantly different from those over 24-hours, apart from whole body protein synthesis and breakdown as calculated from phenylalanine kinetics, which were lower during the 24-hour period. As this possibly resulted from tracer recycling, the average data over 6-hours are possibly even more representative. Hence, shorter duration protocols of 6-hours may be used as proxy for 24-hour protein metabolism.

Amino acids are needed for protein synthesis, but have specific functions too. An important amino acid during critical illness is arginine. It plays a role in immune function, wound healing, cell regeneration and it's the only precursor of the signaling molecule nitric oxide (NO). In **chapter 3** we focused on pathophysiological changes in arginine metabolism in critically ill children. In accordance with our previous study on plasma amino acid concentrations, arginine kinetics indicated that arginine becomes an essential amino acid during critical illness in children, proportional to the severity of inflammation. We found indications that this results from increased utilization and that this is not matched by increased release from protein breakdown, while *de novo* arginine synthesis lags behind. Furthermore, we found that when a protein-energy enriched infant formula was provided, arginine rate of appearance and NO synthesis were increased independently of plasma arginine concentrations. Thus, a protein-energy enriched formula might be a an effective tool to improve arginine availability for its functions. Also, plasma arginine concentrations in the fed state might not give an adequate reflection of arginine availability.

INTERVENTIONAL CHALLENGES

To achieve glucose homeostasis, it is standard care to provide glucose containing maintenance infusions in critically ill children when nutritional intake is not established yet. However, the optimal amount of glucose intake is not known well. In addition, in post-surgical patients the metabolic stress response induces hyperglycemia, of which the severity is age-dependent, and may require insulin therapy to achieve normoglycemia. The latter carries risk of hypoglycemia. An alternative approach to achieve glucose homeostasis might be to reduce glucose intake in the early post-operative course. Therefore, in **chapter 4 and 5** we studied whether reducing glucose intake in post-surgical populations was effective to prevent hyperglycemia, without causing hypoglycemia and increased protein catabolism. Indeed, we found that hyperglycemia was decreased both after craniofacial and cardiac surgery, without occurrence of hypoglycemia, nor increased protein catabolism. However, in the patients after cardiac surgery we were not able to achieve normoglycemia, possibly due to an extended acute stress response as compared with the patients after craniofacial surgery.

Glucose maintenance infusions alone do not provide sufficient energy and protein intake. Therefore, whenever tolerated, nutritional intake should be promoted early after admission to the pediatric intensive care unit to achieve protein anabolism as soon as possible. The enteral route is the preferred route. Protein requirements of critically ill children are considered to be higher than those of healthy children and high protein intakes should be accompanied by high-energy intakes to prevent usage of protein as energy source. In **chapter 6 and 7** we therefore studied whether a protein-energy enriched formula was safe and more effective to achieve protein anabolism than a standard infant formula in a randomized blinded setting in

critically ill infants with viral bronchiolitis. Indeed, in chapter 6 we found that the formula was safe, well tolerated, and resulted in improved energy- and protein balances. In chapter 7 we found, using stable isotope tracer methodology, that both protein breakdown and protein synthesis increased with the protein-energy enriched formula on day 5 after admission. As protein synthesis increased more than protein breakdown, protein anabolism was achieved with the protein-energy enriched formula, whereas the protein balance was approximately zero with the standard infant formula. Hence, we recommend the use of a protein-energy enriched formula in critically ill infants with viral bronchiolitis.

The ultimate goal of nutritional support in critically ill children is to improve nutritional status (or prevent deterioration) and improve clinical outcome. In the Netherlands quality of care is evaluated with performance indicators. One of these assesses achievement of protein and energy intake goals at day 4 after admission in malnourished children during hospital admission. In **chapter 8** we studied which proportion of critically ill children achieved these goals and whether achievement was associated with improved clinical outcome. Only half of the patients that had a minimal length-of-stay of 4 days at the pediatric intensive care unit, achieved both protein and energy intake goals. The prevalence of malnutrition at admission was high (19%). Parenteral nutrition or combined parenteral and enteral nutrition was more effective to achieve goals in neonates than enteral nutrition alone (70%, vs. 69% vs. 23% of patients achieved goals respectively), but in older children the route of nutrition was not a determinant to achieve goals. Furthermore, malnourished patients achieved goals more often than non-malnourished children (73% vs. 48% of patients achieved goals respectively). We did not find an effect of achieving goals, the route of nutrition or malnourished status on length-of-stay at the pediatric intensive care, days on ventilator, number of new infections and days on antibiotics. Possibly, supplemental parenteral nutrition is an effective tool to achieve intake goals in a higher proportion in critically ill children and to improve clinical outcome, but the safety and efficacy should be studied.

MAIN CONCLUSIONS

In **chapter 9** we discuss the results of our studies in context of the available literature and the implications for clinical practice. Furthermore, we make recommendations for nutritional support and to achieve protein anabolism in critically ill children as soon as possible and make suggestions for future research. The main conclusions of this thesis are the following:

- Protein metabolism in relatively stable continuously fed critically ill children does not show an innate pattern or circadian rhythm.
- Short-duration stable isotope tracer protocols of 6-hours are acceptably representative to 24-hour studies.

- Arginine becomes an essential amino acid in critically ill children proportional to the severity of inflammation, irrespective of plasma arginine concentrations.
- Arginine appearance and NO synthesis can be improved with a protein-energy enriched infant formula.
- Plasma arginine concentrations in the fed state may not reflect arginine availability for its functions accurately.
- Reduced glucose intake in the early post-operative phase in infants after craniofacial surgery is safe and effective to achieve normoglycemia and does not increase protein catabolism.
- Reduced glucose intake in the early post-operative phase in infants and young children after cardiothoracic surgery is safe and effective to reduce hyperglycemia and does not increase protein catabolism, although normoglycemia could not be achieved.
- Early enteral intake of a protein-energy enriched formula is safe, well tolerated and improves protein- and energy balances as compared to a standard infant formula in critically ill infants with viral bronchiolitis.
- Intake of a protein-energy enriched formula results in increased protein synthesis, which exceeds the concomitantly increased protein breakdown and thus results in protein anabolism early after admission in critically ill infants with viral bronchiolitis, whereas with a standard infant formula this was not achieved.
- Only half of patients with a minimal length of stay of 4 days at the pedatric intensive care unit achieved both protein and energy goals, although this was not associated with improved clinical outcome.
- The route of nutrition or a malnourished status was not associated with changes in clinical outcome in our study population.

SAMENVATTING

In dit proefschrift beschrijven we veranderingen in de eiwitstofwisseling tijdens kritische ziekte bij kinderen. Kritische ziekte is een levensbedreigende toestand, waarbij over het algemeen opname op een intensive care nodig is, bijvoorbeeld voor beademing aan een beademingsmachine. Het treedt meestal op als gevolg van infectie, bloedvergiftiging (*sepsis*), brandwonden, na een grote operatie of een groot ongeval. Kritische ziekte bij kinderen kenmerkt zich door een stress respons met veranderingen in de lichaamsstofwisseling (*metabolisme*), welke gepaard gaat met netto afbraak van lichaamseiwit, oftewel *eiwitcatabolisme*. Dit leidt vervolgens tot verlies van lichaamsgewicht, wat geassocieerd is met verhoogde ziekte- en sterftecijfers (*morbiditeit en mortaliteit*). Mogelijk geeft het ook aanleiding tot vertragingen in groei en hersenontwikkeling bij kinderen. Het is daarom belangrijk om eiwitcatabolisme zoveel mogelijk te beperken tijdens kritische ziekte bij kinderen en te streven naar netto opbouw van lichaamseiwit, oftewel *eiwitanabolisme*. Adequate voedingsondersteuning is van essentieel belang om deze doelen te bereiken. Echter, het is, behoudens bij te vroeg geboren kinderen, onvoldoende onderzocht wat de precieze eiwitbehoeften van kritisch zieke kinderen zijn. Een ander fenomeen waarmee de metabole stress respons gepaard gaat, is te hoge bloedsuikerspiegels, oftewel *hyperglycemie*. Aangezien hyperglycemie bij kritisch zieke kinderen ook is gerelateerd aan verhoogde ziekte- en sterftecijfers, is het bereiken van een stabiele, evenwichtige toestand in bloedsuikerspiegels (*glucosehomeostase*) een belangrijk aanvullend doel in deze patiëntengroep.

In **hoofdstuk 1** beschrijven we de metabole veranderingen die optreden tijdens kritische ziekte, het belang en de moeilijkheden van voedingsondersteuning tijdens kritische ziekte en geven we een overzicht van de tot nu toe beschikbare data over eiwitbehoeften van kritisch zieke kinderen. Daarnaast bespreken we verschillende methodes om eiwitmetabolisme te onderzoeken. We sluiten af met het doeleinde van dit proefschrift, welke tweeledig is. Ten eerste hebben we in dit proefschrift enkele *pathofysiologische aspecten* (verschijnselen die onder omstandigheden van ziekte optreden) van eiwitmetabolisme tijdens kritische ziekte bij kinderen onderzocht. Ten tweede zochten we naar interventies om enerzijds eiwitcatabolisme te beperken en anderzijds glucosehomeostase te bereiken zolang voedingsinname nog niet mogelijk is. In de fase waarin voedingsinname wel mogelijk is, zochten we naar interventies om eiwitanabolisme zo snel mogelijk te bereiken.

PATHOFYSIOLOGISCHE ASPECTEN

De referentiemethode om eiwitbehoeften te bestuderen is 24-uurs stabiele isotopen tracer methodologie. *Stabiele isotopen tracers* zijn als het ware labels waarmee bepaalde stoffen in de stofwisseling getraceerd kunnen worden, om zo de snelheid van verschillende omzettingen van

stoffen in de stofwisseling te kunnen meten. De snelheid van omzettingen wordt ook wel *kinetiek* genoemd. Op deze manier kan eiwitkinetiek gemeten worden; de snelheden waarmee lichaamseiwit wordt opgebouwd en afgebroken. Deze methode is bij gezonde volwassen vrijwilligers toegepast, echter bij kinderen zijn dergelijke studies niet uitgevoerd. Bij kritisch zieke kinderen worden meestal kortdurende stabiele isotopen studies gebruikt, maar het is niet bekend of deze ook daadwerkelijk representatief zijn voor een 24-uurs periode. Het is ook niet bekend of er een *circadiaan ritme* (een dag-nacht ritme) aanwezig is in het eiwitmetabolisme van kritisch zieke kinderen. In **hoofdstuk 2** hebben we het 24-uurs eitwitmetabolisme van relatief stabiele patiënten op de kinder intensive care bestudeerd, door middel van stabiele isotopen tracer methodologie. We vonden geen circadiaan ritme. Wel waren er, alhoewel niet onverwacht, aanwijzingen voor substantiële tracer recycling gedurende 24-uur, wat mogelijk de kinetiekresultaten beïnvloedt. *Tracer recycling* betekent dat de hiervoor genoemde stoffen met labels in het lichaam gerecycled werden, waardoor de concentraties foutief hoger waren dan in werkelijkheid kan worden verwacht. We raden daarom aan om stabiele isotopen tracerprotocollen niet langer dan 10 uur achtereen te laten duren in studies bij kritisch zieke kinderen. De varatie in kinetiekdata binnen de individuen was hoog, maar waarschijnlijk grotendeels onvermijdelijk in kritisch zieke kinderen, door de verschillende interventies die ze ondergaan als onderdeel van intensieve zorg. Gemiddelde kinietiekwaarden over der eerste 6-uur waren niet significant verschillend van de waarden over de gehele 24-uurs periode, behalve voor lichaamseiwitopbouw en –afbraak. De significante verschillen voor de laatste twee variabelen waren mogelijk het gevolg van tracer recycling. De waarden over de eerste 6-uur zijn daarom waarschijnlijk het meest representatief. Daarom kunnen 6-uur durende protocollen gebruikt worden als benadering voor 24-uurs eiwitmetabolisme.

Aminozuren, de bouwstenen van eiwitten, zijn noodzakelijk voor eiwitopbouw, maar hebben ook specifieke individuele functies. Een belangrijk aminozuur tijdens kritische ziekte is arginine. Arginine heeft een rol in het afweersysteem, de wondgenezing, celvernieuwing en is bovendien de enige voorloper van het signaalmolecuul *stikstofoxide* (NO). In **hoofdstuk 3** richtten we ons op pathofysiologische veranderingen in het argininemetabolisme van kritisch zieke kinderen. In overeenstemming met onze eerdere studie naar aminozuurconcentraties in het bloed (*plasma aminozuurconcentraties*) suggereerden de resultaten van de argininekinetiek dat arginine een essentieel aminozuur wordt tijdens kritische ziekte in kinderen, evenredig aan de ernst van de ontsteking in het lichaam. Een essentieel aminozuur is een aminozuur dat onvoldoende in het lichaam wordt aangemaakt en daarom via de voeding moet worden ingenomen om te voorkomen dat er een gebrek aan het aminozuur optreedt. Aangezien verschillende oorzaken van kritische ziekte bij kinderen gepaard gaan met een verschillende ernst van ontsteking, zou dit betekenen dat de behoefte aan arginine verschilt per onderliggend ziektebeeld aan de hand van de ernst van ontsteking. We vonden aanwijzingen dat dit het gevolg is van verhoogd verbruik van arginine, met toenemende ernst van

ontsteking, welke niet wordt opgevangen door een toename in aanmaak van arginine. Dit laatste kwam doordat de afgifte van arginine uit eiwitafbraak gelijkbleef en de nieuwvorming van arginine (*de novo* arginine synthese) achterbleef met toenemende ernst van ontsteking. Verder vonden we dat als een eiwit-energie verrijkte zuigelingenvoeding wordt gegeven, de snelheid waarmee arginine in het bloed verschijnt (*rate of appearance*) en NO synthese significant stegen ten opzichte van een standaardvoeding. Dit gebeurde onafhankelijk van de hoogte van argininеconcentraties in het bloed. Zodoende zou een eiwit-energie verrijkte voeding een effectief middel kunnen zijn om de beschikbaarheid van arginine te verbeteren. Verder lijken argininеconcentraties in het bloed in de gevoede toestand geen adequate indicatie te geven van de arginine beschikbaarheid.

ZOEKTOCHT NAAR GESCHIKTE INTERVENTIES

Om glucosehomeostase te bereiken, worden standaard glucosebevattende onderhoudsinfusen gegeven aan kritisch zieke kinderen, zolang voedingsinname nog niet mogelijk is in de acute fase van de ziekte. De optimale hoeveelheid glucose is echter niet goed bekend. Bovendien veroorzaakt de metabole stress respons hyperglycemieën bij patiënten na operatie (*post-operatief*). De ernst hiervan is leeftijdsafhankelijk. Vervolgens kan insulinetherapie genoodzaakt zijn om normale bloedsuikerspiegels (*normoglycemie*) te bereiken, wat het risico op te lage bloedsuikerspiegels (*hypoglycemieën*) met zich mee brengt. Een alternatieve aanpak zou kunnen zijn om de glucose-inname in de vroege post-operatieve fase te verlagen. In **hoofdstuk 4 en 5** hebben we daarom bestudeerd of het verminderen van de glucose-inname in post-operatieve patiënten effectief is om hyperglycemieën te voorkomen, zonder hiermee hypoglycemieën en verhoogd eiwitcatabolisme te bewerkstelligen. We vonden inderdaad dat hyperglycemieën afnamen zowel na *craniofaciale* (schedel- en aangezicht) operaties als na hartoperaties, zonder dat hypoglycemie optrad of eiwitcatabolisme werd verhoogd. Echter, in de patiënten na hartoperatie kon normoglycemie niet worden bereikt met deze aanpak, mogelijk door een ernstigere stress respons in vergelijking met de patiënten na craniofaciale operaties.

Glucosebevattende onderhoudsinfusen voorzien onvoldoende in energie- en eiwitinname. Daarom moet voedingsinname, wanneer dit verdragen wordt, vroeg na opname op de kinder intensive care gestimuleerd worden, zodat eiwitanabolisme zo snel mogelijk kan worden bereikt. Voeding via het maagdarmstelsel (*enterale route*) heeft de voorkeur. Eiwitbehoeften van kritisch zieke kinderen worden geacht hoger te zijn dan die van gezonde kinderen. Hoge eiwitinnames dienen daarnaast gepaard te gaan met hoge energie-innames om te voorkomen dat eiwit als energiebron wordt gebruikt. In **hoofdstuk 6 en 7** hebben we daarom in een gerandomiseerde dubbel-blind gecontroleerde studie bij kinderen met een ernstige luchtweginfectie op basis van een virus (*virale bronchiolitis*) onderzocht of een eiwit-energie verreikte voeding

veilig was en effectiever om eiwitanabolisme te bereiken dan een standaard zuigelingenvoeding. Inderdaad vonden we in **hoofdstuk 6** dat de eiwit-energie verrijkte voeding veilig was, goed verdragen werd en leidde tot verhoogde energie- en eiwitbalansen. In **hoofdstuk 7** zagen we, door middel van stabiele isotopen tracer methodology, dat zowel lichaamseiwitafbraak als -opbouw verhoogd waren in de groep die de eiwit-energie verrijkte voeding kreeg op dag 5 na opname. Omdat lichaamseiwitopbouw meer was toegenomen dan -afbraak, werd netto eiwitopbouw bereikt, dus eiwitanabolisme, met de eiwit-energie verrijkte voeding. Met de standaardvoeding was de eiwitbalans daarentegen nul (geen netto opbouw en geen netto afbraak). Daarom bevelen we het gebruik van een eiwit-energie verrijkte voedingen aan in kritisch zieke kinderen met virale bronchiolitis.

Het uiteindelijke doel van voedingsondersteuning bij kritisch zieke kinderen is het verbeteren van de voedingsstatus (of het voorkomen van verslechtering hiervan) en de klinische uitkomst te verbeteren. In Nederland wordt de kwaliteit van zorg geëvalueerd door middel van kwaliteitsindicatoren. Eén van deze inventariseert welk deel van de ondervoede kinderen tijdens ziekenhuisopname eiwit- en energiedoelen op dag 4 na opname behaalt. In **hoofdstuk 8** onderzochten we welk deel van kritisch zieke kinderen deze doelen behaalde op dag 4 na opname en of het behalen van de doelen geassocieerd is met verbeterde klinische uitkomst. Slechts de helft van de patiënten die een minimale opnameduur van 4 dagen had, behaalde zowel eiwit- als energiedoelen. Het voorkomen van ondervoeding was hoog (19%). In neonaten waren voeding via het infuus (*parenterale voeding*) en de combinatie van parenterale voeding met enterale voeding effectiever om de doelen te behalen dan enterale voeding alleen (respectievelijk 70% vs. 69% vs. 23% van de patiënten behaalde doelen), maar in oudere kinderen was de voedingsroute niet bepalend voor het behalen van doelen. Verder behaalden ondervoede patiënten de doelen vaker dan niet-ondervoede patiënten (respectievelijk 73% vs. 48% van de patiënten behaalde doelen). Noch het behalen van de doelen, noch de voedingsroute, noch een ondervoede status leek effect te hebben op de opnameduur op de kinder intensive care, het aantal dagen aan de beademing, het aantal nieuwe infecties en het aantal dagen antibiotische behandeling. Mogelijk is het toepassen van aanvullende parenterale voeding naast enterale voeding een effectief middel om voedingsdoelen in een groter aandeel van patiënten te behalen en om klinische uitkomst te verbeteren, maar de veiligheid en effectiviteit hiervan moet onderzocht worden bij kritisch zieke kinderen.

BELANGRIJKSTE BEVINDINGEN

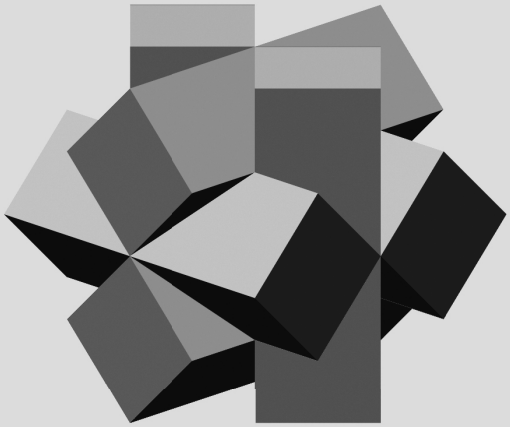
In **hoofdstuk 9** bespreken we de resultaten van de studies in dit proefschrift in de context van de beschikbare literatuur en de implicaties voor de klinische praktijk. Daarnaast geven we aanbevelingen voor voedingsondersteuning en om eiwitanabolisme in kritisch zieke kinderen

zo vroeg mogelijk te bereiken en doen we suggesties voor vervolgonderzoek. De belangrijkste bevindingen in dit proefschrift zijn de volgende:

- Eiwitmetabolisme in relatief stabiele continu gevoede kritisch zieke kinderen laat geen circadiaan ritme zien.
- Kortdurende stabiele isotopen tracer protocollen van 6-uur zijn voldoende representatief voor een 24-uurs periode.
- Arginine wordt evenredig naar de ernst van ontsteking een essentieel aminozuur in kritisch zieke kinderen.
- De snelheid van verschijnen van arginine in het bloed en NO synthese kunnen verhoogd worden met een eiwit-energie verrijkte zuigelingenvoeding, onafhankelijk van de plasma arginine concentraties
- Plasma arginine concentraties in de gevoede staat geven mogelijk geen accurate indicatie van de argininebeschikbaarheid voor de functies van arginine.
- Verminderde glucose-inname in de vroege post-operatieve fase na craniofaciale operaties bij zuigelingen is veilig en effectief om normoglycemie te bereiken, zonder hierbij eiwitcatabolisme te verhogen.
- Verminderde glucose-inname in de vroege post-operatieve fase na hartoperaties bij zuigelingen en jonge kinderen is veilig en effectief om hyperglycemieën te verlagen, zonder hierbij eiwitcatabolisme te verhogen, alhoewel normoglycemie niet kan worden bereikt.
- Vroege enterale eiwit-energie verrijkte voeding is veilig, wordt goed getolereerd en verhoogt eiwit- en energiebalansen in vergelijking met een standaard zuigelingenvoeding in kritisch zieke zuigelingen met virale bronchiolitis.
- Inname van een eiwit-energie verrijkte voeding resulteert in verhoogde eiwitsynthese die meer toeneemt dan de tegelijkertijd verhoogde eiwitafbraak, waardoor eiwitanabolisme vroeg na opname kan worden bereikt in kritisch zieke zuigelingen met virale bronchiolitis. Met een standaard zuigelingenvoeding kon dit niet worden bereikt.
- Slechts de helft van de patiënten met een minimale opnameduur van 4 dagen op de kinder intensive care behaalt zowel eiwit- als energiedoelen, alhoewel het behalen van doelen niet geassocieerd was met verbeterde klinische uitkomst.
- Noch de voedingsroute, noch een ondervoede status was geassocieerd met veranderingen in klinische uitkomst in onze populatie.

PART 6

APPENDICES



Twenty years from now you will be more disappointed by
the things that you didn't do than by the ones you did do.
So throw off the bowlines. Sail away from the safe harbor.
Catch the trade winds in your sails.
Explore. Dream. Discover.

Mark Twain (1835-1910)

CURRICULUM VITAE

Carlijn de Betue was born on the 27th of March 1984 in Leidschendam. She grew up in Zoetermeer, where she completed high school (Gymnasium, cum laude) in 2002 at the Erasmus College Zoetermeer. The same year she moved to Maastricht to study Medicine at the Faculty of Health Medicine and Life Sciences of Maastricht University. After finishing the first three years of the study, she was looking for an additional challenge and became interested in medical research. Therefore, she paused her curriculum of Medicine for one year to do an elective research internship before starting clinical rotations (co-schappen). During this year she was involved in projects on protein and amino acid metabolism in the Pediatric Intensive Care Unit, Department of Pediatrics in conjunction with the Metabolic Research Center, Department of Surgery of Maastricht University Medical Center (supervisor Dr. van Waardenburg and Prof. Dr. Deutz). In 2006 she resumed her studies of Medicine, while continuing to be involved in research activities; among others a four-month research internship at the University of Arkansas for Medical Sciences and the Pediatric and Cardiovascular Intensive Care Unit of Arkansas Children's Hospital, Little Rock, Arkansas, USA (supervisor Prof. Dr. Deutz). As part of the clinical rotations she did elective clinical internships in pediatric surgery at Academic Medical Center Amsterdam and tropical medicine/ primary health care at the rural district's hospital West Gonja Hospital, Damongo, Ghana. She obtained her medical degree in 2009 and as of January 2010 she started working in Rotterdam on her dissertation in the combined function of PhD-student at the Intensive Care (promotor Prof. Dr. Tibboel, promotor Prof. Dr. Deutz, co-promotor Dr. Joosten, co-promotor Dr. van Waardenburg) and resident (ANIOS) at the Department of Pediatric Surgery (head Prof. Dr. Wijnen) of Erasmus MC-Sophia Children's Hospital. During this period she returned to Little Rock, Arkansas, USA for three months to complete the research study that she had previously set up.

Carlijn likes playing piano, running and travelling. She aspires a career in General Surgery.

LIST OF PUBLICATIONS

de Betue CT, Garcia Casal XC, van Waardenburg DA, Schexnayder SS, Joosten KF, Deutz NE, Engelen MP. *24-Hour protein, arginine and citrulline metabolism in fed critically ill children does not show a circadian rhythm – a stable isotope tracer study*. Submitted.

de Betue CT, van Steenselen WN, Hulst JM, Olieman JF, Augustus M, Mohd Din SH, Verbruggen SC, Tibboel D, Joosten KF. *Achieving nutritional goals at day 4 after admission in critically ill children; predictive for outcome?* Submitted.

de Betue CT, Joosten KF, Deutz NE, Vreugdenhil AC, van Waardenburg DA. *Arginine and NO production in critically ill children can be increased with a protein-energy enriched enteral formula*. Submitted.

de Betue CT, Verbruggen SC, Schierbeek H, Chacko SK, Bogers AJ, van Goudoever JB, Joosten KF. *Does a reduced glucose intake prevent hyperglycemia in children early after cardiac surgery? – A randomized controlled crossover study.* Provisionally accepted Crit Care.

de Betue CT, Boersma D, Oomen MW, Benninga MA, de Jong JR. *Volvulus as a complication of chronic intestinal pseudo-obstruction syndrome.* Eur J Pediatr, 2011. **170**(12):1591-5.

Verbruggen SC, **de Betue CT**, Schierbeek H, Chacko S, van Adrichem LN, Verhoeven J, van Goudoever JB, Joosten KF. *Reducing glucose infusion safely prevents hyperglycemia in post-surgical children.* Clin Nutr, 2011. **30**(6):786-92.

de Betue CT, van Waardenburg DA, Deutz NE, van Eijk HM, van Goudoever JB, Luiking YC, Zimmermann LJ, Joosten KF. *Increased protein-energy intake promotes anabolism in critically ill infants with viral bronchiolitis: a double-blind randomised controlled trial.* Arch Dis Child, 2011. **96**(9):817-22.

van Waardenburg DA, **de Betue CT**, Goudoever JB, Zimmermann LJ, Joosten KF. *Critically ill infants benefit from early administration of protein and energy-enriched formula: a randomized controlled trial.* Clin Nutr, 2009. **28**(3): p. 249-55.

van Waardenburg DA, **de Betue CT**, Luiking YC, Engel M, Deutz NE. *Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation.* Am J Clin Nutr, 2007. **86**(5):1438-44.

AWARDS

ESPEN 2011 Travel Fellowship, 33rd European Society of Clinical Nutrition and Metabolism (ESPEN) Congress, September 2011, Gothenborg, Sweden.

Young investigator Award, European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), 44th Annual Meeting of ESPGHAN, May 2011, Sorrento, Italy.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Carlijn T. I. de Betue
Erasmus MC Department: Intensive Care and Pediatric Surgery, Sophia Children's Hospital

PhD period: January 2010 - September 2012
Promotors: Prof. Dr. D. Tibboel. Prof. Dr. N.E.P. Deutz
Supervisors: Dr. K.F.M. Joosten, Dr. D.A. van Waardenburg

	Year	Workload (ECTS)
1. PhD training		
General courses		
- Collaborative Institutional Training Initiative, Human research curriculum, course on Biomedical Research, USA	2010	0.5
- Principles of Research in Medicine, NIHES, Erasmus MC	2010	0.5
- Introduction to data-analysis, NIHES, Erasmus MC	2010	1.2
- Basic Introduction course on SPSS – Molmed, Erasmus MC	2010	0.6
- Biomedical English Writing – Molmed, Erasmus MC	2011	1
- Literature search basic and advanced course and Endnote course, Medical Library, Erasmus MC	2011	0.2
- BROK course, Erasmus MC	2011	1
- Basic Acute Care (ABCDE method, BLS, AED, PBLS), Erasmus MC	2011	0.3
- Basic Surgical Skills, Royal College of Surgeons of Edinburgh, Edinburgh, Scotland	2011	0.5
- Course "Opvang van het Acuut zieke kind", post-academic education Sophia, Erasmus MC-Sophia Children's Hospital	2011	0.3
- Course "Treatment of burns", Rode Kruis Ziekenhuis, Beverwijk	2012	0.3
- Workshop on Photoshop, Illustrator and Indesign CS – Molmed, Erasmus MC	2012	0.4
Specific courses		
- ESPEN Leonardo da Vinci Life Long Learning (LLL) Courses: "Nutrition in paediatric patients" and "Nutritional support of ICU patients"	2011	0.3
Seminars and workshops		
- Workshops at Erasmus MC PhD day	2010	0.1
- Symposium "Current issues in nutritional support for critically ill neonates and children", Erasmus MC-Sophia	2011	0.3
- Symposium "Pre-conference meeting on nutrition", Sorrento, Italy	2011	0.3
- The perfect leadership – Postdoc network Erasmus MC	2011	0.1
Presentations		
- Presentation in research meeting, UAMS, Little Rock, USA	2010	1
- Two poster presentations ISICEM congress	2011	1
- Oral presentation ESPGHAN congress	2011	1
- Presentation in Pharmacology research meeting, Erasmus MC -Sophia Children's Hospital	2011	1
- Oral presentation ESPEN congress 2011	2011	1
- Oral poster presentation ESPNIC congress 2011	2011	1

	Year	Workload (ECTS)
International conferences		
- 24th North American CF Conference, 20-23 October 2010, Baltimore, MD, USA	2010	1
- 31st International Symposium on Intensive Care and Emergency Medicine, 22-25 March 2011, Brussels, Belgium	2011	1
- 44th Annual Meeting of European Society for Paediatric Gastroentrology Hepatology and Nutrition, 25-28 May, Sorrento, Italy	2011	1
- 33rd Congress Clinical Nutrition and Metabolism, European Society of Parenteral and Enteral Nutrition, Gothenborg, Sweden	2011	1
- 22nd Annual Congress European Society of Pediatric and Neonatal Intensive Care, Hannover, Germany	2011	1
- Motility meeting, Department of Pediatric Gastroenterology, AMC, Amsterdam, the Netherlands	2011	1
- Colorectal course, Department of Pediatric Surgery, Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands	2012	0.5
Other		
- Various research meetings at University of Arkansas for Medical Sciences, Little Rock, USA and Erasmus MC-Sophia Children's Hospital, Rotterdam	2010-2012	2
2. Teaching		
Lecturing		
- Educational lectures for nurse practitioners and residents, Department of Pediatric Surgery, Erasmus MC - Sophia Children's Hospital	2010-2012	1
Supervising practicals and excursions, Tutoring		
- Tutoring/ supervising nurse practitioners in training, Department of Pediatric Surgery, Erasmus MC - Sophia Children's Hospital	2010-2012	3
Supervising Master's theses		
- Supervising research internship of medical student W.N. van Steenselen	2011	4
- Supervising research internship of medical student B.C. Oosterloo	2012	3

DANKWOORD

Het is eindelijk zover, het proefschrift is klaar, de puzzel is af! Ik kijk terug op mooie, leerzame, waardevolle jaren, waarin ik veel bijzondere mensen heb ontmoet. Iedereen die mij de afgelopen jaren geholpen heeft, wil ik hartelijk bedanken en in het bijzonder de volgende mensen.

Allereerst wil ik de kinderen en ouders die hebben meegedaan in één van de studies hartelijk bedanken voor hun deelname. Ik heb mij vaak verwonderd over de bereidheid van ouders om in zeer moeilijke tijden open te staan voor onderzoek. Zonder hen had dit proefschrift niet tot stand kunnen komen.

Tijdens mijn tocht langs de Maas naar het noorden heb ik het uitzonderlijke aantal van vier supervisors mogen genieten. Ik ben blij dat jullie alle vier in de functie van promotor of co-promotor bij mijn promotie aanwezig zijn.

Prof. Dr. Tibboel, beste Professor, u bood mij de kans om de onderzoeksprojecten die al liepen tot een promotie te voltooien. Uw andere invalshoek en kritische blik hebben mijn stukken zeker verbeterd en waren soms een eye-opener waar ik veel van heb geleerd. Ik heb waardering voor de manier waarop u uw promovendi-bataljon door de strijd helpt en dat u precies op de juiste momenten mentale support weet te bieden.

Prof. Dr. Deutz, beste Mick. Je was er vanaf het eerste moment bij: in Maastricht, later in Little Rock en ook in Rotterdam bleef je betrokken bij mijn onderzoek. Hartelijk dank voor je eeuwige bereidheid en beschikbaarheid voor overleg, je steun, de aansporing om onderzoek te (blijven) doen en de tijd in Little Rock. Ik heb veel van je geleerd en ik hoop dat onze samenwerking nog lang voort zal duren.

Dr. Joosten, beste Koen. Jij had het vertrouwen om de samenwerking die we hadden in Maastricht, uit te bouwen tot een promotie en wierp je als directe begeleider op. Dank daarvoor! Van jouw praktische instelling heb ik een hoop kunnen leren en je stond altijd klaar om me aan te sporen, af te spreken voor overleg en stukken te bekijken; dat alles tussen je vele reizen door. Ik heb bewondering voor hoe je dit combineert. Ik hoop dat we nog lang kunnen samenwerken.

Dr. van Waardenburg, beste Dick. Wat mooi is het, dat jouw promotie tot de mijne heeft geleid en dat je nu hier mijn co-promotor kan zijn! Met veel plezier kijk ik terug op onze samenwerking vanaf het allereerste moment, de studies op de PICU, de brainstormsessies over de bevindingen en de congresbezoeken. Jouw kritische blik met oog voor detail heeft me tot verder nadenken aangezet tijdens het schrijven van de stukken. Bedankt voor je vertrouwen al die jaren. Hopelijk kunnen we onze samenwerking nog lang blijven voortzetten.

De leden van de kleine commissie, Prof. Dr. Groeneveld, Prof. Dr. van Goudoever en Prof. Dr. Van den Berghe wil ik hartelijk danken voor het (snelle) beoordelen van het manuscript.

Prof. Dr. van Goudoever, beste Hans, bedankt voor het te gast zijn in je onderzoeksgroep. De inhoudelijke discussies waren een waardevolle aanvulling op mijn promotietijd. Hartelijke dank ook, dat ik met jullie mocht verblijven in Schiazzano en kon deelnemen aan de meeting voor het congres in Sorrento; ik kijk er met veel plezier op terug!

Prof. Dr. Wijnen en Prof. Dr. Bakker wil ik bedanken voor het zitting nemen in de grote commissie. Prof. Dr. Wijnen, beste René, hartelijk dank voor de morele steun en het vertrouwen in mijn werk als arts. Ik vind het een mooie afsluiting dat je, na het zijn van mijn klinische supervisor, nu in de grote commissie zitting neemt.

De kinder-intensivisten, fellows en verpleegkundigen van de PICU in Maastricht en de Intensive Care Kinderen (in het bijzonder ICK2) in Rotterdam wil ik bedanken voor de ondersteuning bij het uitvoeren van mijn studies, evenals de anesthesisten van het Thoraxcentrum Rotterdam. In het bijzonder, bedankt Dr. Gijs Vos. Jij bent de aanstichter van dit alles. Dankzij de mentorgesprekken realiseerde ik me dat ik onderzoek wilde gaan doen en zonder je hulp bij het zoeken naar een passende plek, stond ik nu niet hier.

Prof. Dr. Bogers, hartelijk dank voor de prettige samenwerking in de glucosestudie. Jessie Hulst, Joanne Olieman, Marjolein Augustus, Anita Vreugdenhil en Marlou Adriaanse ook jullie bedankt voor de samenwerking; Jessie, speciaal bedankt voor de uurtjes achter SPSS.

Dr. Schexnayder, thanks for giving me the opportunity to conduct my study at the PICU of Arkansas Children's Hospital. Dr. Garcia, dear Xiomara, thank you for working on the 24-hour study together; your enthousiasm is really inspiring and your support during the studies wonderful. Also I would like to thank Brandy Fuller, all PICU and CVICU medical and nursing staff of Arkansas Children's Hospital, Little Rock, AR, USA for helping me to conduct my studies and making the 24-hours a little bit shorter. Thanks to Scott Schutzler and pharmacist Billy Furgerson. Glenda and Amy, thanks for support and for laughing with this crazy Dutchie. Dr. Gülnür Com, thanks for the pleasant time at the conference in Baltimore.

Annemarie, bedankt dat je naast je werk voor Prof. Tibboel ook een beetje secretaresse van de promovendi bent en altijd klaar staat voor ondersteuning.

Mijn onderzoekscollega's van de drie isotopenlabs in Maastricht, Little Rock en Rotterdam wil ik bedanken voor de leerzame tijd en de ondersteuning. Mariëlle bedankt voor je gastvrijheid, je betrokkenheid en vooral voor het mogelijk maken van de 24-uursstudie. Gabrie, bedankt voor je hulp op het lab en bij het uitvoeren van de studies, maar bovenal voor de gastvrijheid waarmee André en jij me in Little Rock ontvingen. Ik hoop dat jullie en jullie beide gezinnen een mooie en interessante tijd in College Station tegemoet gaan. Yvette Luiking, hartelijk bedankt voor de begeleiding in de eerste isotopen-uurtjes.

Hans van Eijk, Roland Meesters, Henk Schierbeek, Gardi Minderman-Voortman en Kristien Dorst-Lagerwerf, bedankt voor de labbepalingen en bereidheid tot hulp. Also, John

Thaden and Joshua Spore in Little Rock and Shaji Chacko in Houston, thanks for sample analysis.

Ko Hagoort, bedankt voor het editen van de manuscripten. Dr. Wim Hop en Siti Haslinda Mohd Din, bedankt voor de statistische hulp. Marianne Maliepaard, bedankt voor je ondersteunende rol bij het uitvoeren van de studies. Annette van Hoek-Tummers, bedankt voor je hulp met PICE.

Willeke van Steenselen en Neelke Oosterloo, hartelijk dank voor jullie hulp in de verschillende projecten en met name in het uitzoeken van data. Veel succes met het afronden van jullie Geneeskunde-studie en verdere loopbaan!

Thanks to all other people who made my stay in Little Rock a great one! Anna, Maaike, Ashley, Wassim, Ruud, Renate, Nikkie, Fari, Arianna, Veronica are only just some of the amazing people I met. Thanks to you and all other co-workers of the lab, students of the dorm, LR climbers and other friends, for the great dinners, pianobar evenings, get-togethers and trips. Julia and Tony, thanks for introducing me to the real rural Arkansas' life and for organizing great parties at your ranch! Prof. Dr. Griffin, Steve, Pam and other co-workers, thanks for keeping our brain working during lunch, invitations to parties and other after-work activities.

Alle kinderchirurgen, bedankt voor de leerzame en leuke tijd in de kliniek. Alhoewel onderzoek en kliniek niet altijd optimaal te combineren waren, was het werk op 1 zuid een verrijking van mijn tijd in het Erasmus MC-Sophia. Ik heb het met veel plezier gedaan, mede door de luchtige omgang met elkaar; ik heb bewondering voor jullie harde werken en betrokkenheid bij de patiënten. Conny, bedankt voor de gezelligheid na het werk, hopelijk drinken we nog vaker eens een koffie (of een drankje)! Lieve collega-arts-assistenten en nurse practitioners, bedankt voor de samenwerking en het bereid zijn diensten over te nemen als de nood hoog was. Veel succes in jullie verdere loopbaan! Ook de verpleegkundigen van 1 zuid wil ik bedanken voor de fijne tijd en goede samenwerking. Het weekendje Winterberg heeft daar zeker aan bijgedragen. Marja, Marie-Louise, Anita en Margaret, ook jullie bedankt voor het altijd klaar staan om te helpen en een vrolijke noot op de afdeling!

Lieve collega-promovendi/onderzoekers, sommigen al klaar, anderen nog hard op weg naar "de grote dag", bedankt voor de mooie tijd: Sascha, Sara, Sanne, Marie-Chantal, Alexandra, Nienke, Bram, Miriam, Erik, Hendt, Gerbrich, Joke, Anneke, Marlous, Violette, Ilse, Lieke, Ibrahim, Ilona, Danielle, Anniek, Margriet, Hester, Denise, Patrycja, Willemijn, Femke, Lisha, Jacomine en anderen van de groep van Prof. Tibboel en Prof. van Goudoever.

Sascha, bedankt voor de fijne samenwerking aan de projecten. Jouw enthousiasme en gedrevenheid zijn bewonderingswaardig en aanstekelijk; heel veel succes met je mooie

nieuwe project! Sara, bedankt voor de leerzame samenwerking op de high care en succes met je eigen promotie. Lieve MC, met jou kan ik altijd lachen. Bedankt voor de leuke tripjes, op de Kronan-fiets, ons (niet alleen) leerzame uitje naar Edinburgh, en de memorabele avonden op 505. Succes met je opleiding en op naar meer mooie tijden! Lieve SK-1324-ers, bedankt voor de gezelligheid, koffiemomentjes en me een beetje adopteren op jullie kamer. Altijd lachen geblazen, vooral als er een filmpje gemaakt moet worden. Alex, bedankt voor je hulp, met jouw gedrevenheid ga je het nog ver schoppen, veel plezier in de VS! Nienke, jij weet als geen ander wat combineren van onderzoek en kliniek betekent, veel succes nog! Miriam, bedankt voor de Maastrichtse noot, heel veel succes in je promotietraject en geniet van Canada. Bram, ik heb bewondering voor je georganiseerdheid en je ambitie, veel plezier nog in Ierland en succes binnenkort met de grote dag! Erik, met jou heb ik altijd lol, vooral ook op wintersport, veel succes met het afronden van je boekje! Hendt, wat extra leven in de brouwerij en altijd in voor een drankje, veel succes met je co-schappen en het voortzetten van je onderzoek. Lieve Westzeedijkers, ondanks mijn onregelmatige aanwezigheid bedankt voor de fijne tijd! Lieve Ger, mijn arts-maatje op WK-208 en onovertroffen scooter-fanaat, ik zal de AD-momenten missen; succes met het afronden van je mooie MRI-project! Lieve Joke, ik ben blij dat we ons enthousiasme over ene Zoutmans konden delen, net als onze opgeruimdheid en dat Einstein daar ooit ook al iets goeds over zei. Zet 'm op, het komt goed! Lieve Marlous, je bent een ontzettende lieverd en van jouw gedrevenheid en discipline kan ik nog veel leren; bedankt ook voor het uitdossen en de leuke tijd op de piste. Lieve Anneke, inmiddels zag ik je vaker op 1zuid dan op de WZD, heel veel succes met je opleiding (waar je geknipt voor bent). Ik heb bewondering voor wat je allemaal al bereikt hebt! Lieve Violette, veel succes met de laatste loodjes en alvast met de co-schappen. Bedankt voor het overnemen van de functie als ICT-helpdesk op WK-208! Lieve Ilse, nog op de valreep jouw opgewektheid mogen meemaken en zelfs even klinische collega geweest, succes in Leiden! Lieve Lieke, zoveel life-events in 1 jaar is bizar, heel knap en geniet van je klein man! Lieve isotopen-collega's, bedankt voor de mooie tijd in Schiazzano en het kunnen sparren over ons onderzoek; jullie ook veel succes met het afronden van jullie boekjes en/of projecten in Rotterdam en Amsterdam!

Lieve vrienden en vriendinnen. Bedankt dat jullie er altijd voor me zijn, voor de mooie momenten samen als welkome afleiding van de promotie en dat jullie me door de moeilijkere momenten hebben heen geholpen. In het bijzonder Jos, Meik, Bella, Isa, Gees, Paul, Maaik, Ingeee; ik hoop dat ik jullie nu weer wat vaker kan gaan zien! Lieve bakkie-(deluxe)-vrienden, het Rotterdamse leven werd een groter feest met ons clubje; bedankt voor de talloze ultra mega afleidingsbakkies en op naar nog meer! Lieve Willem, bedankt voor je steun de afgelopen jaren, de feedback op presentaties en stukken en het opbeuren tijdens promotie-dips. Veel succes met je eigen proefschrift, ik twijfel er niet aan dat het een prachtig boek wordt.

Lieve paranimfen, ik had me geen beter team kunnen wensen, bedankt dat jullie me op deze belangrijke dag willen bijstaan!! Lieve Nic, daar staan we weer zij aan zij. Ik ben ontzettend blij dat we al 10 jaar vriendinnen zijn en dat ik met jou alle fases van beginnen te studeren tot aan promotie heb doorlopen! Bedankt voor je eeuwige steun op welk vlak dan ook en je hulp bij het regelen van allerlei zaken voor de promotie! Bij jou gaat het ook helemaal goed komen. Hou vol! Lieve San, bizar, maar zo fijn, dat je in zo'n korte zo'n dierbare vriendin bent geworden! Bedankt voor je steun de afgelopen tijd en alle mooie momenten die we al hebben meegemaakt. Ik vind het fantastisch dat je in opleiding bent en met jouw promotie gaat het hoe dan ook goed komen. Ondanks de afstand weet ik zeker dat er nog heel veel mooie momenten bij gaan komen!

Lieve Roeland, ontzettend bedankt voor de prachtige illustraties in mijn proefschrift! Ik ben onwijs trots op wat je dit jaar allemaal bereikt hebt en vind het helemaal geweldig dat jij deze bijdrage hebt geleverd aan mijn boekje, ondanks je eigen drukke bezigheden. Lieve Queen, bedankt dat je al zo lang bij ons gezin hoort; ik hoop dat we elkaar volgend jaar meer gaan zien!

Lieve papa en mama. Dat dit proefschrift hier ligt heb ik, meer dan aan wie dan ook, aan jullie te danken! Bedankt dat jullie me altijd gestimuleerd hebben om dat te doen wat ik ambieerde en daarvoor ook de mogelijkheden hebben geboden. Zonder jullie had ik dit alles niet kunnen bereiken. De onvoorwaardelijke steun en liefde die jullie geven is het mooiste wat een kind zich kan wensen. Dank jullie wel!!!

Carlijn