Protein, Energy and Their Interaction in Critically Ill Children

Sascha Cornelis Antonius Theodorus Verbruggen

The studies as presented in this thesis were financially supported by the Sophia Children's Hospital Fund (SSWO; grant 537; institutional grant, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands). The grant supplier had no involvement whatsoever in the study design, in the collection, analysis, and the interpretation of data, and in the decision to submit the reports for publication.

The printing of this thesis was financially supported by Nutricia Advanced Medical Nutrition, Nestlé Nutrition and Cambridge Isotope Laboratories, Inc.

ISBN: 978-90-8559-168-9

Layout: Optima Grafische Communicatie, Rotterdam, The Netherlands
Cover: Sascha Verbruggen, Arnold van den Berg; www.fotoberg010.nl
Printed by: Optima Grafische Communicatie, Rotterdam, The Netherlands

Protein, Energy and Their Interaction in Critically Ill Children

Eiwit, Energie en Hun Interactie in Kritisch Zieke Kinderen

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
woensdag 22 december 2010 om 15.30 uur

door

Sascha Cornelis Antonius Theodorus Verbruggen

geboren te Nijmegen

Promotiecommissie:

Promotor: Prof. Dr. J.B. van Goudoever

Co-promotor: Dr. K.F.M. Joosten

Overige leden: Prof. Dr. A.J. van der Heijden
Prof. Dr. D. Tibboel
Prof. Dr. P.J.J. Sauer

“The less you know, the more you believe...”
‘last night on earth’
Bono, U2

Dit boek is voor mijn ouders

Table of contents

Chapter 1

General introduction and Outline of the thesis

"In all maladies, those who are well nourished do best"
Hippocrates, (460-377 BC.)

Metabolism during critical illness

Critically ill patients are in a catabolic state, characterized by three major metabolic changes. First, there is an increased protein turnover with enhanced hepatic protein synthesis and muscle protein breakdown[1-3]. Second, during critical illness there is increased lipolysis, or the breakdown of triglycerides to free fatty acids (FFA) and glycerol[2-5]. And third, insulin resistance causes hyperglycemia due to ongoing endogenous glucose production (glycogenolysis and gluconeogenesis) and blunted peripheral uptake[6-9]. These metabolic derangements are caused by various endogenous and exogenous triggers, including increased inflammatory cytokines (Tumor Necrosis Factor α, interleukin-1, interleukin-6, and interleukin-8), catecholamines and glucocorticoids, all in which insulin resistance plays a central role[9-11]. This response to injury is universal and has been beneficial all through evolution at the acute onset of severe disease or trauma. However, modern medicine has improved survival rate and critical illness has become a process which lasts not just mere hours but can last for days or even weeks. Early last century Sir David P. Cuthbertson described the short initial hypometabolic "ebb" phase, followed by the prolonged hypermetabolic "flow" phase during adult critical illness[12]. Persistent glucose overload and breakdown of skeletal muscle and adipose tissue releasing large amounts of amino acids and FFA are the result. Unfortunately, although plasma substrate levels may be increased, their availability to peripheral tissues may be blunted (because of factors such as insulin resistance and inhibition of lipoprotein lipase), while plasma levels of other substrates (e.g. specific amino acids, cholesterol) may be insufficient to meet metabolic demands[13-15]. As a result, these metabolic changes, beneficial in the initial phase from a teleological viewpoint, become detrimental during prolonged critical illness.

Nutritional challenges on the PICU

Pediatric patients are particularly vulnerable to prolonged metabolic stress, as their muscle and fat mass is lower than in adults[16], while they have higher resting energy requirements[17-19]. It has been shown extensively that children admitted to the pediatric intensive care unit (PICU) accumulate substantial energy and protein deficits[18, 20-23], and nutritional goals[24] as well as glycemic control[25] are still not met. The factors contributing to inadequate nutritional and metabolic support are 1) poor knowledge and prescription of adequate energy and protein amounts, 2) the lack of nutritional assessment, 3) inability of adequate nutrient delivery, and 4) fear of causing iatrogenic complications[25-28]. The lack of optimal nutritional and metabolic support does not acutely affect the patient's condition as obvious as inadequate mechanical ventilation, insufficient

inotropic support or withholding the proper medication. However there is compelling evidence that nutrient deficiencies and metabolic deteriorations have both short- as well as long-term consequences. They lead to muscular and physical inability[29, 30], higher risk of infections[30], prolonged mechanical ventilation[31] and prolonged hospital stay[32, 33], and are associated with increased mortality[33]. Timely and adequate initiation of nutritional and metabolic support in the PICU is therefore essential. Nonetheless, it remains a poorly investigated area with little evidence for specific nutritional management. Many recommendations and guidelines are based on small pediatric studies or data extrapolated from adult studies[34-36].

Hence, a clear definition of optimal nutritional and metabolic support for critically ill children is necessary. The overall goal of nutrition in critically ill conditions reaches beyond the replacement of nutrient losses enabling individuals to maintain basal physiological functions. Critically ill children should be fed the amount of nutrients to additionally counteract catabolism and supply sufficient substrate to help recover from the disease process, while enabling normal growth and development. The macronutrient requirements can be roughly divided between energy (glucose and fat) and protein (in essence amino acid) requirements. To be able to create guidelines for these children, nutritional goals should be based upon[37]; 1) nutritional therapy aiming to providing adequate amounts of energy, especially when energy stores are depleted, 2) nutritional therapy manipulating insulin metabolism, and 3) nutritional therapy aiming to conserve or restore the body protein mass to enable growth and development.

The nutritional support is not simply providing *more* nutrients. Although inadequate supply of nutrients can potentially lead to catabolism of protein and/or adipose tissue, “overfeeding” also has several potential detrimental side effects. Providing excessive amounts of energy can cause fat accumulation, dyslipidemia and exacerbation of insulin resistance leading to hyperglycemia. Furthermore, excessive amounts of free amino acids may also result in toxicity and long-term use of parenteral nutrition results on cholestasis and liver failure[38-41]. It has been demonstrated that L-methionine is hepatotoxic[42], whereas L-arginine, through L-ornithine production induces necrotizing pancreatitis in rats[43], and L-Lysine at large doses induces renal failure in dogs[44]. Providing the requirements in a tight balance to prevent underfeeding as well as overfeeding is a major challenge.

Glucose metabolism and control

Energy requirements of the human body, and especially the brain, depend on glucose as the major fuel. Plasma glucose levels are the resultant of a balance between exogenous glucose intake and endogenous glucose production (glycogenolysis and

gluconeogenesis) on the one hand and glucose utilization (oxidation or storage as glycogen and triglycerides) on the other.

Of the metabolic derangements occurring during critical illness, hyperglycemia has gained the most attention in recent years. Hyperglycemia (> 110 mg.dL^{-1} ~ > 6.1 mmol.L^{-1}) was considered a relatively benign response to stress and, although associated with increased morbidity and mortality in critically ill patients, no clear causality was shown[45, 46]. Critical illness hyperglycemia is predominantly caused by insulin resistance, due to suppression of insulin receptor signalling induced by an increased cytokine release[11], which results in increased endogenous glucose production and decreased peripheral glucose uptake. Additionally, the amount of glucose intake provided on the intensive care unit is also independently related to hyperglycemia and poor outcome[47]. Since a landmark paper showed that a tight glucose regimen with insulin improved morbidity and mortality in an adult surgical Intensive Care Unit (ICU)[48], and subsequently in other populations[49], the implementation of insulin administration has been widely adopted on ICU's[50]. Also in PICU's, hyperglycemia is a frequent complication which is associated with increased morbidity[33, 51, 52]. Large randomized outcome studies of a tight glucose regimen with insulin therapy in the critically ill pediatric population are limited to one recently published trial[53] and one other trial which started this year[54], of which the results are eagerly anticipated for. The recently published study showed an improved outcome[53], despite an increase in hypoglycemia (defined as blood glucose ≤ 2.2 mmol.L^{-1}) and severe hypoglycemia (defined as blood glucose ≤ 1.7 mmol.L^{-1}).

Even though the evidence in favour of a tight glucose regimen in adult ICU's is more robust than the single study in children, especially in surgical ICU's, there still remains wide controversy regarding the replicability of earlier results and the increased incidence of hypoglycemia[49, 55, 56]. Hypoglycemia as a consequence of a tight glucose regimen is of even greater importance in the critically ill pediatric population. The child's developing brain is more susceptible to hypoglycemia which can result in permanent damage[57-59]. Furthermore, young age is a risk factor for developing hypoglycemia, especially when the child is ill[60-62]. It is therefore essential to prevent hypoglycemia in these children. Studies which present safe glucose control protocols or alternative approaches to treat hyperglycemia in children are scarce[63-65].

In the mean time, fear of hypoglycemia discourages actual practice habits regarding glycemic control in the PICU[25]. More insight in the risks of hyperglycemia, the feasibility and safety of tight glucose protocols in the PICU, and strategies to prevent hypoglycemia might help increase the awareness of the potential benefits of glycemic control in critically ill children.

Amino acid metabolism and requirements

Proteins are made of amino acids as their building blocks. Almost the entire amino acid pool (98%) resides in proteins, which are in a constant process of degradation and synthesis, the so-called protein turnover. Protein turnover allows for a continuous flow of amino acids available for necessary new proteins. In addition, specific amino acids serve so-called "non-protein" actions. Amino acids act as precursors for the biosynthesis of substrates such as nitric oxide and polyamines[66], and as signalling molecules in signal transduction pathways[67, 68]. They also regulate energy metabolism[69], and help protect against oxidative stress and protect endothelial function cells[70, 71].

During critical illness, protein turnover is markedly increased to maintain the enhanced hepatic protein synthesis, triggered by pro-inflammatory cytokines causing a cascade stimulating the production of inflammatory/immune proteins[72-74]. To provide substrate for these proteins, amino acids are being mobilized from muscle, which is the largest reservoir of peptide-bound and free amino acids[15]. Activation of the ubiquitin-proteasome proteolytic pathway (UPP) triggers a profound catabolic response characterized by increased muscle protein breakdown[75, 76]. Simultaneously, the synthesis of myofibrillar and sarcoplasmic muscle proteins is decreased due to changes in translation initiation and reduction of translation efficiency[77-79]. Despite the increased hepatic protein synthesis, the disproportionate high loss of muscle protein results in a negative whole body protein balance.

Currently, there is consensus on the total amount of protein that critically ill children should receive[35, 36]. This is however based on limited evidence and is still subject to debate[28]. To be able to develop guidelines for protein and amino acids requirements in critically ill children various aspects need to be taken into consideration.

First, the ideal nutrition would provide every individual amino acid in amounts specifically required during a state of critically illness, not only to maintain protein balance but also to maintain the abovementioned "non-protein" functions.

Second, amino acid utilization and metabolism is directed to different pathways depending on the anatomical presentation[80]. Via the enteral route there is a first-pass utilization of amino acids in the splanchnic area (liver, stomach, intestines and their microbiota, pancreas and spleen), the so-called splanchnic uptake. Amino acids provided through parenteral nutrition are not subjected to splanchnic uptake and are presented to the liver through the arterial circulation instead of the portal venous circulation. Therefore, requirements are different when provided via the enteral vs. the parenteral route. Finally, protein and amino acid requirements differ with age. The youngest individuals have the highest requirements even in health[81-83]. Due to the age-dependent difference in body composition, the mass of protein/amino acids available through catabolism triggered during critical illness is lower in young children.

To summarize, protein/amino acid requirements depend on the clinical condition, route of administration and developmental stage of the patient. There is limited knowledge of the specific amino acid requirements in critically ill children of different age groups and on the amounts they are currently provided with.

Protein and energy interactions

A close interrelationship exists between protein and energy metabolism. The World Health Organisation has provided guidelines on protein-energy ratios to prevent stunting and wasting in acute (9-11.5 energy% protein) and chronically malnourished children (11-15% energy% protein)[84]. However, it is not known whether these interactions change during critical illness.

A lack in energy supply (such as glucose) might enhance an already increased protein catabolism during critical illness. However, an increase in the energy supply will not promote nitrogen retention unless the protein supply is adequate, and conversely an increased protein supply will be useless if energy is limiting. Moreover, free amino acids provided in excessive amounts or released from proteolysis, and exceeding the incorporation into proteins are oxidized and/or channelled into the gluconeogenic pathway. So, although an increased amino acid intake might improve whole body protein balance in critically ill children[20], potentially there is a detrimental influence on glucose metabolism. Finally, the increased lipolysis during critical illness results in the excessive release of FFA, which exceeds their oxidation rates[4, 5]. Furthermore, the ability of the utilization of substrates, such as glucose and FFA, in the peripheral tissue is diminished due to insulin resistance and lipoprotein lipase inhibition[4, 7, 13, 14]. The consequences of excessive FFA are decreased mitochondrial function, increased insulin resistance, as well as an inhibition of glucose oxidation[4, 85]. The resulting effect on the hampered energy metabolism potentially also influences protein metabolism. These close interactions between the metabolism of the macronutrients, also in relation to insulin resistance and metabolism during critical illness, are not fully understood.

The role of insulin

Under physiological circumstances, the anabolic hormone insulin inhibits endogenous glucose production and lipolysis, and improves whole body protein balance[86, 87]. Moreover, maintaining normoglycemia improves muscle protein anabolism independent of insulin plasma levels[88]. It has been hypothesized that the anti-catabolic effects exerted during a tight glucose regimen with intravenous insulin partially explain the beneficial effects in critically ill patients. In critically ill adults insulin administration decreased lipolysis and limited endogenous glucose production[89, 90], but had no protein-sparing

effects[90]. However, these studies were performed without providing amino acids to the patients. Furthermore, there are age specific differences in insulin resistance following an inflammatory or traumatic insult[91].
Therefore, it remains unclear whether the anabolic response to insulin and nutrients differs in the pediatric population, although it has been shown that there is a clear age-related response to both nutrients and insulin on muscle protein synthesis in favour of younger individuals[92].
There is limited knowledge on the effects of different intakes of either glucose or amino acids on one each other's metabolism in critically ill children. Furthermore, whether substrate metabolism differs in insulin resistant children treated with a tight glucose regimen is currently unknown. These questions evidently contain essential information to provide tools for nutritional and metabolic support in the PICU.

Outline of the thesis

The ultimate goal of research related to pediatric intensive care is to improve morbidity and mortality. As can be deducted from the introduction, nutrition for the critically ill child is a major uncharted field in pediatric intensive care. Inherently, this means that extra insight into the requirements and interactions of macronutrients potentially will have widespread implications on the support given on the PICU. This notion has lead to the following studies presented in this thesis.
The overall aim is to optimize nutritional and metabolic support in critically ill children. With this in mind, the following hypotheses are tested:

- Hyperglycemia in critically ill children is caused by insulin resistance and can be treated according to age-related glucose control protocols.
- Glucose and protein/amino acids are currently provided in inadequate amounts to critically ill children of different age groups.
- Insulin exerts its anabolic properties also in critically ill children and can be used as an additive tool to deflect catabolism.

Part I Glucose metabolism

The first part of this thesis aimed to describe the consequences of hyperglycemia and its treatment. **Chapter 2** aimed to evaluate the causes and complications of hyperglycemia in critically ill children. Additionally we wanted to provide an overview on the outcome and (non-)metabolic effects of insulin therapy in the PICU.
The following two chapters focus on the treatment and/or prevention of hyperglycemia in infants. **Chapter 3** sets out to evaluate the effectiveness and safety of insulin therapy by means of a tight glucose protocol in infants less than one month old. **Chapter 4**

describes a study investigating the effects of a reduced glucose intake in post-surgical infants. The aim of this study was to determine whether this approach is a safe alternative for insulin therapy to prevent and/or treat hyperglycemia. Glucose homeostasis, glucose kinetics and protein/amino acid catabolism were determined by means of stable isotope tracer techniques.

Part II Protein and amino acid metabolism

After the first part of the thesis the focus shifts from glucose to protein and amino acid metabolism. In the two following chapters the effect of route of administration are evaluated, and the age related differences are highlighted. **Chapter 5** aimed to provide insight into the ontogeny of the splanchnic uptake of the essential amino acid methionine in three different age groups of critically ill children using stable isotope tracer techniques. In **chapter 6** we aimed to evaluate the actual parenteral intake of all amino acids provided to children admitted to the PICU and to compare these with the recommended amino acid intakes by the Institute of Medicine (IOM)[93].

Part III Protein and energy interactions

In the third part of this thesis part I and part II are taken a step further as it describes the close interaction between the metabolism of glucose and protein/amino acids. Two studies are presented which show how different amounts of glucose and amino acid intake affect one another's metabolism. Furthermore, these studies shed light on the role of insulin resistance and insulin treatment on substrate metabolism. **Chapter 7** aimed to describe the effects of increased amino acids and hyperinsulinemia on glucose, lipid and protein kinetics in insulin resistant septic adolescents. They were provided total parenteral nutrition with two different amounts of amino acids. The study used a combination of stable isotope tracer techniques and hyperinsulinemic euglycemic clamps. In **chapter 8** we set out to investigate the effect of different amounts of glucose, amino acids and insulin infusion on albumin synthesis rates in post-surgical infants and septic adolescents.

Part IV General discussion and future perspectives

The final part of this thesis provides a general discussion and recommendations for nutritional and metabolic support in addition to suggestions for future research in **chapter 9**. A summary of our studies and major findings completes this thesis in **chapters 10 and 11**.

References

1. Biolo G, Toigo G, Ciocchi B, et al. Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition 1997;13(9 Suppl):52S-7S.
2. Cogo PE, Carnielli VP, Rosso F, et al. Protein turnover, lipolysis, and endogenous hormonal secretion in critically ill children. Crit Care Med 2002;30(1):65-70.
3. Mizock BA. Metabolic derangements in sepsis and septic shock. Crit Care Clin 2000;16(2):319-36, vii.
4. Krogh-Madsen R, Moller K, Dela F, Kronborg G, Jauffred S, Pedersen BK. Effect of hyperglycemia and hyperinsulinemia on the response of IL-6, TNF-alpha, and FFAs to low-dose endotoxemia in humans. Am J Physiol Endocrinol Metab 2004;286(5):E766-72.
5. Khovidhunkit W, Kim MS, Memon RA, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45(7):1169-96.
6. Klein GW, Hojsak JM, Rapaport R. Hyperglycemia in the pediatric intensive care unit. Curr Opin Clin Nutr Metab Care 2007;10(2):187-92.
7. Mizock BA. Alterations in fuel metabolism in critical illness: hyperglycaemia. Best Pract Res Clin Endocrinol Metab 2001; 15(4):533-51.
8. Montori VM, Bistrian BR, McMahon MM. Hyperglycemia in acutely ill patients. Jama 2002;288(17):2167-9.
9. McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. Crit Care Clin 2001;17(1):107-24.
10. Agwunobi AO, Reid C, Maycock P, Little RA, Carlson GL. Insulin resistance and substrate utilization in human endotoxemia. J Clin Endocrinol Metab 2000; 85(10):3770-8.
11. Matsuda N, Yamamoto S, Yokoo H, Tobe K, Hattori Y. Nuclear factor-kappaB decoy oligodeoxynucleotides ameliorate impaired glucose tolerance and insulin resistance in mice with cecal ligation and puncture-induced sepsis. Crit Care Med 2009;37(10):2791-9.
12. Cuthbertson DP, Angeles Valero Zanuy MA, Leon Sanz ML. Post-shock metabolic response. 1942. Nutr Hosp 2001; 16(5):176-82; discussion 5-6.
13. Cree MG, Wolfe RR. Postburn trauma insulin resistance and fat metabolism. Am J Physiol Endocrinol Metab 2008; 294(1):E1-9.
14. van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003; 31(5):1359-66.
15. 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.
16. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am J Clin Nutr 1982;35(5 Suppl):1169-75.
17. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39 Suppl 1:5-41.
18. Coss-Bu JA, Jefferson LS, Walding D, David Y, Smith EO, Klish WJ. Resting energy expenditure and nitrogen balance in critically ill pediatric patients on mechanical ventilation. Nutrition 1998; 14(9):649-52.
19. Joosten KF, Verhoeven JJ, Hazelzet JA. Energy expenditure and substrate utilization in mechanically ventilated children. Nutrition 1999;15(6):444-8.
20. Coss-Bu JA, Klish WJ, Walding D, Stein F, Smith EO, Jefferson LS. Energy

metabolism, nitrogen balance, and substrate utilization in critically ill children. Am J Clin Nutr 2001;74(5):664-9.
21. Hulst J, Joosten K, Zimmermann L, et al. Malnutrition in critically ill children: from admission to 6 months after discharge. Clin Nutr 2004;23(2):223-32.
22. 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(6):1381-9.
23. Oosterveld MJ, Van Der Kuip M, De Meer K, De Greef HJ, Gemke RJ. Energy expenditure and balance following pediatric intensive care unit admission: a longitudinal study of critically ill children. Pediatr Crit Care Med 2006;7(2):147-53.
24. de Neef M, Geukers VG, Dral A, Lindeboom R, Sauerwein HP, Bos AP. Nutritional goals, prescription and delivery in a pediatric intensive care unit. Clin Nutr 2008;27(1):65-71.
25. Preissig CM, Rigby MR. A disparity between physician attitudes and practice regarding hyperglycemia in pediatric intensive care units in the United States: a survey on actual practice habits. Crit Care 2010;14(1):R11.
26. 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.
27. Taylor B, Krenitsky J. Nutrition in the intensive care unit: year in review 2008-2009. JPEN J Parenter Enteral Nutr 2010; 34(1):21-31.
28. Stroud M. Protein and the critically ill; do we know what to give? Proc Nutr Soc 2007;66(3):378-83.
29. 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.
30. Villet S, Chiolero RL, Bollmann MD, et al. Negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients. Clin Nutr 2005;24(4): 502-9.
31. Pichard C, Kyle UG, Morabia A, Perrier A, Vermeulen B, Unger P. Nutritional assessment: lean body mass depletion at hospital admission is associated with an increased length of stay. Am J Clin Nutr 2004;79(4):613-8.
32. Heyland DK, MacDonald S, Keefe L, Drover JW. Total parenteral nutrition in the critically ill patient: a meta-analysis. Jama 1998;280(23):2013-9.
33. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, Kache S, Wilson DM. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. Pediatrics 2006;118(1):173-9.
34. Skillman HE, Wischmeyer PE. Nutrition therapy in critically ill infants and children. JPEN J Parenter Enteral Nutr 2008; 32(5):520-34.
35. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 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.
36. 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.
37. 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

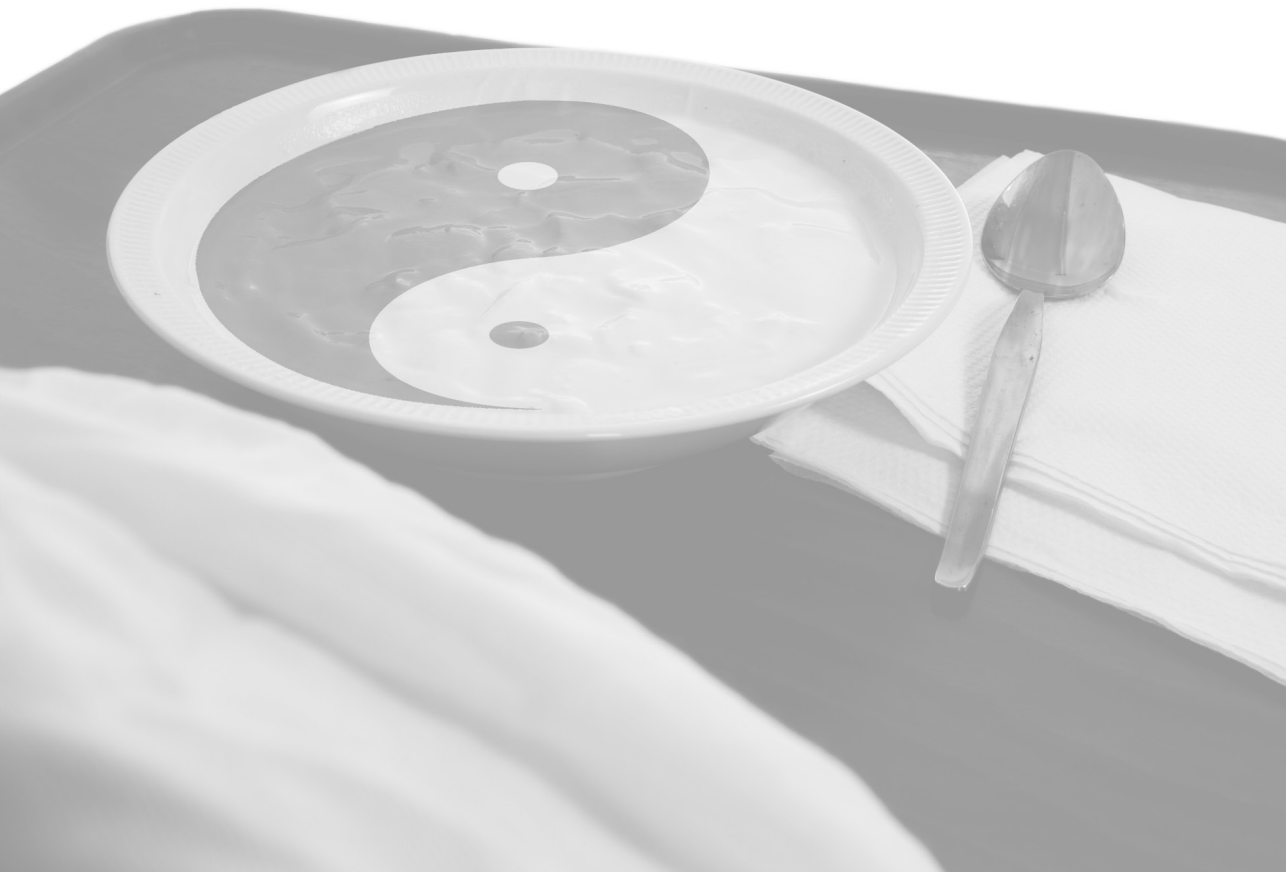
nutritional intervention. Clin Nutr 2007; 26(1):154-8.
38. Kelly DA. Intestinal failure-associated liver disease: what do we know today? Gastroenterology 2006;130(2 Suppl 1): S70-7.
39. Baker DH. Comparative species utilization and toxicity of sulfur amino acids. J Nutr 2006;136(6 Suppl):1670S-5S.
40. Garlick PJ. Assessment of the safety of glutamine and other amino acids. J Nutr 2001;131(9 Suppl):2556S-61S.
41. Munro IC, Renwick AG. The 5th workshop on the assessment of adequate intake of dietary amino acids: general discussion 2. J Nutr 2006;136(6 Suppl):1755S-7S.
42. Moss RL, Haynes AL, Pastuszyn A, Glew RH. Methionine infusion reproduces liver injury of parenteral nutrition cholestasis. Pediatr Res 1999;45(5 Pt 1):664-8.
43. Rakonczay Z, Jr., Hegyi P, Dosa S, et al. A new severe acute necrotizing pancreatitis model induced by L-ornithine in rats. Crit Care Med 2008;36(7):2117-27.
44. Asanuma K, Adachi K, Sugimoto T, Chiba S. Effects of lysine-induced acute renal failure in dogs. J Toxicol Sci 2006;31(2): 87-98.
45. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet 2000;355(9206):773-8.
46. Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. Stroke 2001;32(10):2426-32.
47. der Voort PH, Feenstra RA, Bakker AJ, Heide L, Boerma EC, der Horst IC. Intravenous glucose intake independently related to intensive care unit and hospital mortality: an argument for glucose toxicity in critically ill patients. Clin Endocrinol (Oxf) 2006;64(2):141-5.
48. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001; 345(19):1359-67.
49. Griesdale DE, de Souza RJ, van Dam RM, et al. Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data. Cmaj 2009;180(8):821-7.
50. Wilson M, Weinreb J, Hoo GW. Intensive insulin therapy in critical care: a review of 12 protocols. Diabetes Care 2007;30(4): 1005-11.
51. Faustino EV, Apkon M. Persistent hyperglycemia in critically ill children. J Pediatr 2005;146(1):30-4.
52. Srinivasan V, Spinella PC, Drott HR, Roth CL, Helfaer MA, Nadkarni V. 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.
53. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373(9663):547-56.
54. Macrae D, Pappachan J, Grieve R, et al. Control of hyperglycaemia in paediatric intensive care (CHiP): study protocol. BMC Pediatr 2010;10:5.
55. Finfer S, Chittock DR, Su SY, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med 2009;360(13):1283-97.
56. 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.
57. 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.
58. Vannucci RC, Vannucci SJ. Glucose metabolism in the developing brain. Semin Perinatol 2000;24(2):107-15.

59. Flykanaka-Gantenbein C. Hypoglycemia in childhood: long-term effects. Pediatr Endocrinol Rev 2004;1 Suppl 3:530-6.
60. Solomon T, Felix JM, Samuel M, et al. Hypoglycaemia in paediatric admissions in Mozambique. Lancet 1994;343(8890): 149-50.
61. 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.
62. Faustino EV, Bogue CW. Relationship between hypoglycemia and mortality in critically ill children. Pediatr Crit Care Med 2010.
63. Preissig CM, Hansen I, Roerig PL, Rigby MR. A protocolized approach to identify and manage hyperglycemia in a pediatric critical care unit. Pediatr Crit Care Med 2008;9(6):581-8.
64. Preissig CM, Rigby MR, Maher KO. Glycemic control for postoperative pediatric cardiac patients. Pediatr Cardiol 2009; 30(8):1098-104.
65. Verhoeven JJ, Brand JB, van de Polder MM, Joosten KF. Management of hyperglycemia in the pediatric intensive care unit; implementation of a glucose control protocol. Pediatr Crit Care Med 2009; 10(6):648-52.
66. Morris SM, Jr. Arginine: beyond protein. Am J Clin Nutr 2006;83(2):508S-12S.
67. Tokunaga C, Yoshino K, Yonezawa K. mTOR integrates amino acid- and energy-sensing pathways. Biochem Biophys Res Commun 2004;313(2):443-6.
68. Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr 2007; 27:293-310.
69. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J Nutr Biochem 2006;17(9):571-88.
70. Biolo G, Antonione R, De Cicco M. Glutathione metabolism in sepsis. Crit Care Med 2007;35(9 Suppl):S591-5.
71. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009; 37(1):1-17.
72. Hasselgren PO, Pedersen P, Sax HC, Warner BW, Fischer JE. Current concepts of protein turnover and amino acid transport in liver and skeletal muscle during sepsis. Arch Surg 1988;123(8):992-9.
73. Vary TC. Regulation of skeletal muscle protein turnover during sepsis. Curr Opin Clin Nutr Metab Care 1998;1(2):217-24.
74. Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol 1992; 262(2 Pt 1):C445-52.
75. 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-37S.
76. Voisin L, Breuille D, Combaret L, 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.
77. Lang CH, Frost RA, Vary TC. Regulation of muscle protein synthesis during sepsis and inflammation. Am J Physiol Endocrinol Metab 2007;293(2):E453-9.
78. Cooney RN, Kimball SR, Vary TC. Regulation of skeletal muscle protein turnover during sepsis: mechanisms and mediators. Shock 1997;7(1):1-16.
79. Vary TC, Kimball SR. Sepsis-induced changes in protein synthesis: differential effects on fast- and slow-twitch muscles. Am J Physiol 1992;262(6 Pt 1):C1513-9.
80. Stoll B, Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 2006; 84 Suppl:E60-72.

81. Dewey KG, Beaton G, Fjeld C, Lonnerdal B, Reeds P. Protein requirements of infants and children. Eur J Clin Nutr 1996; 50 Suppl 1:S119-47; discussion S47-50.
82. Pencharz PB, Ball RO. Amino acid needs for early growth and development. J Nutr 2004;134(6 Suppl):1566S-8S.
83. te Braake FW, van den Akker CH, Riedijk MA, van Goudoever JB. Parenteral amino acid and energy administration to premature infants in early life. Semin Fetal Neonatal Med 2007;12(1):11-8.
84. WHO. Management of severe malnutrition: a manual for physicians and other senior health workers. Geneva: World Health Organization 1999.
85. Delarue J, Magnan C. Free fatty acids and insulin resistance. Curr Opin Clin Nutr Metab Care 2007;10(2):142-8.
86. Bell JA, Fujita S, Volpi E, Cadenas JG, Rasmussen BB. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. Am J Physiol Endocrinol Metab 2005;289(6): E999-1006.
87. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab 2006;291(4):E745-54.
88. Biolo G, De Cicco M, Lorenzon S, et al. Treating hyperglycemia improves skeletal muscle protein metabolism in cancer patients after major surgery. Crit Care Med 2008;36(6):1768-75.
89. Chambrier C, Laville M, Rhzioual Berrada K, Odeon M, Bouletreau P, Beylot M. Insulin sensitivity of glucose and fat metabolism in severe sepsis. Clin Sci (Lond) 2000;99(4):321-8.
90. Whyte MB, Jackson NC, Shojaee-Moradie F, et al. Metabolic effects of intensive insulin therapy in critically ill patients. Am J Physiol Endocrinol Metab 2009;298(3):E697-705.
91. Zhai L, Messina JL. Age and tissue specific differences in the development of acute insulin resistance following injury. J Endocrinol 2009;203(3):365-74.
92. Wilkes EA, Selby AL, Atherton PJ, et al. Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. Am J Clin Nutr 2009;90(5):1343-50.
93. Food and Nutrition Board Institute of Medicine. Panel on Macronutrients. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty acids, Cholesterol, Proteins and Amino Acids. Washington DC: The National Press; 2005.

Part I

Glucose metabolism



Chapter 2

Insulin therapy in the Pediatric Intensive Care Unit

Sascha CAT Verbruggen
Koen FM Joosten
Leticia Castillo
Johannes B van Goudoever

Clin Nutr. 2007;26(6):677-90.

Koen FM Joosten
Sascha CAT Verbruggen
Jennifer J Verhoeven

Lancet. 2009; 373(9673):1423-4

Abstract

Background & aims

Hyperglycemia is a major risk factor for increased morbidity and mortality in the intensive care unit. Insulin therapy has emerged in adult intensive care units and several pediatric studies are currently being conducted. This review discusses hyperglycemia and the effects of insulin on metabolic and non-metabolic pathways, with a focus on pediatric critical illness.

Methods

A PubMed search was performed by a using the following keywords and limits ((“hyperglycemia”[MeSH Terms] OR (“insulin resistance”[MeSH Major Topic]) AND (“critical care”[MeSH Terms] OR “critical illness”[MeSH Terms])) in different combinations with (“metabolism”[MeSH Terms] OR “metabolic networks and pathways”[MeSH Terms]) and (“outcome”[All Fields]) and (“infant”[MeSH Terms] OR “child”[MeSH Terms] OR “adolescent”[MeSH Terms]). Quality assessment of selected studies included clinical pertinence, publication in peer-reviewed journals, objectivity of measurements and techniques used to minimize bias. Reference lists of such studies were included.

Results

The magnitude and duration of hyperglycemia are associated with increased morbidity and mortality in the Pediatric Intensive Care Unit (PICU). Although a large randomized trial showed clear benefit of insulin therapy, (severe) hypoglycemia occurred frequently and warrants other multi-center studies and long-term follow-up. Evidence concerning the mechanism and effect of insulin on glucose and lipid metabolism in pediatric critical illness is scarce. More is known about the positive effect on protein homeostasis, especially in severely burned children. The effect in septic children is less clear and seems age dependent. Some non-metabolic properties of insulin such as modulation of inflammation, endothelial dysfunction and coagulopathy have not been fully investigated in children.

Conclusion

Further studies on the effect of insulin on morbidity and mortality as well as on the mechanisms through which insulin exerts these effects are necessary in critically ill children. We propose these studies to be conducted under standardized conditions including precise definitions of hyperglycemia and rates of glucose intake.

Introduction

Critical illness triggers an acute phase response, which is associated with severe metabolic abnormalities. These changes are characterized by hyperglycemia, dyslipidemia and increased protein turnover[1-8]. Recently, hyperglycemia has become the focus of interest due to its relationship to outcome. The onset of "stress hyperglycemia" in previously non-diabetic critically ill patients has been attributed to peripheral and hepatic insulin resistance, certain drugs (steroids, catecholamines, thiazides), increased stress hormone release and excessive dextrose administration via total parenteral nutrition[3, 5, 6].

Previously, hyperglycemia in these patients was considered to be part of the adaptive stress response and even to be beneficial for the patient. It was thought to provide glucose-dependent organs adequate energy supply and to compensate for volume loss by increasing the osmotic pressure and so promote fluid movement into the intravascular compartment[5, 9, 10]. As such, most clinicians accepted moderate hyperglycemia in these patients. In 2001 van den Berghe et al. described a regimen of tight control of glucose levels in a surgical Intensive Care Unit. Strict insulin therapy reduced overall in-hospital mortality (by 34 percent), and also reduced bacteremia, acute renal failure, red-cell transfusions and critical-illness polyneuropathy[11].

Since then, the use of insulin therapy has increased as standard therapy in adult intensive care units and several studies are currently in progress in pediatric and neonatal intensive care units. There is a definitive association between hyperglycemia and outcome in the Pediatric Intensive Care Unit (PICU)[12-20]. However, some questions need to be answered before we can accept insulin administration as standard care in pediatric critical illness. Are the beneficial effects and the safety of tight glucose control with insulin in critically ill children the same as in adults? What specific mechanisms in the developing pediatric population are responsible for the beneficial of glycemic control? Finally, what metabolic and non-metabolic effects, other than glycemic control, does insulin exert in critical illness? With this review we want to address these issues by summarizing the pediatric literature concerning hyperglycemia in the PICU and the relevant adult and animal studies concerning the different properties of insulin in critical illness.

Pediatric studies

Hyperglycemia in critical illness

Several studies have investigated hyperglycemia in pediatric and neonatal ICU's in relation to morbidity and mortality. Most of these studies were done in the PICU

with a diverse spectrum of diseases and injuries (Table 1)[12-17, 20]. Except for the study performed by Branco and colleagues all studies were retrospective. A total of 2599 children were studied with an average age of approximately 3 years, ranging from 0 – 21 years. All studies report an association between hyperglycemia and morbidity

Table 1. Summary of studies addressing hyperglycemia in Pediatric Intensive Care Unit.

Author	Study type	Year	Diagnosis	Median age yrs (range)	N=	Glucose intake mg/kg/min (range)	Threshold mg/dl (mmol/l)	Outcome
Branco [12]	Prospective	2005	Septic shock	2.8 (0 – 7.1)	57	2.8 (1.8 – 3.9)	178 (9.9)	Peak glucose > 178 mg/dl was associated with 2.59 fold increase in risk of death
Cochran [13]	Retrospective	2003	Head trauma	4.0 (0.1 – 17)	170	n.m.	135, 267 (7.5, 14.8)	Nonsurvivors had higher admission glucose levels than survivors (267 mg/dl vs. 135 mg/dl, p = 0.000) and an admission blood glucose level 200 mg/dl associated with worse neurological outcome
Faustino [14]	Retrospective	2005	Mixed PICU	3.2 (0.3 – 10.8)	942	n.m.	120, 150 and 200 (6.7, 8.3 and 11.1)	Maximum glucose levels increased relative risk (RR) for dying (within 24 hrs > 150 mg/dl; RR 2.50; 95% confidence interval (CI) 1.26 – 4.93 and within 10 days >120 mg/dl: RR 5.68; 95% CI 1.38 – 23.47).
Srinivasan [15]	Retrospective	2004	Mixed PICU	6 (1 – 12)	152	n.m.	126 (7.0)	Peak blood glucose in nonsurvivors were higher (311 ± 115 vs. 205 ± 80 mg/dl, p < 0.001) and lasted longer (71% ± 14% vs. 37% ± 5% of PICU days, p < 0.001)
Wintergerst [16]	Retrospective	2006	Mixed PICU	2.8 (0 – 21)	1094	n.m.	65, 120, 150, 200 (3.6, 6.7, 8.3, 11.1)	Both high peak glucose levels (mortality rate 9.9% at > 200 mg/dl, p < 0.0001 $_{x}^{2}$ test) and low peak glucose levels (mortality rate 16.5% at < 65 mg/dl, p < 0.0001 $_{x}^{2}$ test) worsened outcome. LOS was associated with hyper- and hypoglycemia. Increased glucose variability had the strongest association with increased mortality and LOS.
Yates [17]	Retrospective	2006	Post cardiac surgery	0.3 (0.1 – 0.6)	184	n.m.	126 (7)	Nonsurvivors had higher peak glucose levels (256 ± 196 vs. 179 ± 87 mg/dl, p < 0.001) and longer duration of hyperglycemia > 126 mg/dl (2.95 ± 2.28 vs. 1.19 ±1.3 days, p < 0.001). Duration of hyperglycemia was associated with longer ventilator use (p < 0.001, R^2 = 0.10) and LOS (p < 0.001, R^2 = 0.21).

Table 2. Summary of studies addressing hyperglycemia in Neonatal Intensive Care Unit

Author	Study type	Year	Diagnosis	Gestational age	N=	Intake kcal/kg/day	Threshold mg/dl (mmol/l)	Outcome
Alaedeen [18]	Retro-spective	2006	Septic Very Low Birth Weight infants	Median 26 (23 – 34)	37	83 (64 – 102)	120 (6.7)	Peak glucose levels over 120 mg/dl associated with longer LOS (p = 0.006). Nonsurvivors had higher peak glucose levels (241 ± 46 mg/dl vs. 141 ± 47 mg/dl, p < 0.0001).
Hall [19]	Retro-spective	2004	Necrotizing enterocolitis	Mean 29.1 SEM 0.5	88	n.m.	214 (11.9)	Peak glucose levels over 214 mg/dl associated with a higher late mortality > 10 admission (29% vs. 2%, p = 0.0009) and LOS (p < 0.0001).

(e.g. Length of stay (LOS), length of ventilator use, neurological outcome). Altogether peak glucose levels and duration of hyperglycemia were higher in non-survivors and two studies found hyperglycemia to be associated with a 2.5 fold increase in mortality risk. Wintergerst et al. also reported an increased mortality rate in children with hypoglycemia[16].

Two retrospective studies were performed in NICU patients, involving respectively neonates with sepsis and necrotizing enterocolitis (Table 2)[18, 19]. A total of 125 neonates were studied with an average gestational age of 28.2 weeks. Both studies show an association between hyperglycemia, longer LOS and higher mortality rate.

Overall one can say that hyperglycemia is frequently present in critically ill children and it is associated with an increased morbidity and mortality rate (Table 1, 2). However, there are some controversial issues on hyperglycemia in critically ill children. First, most studies are retrospective and could not demonstrate causality between glucose levels and outcome measures, only associations were demonstrated. Second, only the study performed by Branco and colleagues documented data on glucose intake (Table 1)[12]. Moreover, excessive glucose intake might partially be responsible for hyperglycemia. Finally, these retrospective studies mention different glucose thresholds, most of them above 120 mg.dL^{-1} (6.7 mmol.L^{-1}) (Table 1, 2). This makes it difficult to compare the data and draw conclusions. Although various thresholds are reported, a glucose level of 150 mg.dL^{-1} (8.3 mmol.L^{-1}) seems to have the strongest association between hyperglycemia and increased morbidity and mortality[14-16]. A standardized approach including definition of hyperglycemia, values that require intervention, length of hyperglycemia and rates of glucose intake, is necessary to make statements concerning the risks of hyperglycemia and the necessity of insulin therapy. In anticipation for more evidence we propose to use 150 mg.dL^{-1} (8.3 mmol.L^{-1}) as a limit to study and treat hyperglycemia children in the ICU with insulin.

Insulin therapy

Initially studies on insulin therapy in the PICU focused primarily on burn patients. Pham et al. compared in a retrospective study conventional treatment (n = 31) versus intensive insulin treatment (n = 33) in severely burned children (Table 3). Their study reported lower infection rates and even a positive association with survival in the intensive treatment group. Patients who received intensive insulin therapy were more than five times more likely to survive than those receiving conventional therapy (adjusted Odds Ratio = 5.52; p = 0.06)[21].

In the neonatal ICU there have been several smaller studies which looked at the effect and safety of insulin therapy[22-27]. These studies showed improved glycemic control and all, except the study performed by Ng and colleagues, reported improved caloric intake and even growth[22-27]. Hypoglycemia (glucose < 40 $mg.dL^{-1}$ (< 2.2 $mmol.L^{-1}$)) was the major complication, but this happened infrequently (0 – 5%) and no other serious adverse effects were noted. To date, two large randomized studies have investigated the effect of tight glucose control with insulin on morbidity and mortality in the pediatric population[20, 28]. The study by Beardsall and colleagues in very low birth weight neonates where hyperglycemia was treated with insulin was discontinued early because of an increased incidence of hypoglycemia and parenchymal abnormalities detected on cranial ultrasound images in the infants treated with insulin[28]. Vlasselaers and colleagues showed an improved short-term outcome, by decreasing PICU length of stay (PICU LOS), attenuating the inflammatory response and even a decrease

Table 3. Prospective studies of insulin therapy on critically ill children

Author	Year	Diagnosis	Age yrs	N=	Intake	Threshold mg/dl (mmol/l)	Outcome
Vlasselaers [20]	2009	Various	1.3 (0.3 – 5.5)	700	20 – 80 kcal/kg/day	Conservative 11.9 (214) Intensive Infants 0 - 1 y 2.8 - 4.4 (50 - 79) Children 1 - 16 y 3.9 - 5.5 (70 - 99)	Intensive insulin therapy decreased duration of PICU stay, attenuated the inflammatory response as measured with C-reactive protein on day 5, and decreased mortality from 6% in the conservative group to 3% in the treatment group
Pham [21]	2005	Burn	6.0 (1.0 – 11.2)	64	35 kcal/kg/day	Conservative 200 (11.1) Intensive 140 (7.8)	Patients who received intensive insulin therapy were more than five times more likely to survive than those receiving conventional therapy Intensive insulin therapy showed a trend toward lower infection rates.
Jeschke [69]	2004	Burn	5.4 (0.4 – 10.4)	28	1500 kcal/m^2 body surface + 1500 kcal/m^2 area burn	180 mg/dl (10) in the treatment group and non-treatment group	Insulin therapy improved lipid metabolism and had a positive effect on inflammatory response and hepatic constitutive proteins.
Wu [114]	2004	Burn	9.5 (2 – 17)	18	1500 kcal/m^2 body surface + 1500 kcal/m^2 area burn	140 mg/dl (7.8)	Prolonged hepatic acute phase response decreased, no effect on constitutive proteins

in mortality rate (3% vs. 6%)[20]. However, insulin therapy 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. The high incidence of hypoglycemia in the various studies with tight glucose control and insulin therapy remains a matter of major concern. Neonates and young children are at particular risk for developing hypoglycemia. Furthermore, as brain development is most profound in younger children the complications caused by hypoglycemia are potentially highest in this patient group. Hypoglycemia is a risk factor of thalamic infarction in neonates[29]. Hypoglycemia has also been reported in the adult population during insulin therapy[30-33]. In a large Australian cohort, a U-shaped outcome curve showed that both high and low blood glucose concentrations worsen outcome[34]. The reasons for hypoglycemia in the single large randomized tight glucose study on the PICU are diverse. First, the target ranges for plasma glucose levels concentrations were low (2.8 – 4.4 mmol.L^{-1} for infants and 3.9 – 5.6 mmol.L^{-1} for children) in the study of Valsselaers and colleagues[20]. Second, the infants' glucose intake was lower than recommended (median 3.5 mg.kg^{-1}.min^{-1} on day 1 compared with 5.5 mg.kg^{-1}.min^{-1} according to the guidelines from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN))[35]. Third, the treatment algorithm for insulin was adjusted by the nurses on the basis of their experience. In our institution, hyperglycemia is treated (target glucose concentration 4-8 mmol.L^{-1}) by use of a detailed stepwise algorithm to adjust insulin therapy without occurrence of hypoglycemia (< 2.2 mmol.L^{-1})[36]. Finally, the starting dose of insulin was high (0.1 – 0.2 IU.kg^{-1}.h^{-1}), whereas a lower starting dose of 0.02 – 0.05 IU.kg^{-1}.h^{-1} would seem safer. An appropriate glucose intake, especially in infants, according to weight and age, in combination with a step-wise (computer-assisted, or nurse driven) protocol and slightly higher glucose target concentrations might decrease the incidence of hypoglycemia without losing the beneficial effects of insulin therapy in critically ill children. However, in critically ill children, hypoglycemia is a frequent complication even in the absence of insulin therapy[15, 16, 37, 38]. In a heterogeneous group of 1094 PICU patients a relatively higher prevalence of hypoglycemia of 18.6% was associated with mortality[16]. In one study with 26 children with meningococcal septic shock (MSS) they found lower glucose levels of 3.9 mmol.L^{-1} (70 mg.dL^{-1}) with a range from 2.5 to 6.5 mmol.L^{-1} (45 to 117 mg.dL^{-1}) on admission in the non-surviving group, whereas in both the surviving and non-surviving group the insulin-glucose ratios were normal[37]. In another smaller study on 10 children diagnosed with meningococcal septic shock (MSS) compared to a group diagnosed with meningococcal sepsis (MS), insulin and insulin-glucose ratios remained lower in the meningococcal septic shock patients, although both groups had hyperglycemia[38]. These studies indicate that in the acute phase of septic shock in children hypoglycemia can occur and subsequently that the mechanism of hyperglycemia is more complicated than mere insulin resistance.

Hypoglycemia is a realistic complication as it occurs in a relatively high prevalence in critically ill children without insulin therapy, especially in the acute phase of septic shock. More large multi-center prospective studies are warranted to determine whether the beneficial effects and safety of a tight glucose control shown in adults can be extrapolated to the pediatric population. Adequate glucose intake depends on age and clinical situation, such as prematurity and critical illness[35]. Therefore, especially in the pediatric situation glucose intake is essential information to adequately interpret the literature. Younger children and neonates should be provided with higher glucose intake. The ESPHGAN guidelines also state that critically ill children probably should receive lower amounts of glucose (limited to 5 mg/kg/min) than their healthy peers[35]. However, this recommendation was based on one study performed in burned children[39]. For other disease processes (e.g. sepsis, trauma) in critically ill children no extensive studies regarding glucose requirements have been performed. These guidelines encourage to correct caloric needs according to various disease processes[35]. We propose to use standardized parenteral glucose intake in critically ill children on admission. In critically ill children below 30 kg we propose to use a glucose intake of 4 – 6 $mg.kg^{-1}.min^{-1}$ and in children above 30 kg we propose to use 2 – 4 $mg.kg^{-1}.min^{-1}$. Once tolerated in the succeeding days enteral nutrition is preferred according to the child's condition.

Metabolic properties of insulin

Lowering blood glucose levels

Critical illness is a condition where both hepatic and peripheral insulin resistance contribute to the development of hyperglycemia. Although the effects of insulin are peripheral (muscle and adipose tissue) and hepatic, the liver becomes more insulin resistant than the peripheral tissues in critical illness (Figure 1)[40, 41]. The expression of phosphoenolpyruvate carboxy kinase (PEPCK), which is the rate-limiting enzyme of gluconeogenesis, is increased in critical illness due to elevated levels of cortisol and catecholamines. Insulin is one of the most potent inhibitors of PEPCK[42]. However in critically ill patients both the expression of PEPCK and hepatic glucokinase, which controls glucose uptake and glycogen synthesis, remains unaltered by intensive insulin therapy[41, 43, 44]. In contrast, insulin therapy increases expression in muscle of an insulin dependent glucose transporter (GLUT-4), and of hexokinase–II, which is the rate limiting enzyme of intracellular insulin-mediated glucose metabolism[44]. This suggests that in critically ill patients exogenous insulin does not affect hepatic insulin resistance, but lowers blood glucose levels mainly through stimulation of skeletal muscle glucose uptake. The explanation for the difference in insulin sensitivity between tissues remains unclear. In order to understand how glycemic control with insulin proves to be beneficial in critical illness one has to start with the effect of hyperglycemia in these patients.

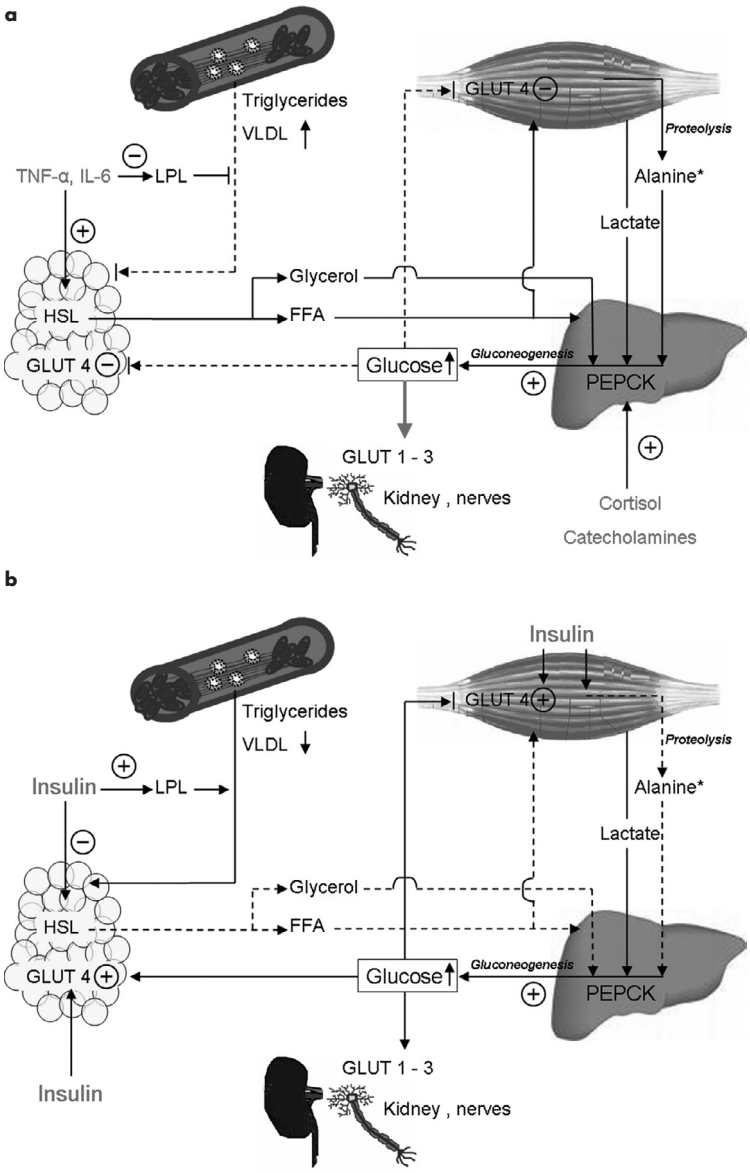


Figure 1. Glucose and lipid metabolism during critical illness (Panel A) and the effect of insulin therapy on metabolism during critical illness (Panel B). * Gluconeogenic amino acids such as Alanine. HSL = hormone sensitive lipase; LPL = lipoprotein lipase; VLDL = very-low-density lipoprotein; GLUT = glucose transporters; FFA = free fatty acids; PEPCK = phosphoenolpyruvate carboxylase

Hyperglycemia has been shown to be more acutely toxic in critically ill patients than in healthy individuals or even patients with diabetes[41]. Under physiological circumstances glucose uptake in the liver is directly proportional to blood glucose concentration, while peripheral uptake is insulin dependent. Hyperglycemia down regulates insulin

independent glucose transporters (GLUT-1, GLUT-2 and GLUT-3). In contrast, critical illness causes an over expression of these transporters and leads to glucose overload in organ systems that express these transporters. The upregulation of these insulin independent glucose transporters is seen in central and peripheral nervous system, as well as in endothelial, hepatic and immune cells, renal tubules and gastrointestinal mucosa[41]. Glucose overload causes more excessive glycolysis and oxidative phosphorylation resulting in larger generation of oxygen radicals such as peroxynitrite and superoxide generation in these cells. These reactive species cause mitochondrial dysfunction and disturbed energy metabolism which leads to increased apoptosis and might explain cellular and organ system failure in critically ill patients[41, 45, 46].

Insulin therapy has been shown to protect the function and structure of the mitochondrial compartment[45, 47]. The mitochondrial changes described were seen in hepatic and not in muscular cells. These data suggest that maintaining normoglycemia, rather than a direct effect of the administered insulin, is responsible for the major beneficial effects of a tight glucose control[45, 48-50]. However, many of the proposed benefits of normoglycemic control in critical illness are difficult to separate from the direct effects of insulin on several cell and organ systems. Insulin administration in adults show a definitive direct effect on critical illness polyneuropathy and bacteremia[51]. The potential improvement of lipid and protein metabolism as we will describe next are also direct effects of insulin.

The abovementioned data are from adult studies. Pediatric ICU patients in general have a lower morbidity and mortality rate than adults admitted to an ICU[52-56]. Still, it is likely that the mechanisms of hyperglycemia also apply for the PICU population.

Insulin improves lipid metabolism

Critical illness is a condition accompanied by dyslipidemia (Figure 1). This is characterized by two main disturbances in the serum lipid profile. The first is an increase in plasma triglycerides and very low density lipoproteins (VLDL)[57-59]. Increased lipolysis, de novo fatty acid synthesis and decreased oxidation of fatty acids increase hepatic triglyceride production and VLDL secretion. Additionally, inflammatory responses inhibit the activity of endothelial lipoprotein lipase, an enzyme responsible for triglyceride clearance[57-59]. In critical illness there is lipolysis, or the breakdown of triglycerides to free fatty acids (FFA) and glycerol, and this exceeds fatty acid oxidation rates. The consequences of excessive FFA are decreased mitochondrial function and increased oxidative stress[60]. Moreover, elevated FFA are implicated in generating insulin resistance, as well as diminishing insulin secretion by the pancreas and inhibiting glucose oxidation[8, 42, 60, 61]. Furthermore, there is a decreased cholesterol content of high density lipoprotein (HDL) and low density lipoprotein (LDL)[62, 63]. The etiology of hypocholesterolemia in critical illness is not completely understood and is probably multifactorial. Caloric and protein

deficiency, decreased hepatic synthesis, redistribution due to capillary leakage and an increased utilization in cell recovery and endotoxin scavenging are potential explanations[7, 62, 64, 65].

In physiological conditions triglycerides have an important role in energy provision and the lipoproteins play a key role in innate immunity, in endotoxin scavenging and transportation of lipid products[64, 66]. Besides decreasing the HDL and LDL concentrations, acute illness also changes the composition of these lipoproteins[57, 59]. Modified LDL particles are directly cytotoxic to the endothelium, whereas modified HDL particles lose their anti-atherogenic and anti-inflammatory conditions[57, 59]. The substantial change in lipid metabolism in critical illness has a potential negative effect on outcome[50, 62, 63, 67]. Specifically, hypocholesterolemia in critically ill patients is associated with increased mortality[62, 63]. The exact role of circulating triglycerides in critical illness remains ambiguous as they reflect severity of illness, but also play an important role in energy provision and altering endotoxins[61, 68].

Intensive insulin therapy corrects the disturbed serum lipid profile in critical illness (Figure 1). Foremost the increased triglycerides and FFA are almost completely suppressed[44, 60]. Insulin administration in critically ill adults increased, but not fully restored, concentrations of LDL and HDL cholesterol[44]. The effect was ascribed to a direct effect of insulin. Moreover, a multivariate logistic regression analysis showed that the positive effect of insulin therapy on dyslipidemia in critically ill patients surpassed the effect of glycemic control on morbidity and mortality[44]. Future investigations are necessary to fully understand the mechanism(s) responsible for the effects of insulin on dyslipidemia.

The dyslipidemia seen in critically ill adults is also seen in septic children and correlates with disease severity and outcome. In fifty-seven children with severe meningococcal disease (MS or MSS) very low levels of total cholesterol, HDL and LDL were found[65]. Another study in ICU patients reported increased rates of lipolysis comparable with adult studies[2]. In severely burned children insulin therapy was reported to decrease serum triglycerides and free fatty acids (FFA). Because glucose levels were between 120 to 180 mg.dL^{-1} (6.7 - 10 mmol.L^{-1}), both in the insulin and in the control groups, the decreased triglycerides and FFA can be ascribed to insulin per se (Table 3)[69]. The effect of insulin on pediatric lipid metabolism needs to be further investigated.

Improve anabolism and protein balance

Critical illness is a highly catabolic state leading to loss of lean body mass mainly due to muscle wasting and a negative protein balance. In pediatric critical illness the increase in catabolism and subsequent loss of lean body mass has been extensively reported[70-76]. Often PICU patients have already a deprived nutritional status compared to healthy children[75, 77]. Malnutrition and muscle wasting lead to prolonged mechanical ventilation, weakness and organ dysfunction[1, 75, 76, 78, 79]. After ICU discharge malnu-

trition and loss of lean body mass persists, especially in younger children and neonates[73, 75-77, 80, 81]. Development of enteral and parenteral nutrition has made it possible to provide large amounts of energy along with proteins to critically ill patients. However, increasing caloric intake or protein supply alone seems insufficient to prevent or reverse muscle breakdown[76, 82, 83]. The catabolic state and the negative protein balance are not solely explained by insufficient nutritional support but is aggravated by both hormonal and inflammatory factors. The catabolic state in critical illness is due in part to dysregulation of growth hormone (GH), insulin like growth factor (IGF) and insulin like growth factor binding protein (IGFBP). Pro-inflammatory cytokines play a significant role, inducing growth hormone resistance, decreased insulin like growth factor-I (IGF-I) and muscle sensitivity to IGF-I[84, 85]. In pediatric critical illness comparable derangements are reported[2, 69, 74, 86, 87]. These changes are not related to nutritional support and a lack of IGF-I recovery was associated with poor outcome[74, 86-88]. Insulin has anabolic properties mediated largely by its suppressive effect of insulin growth factor binding protein-1 (IGFBP-I), whereby increasing IGF-I concentration[41]. Insulin normalized low IGF-I levels in patients with diabetes mellitus[89]. In pediatric burn patients, serum IGF-I concentration was found to decrease 3–4 fold. Insulin therapy increased IGF-I and its major binding protein IGFBP-3 but had no significant effect on serum IGFBP-1 (Table 3)[69]. Critically ill adults treated with insulin had a reduced incidence of critical illness myopathy and prolonged mechanical ventilation occurred less frequently[90]. However, the somatotropic axis in these adult patients was further suppressed with insulin therapy and no obvious anabolic effects were observed, despite the beneficial effects on outcome[91]. Others have reported no effect of insulin therapy on IGFBP-I serum concentrations, which might explain why anabolic effects of insulin were not obvious in these patients[43]. The mechanisms through which insulin therapy affects the somatotropic axis remain to be established.

In critical illness whole body protein synthesis is enhanced although there is a net negative protein balance due to increased protein breakdown, which aggravates muscle wasting. There is an increased visceral (especially hepatic) protein synthesis of acute phase proteins. Pro-inflammatory cytokines stimulate the production of acute phase proteins at the expense of constitutive proteins (e.g. albumin, transferrin)[92-95]. Simultaneously, critical illness reduces protein synthesis and increases proteolysis in muscle[85, 96]. Reduced muscular protein synthesis is caused by a profound reduction of the translation initiation pathway[85, 96-98]. Mobilization of amino acids through protein breakdown serves to provide substrate for gluconeogenesis, oxidation and acute phase protein synthesis[93, 94]. In contrast with glucose and fat, no storage pool for protein exists. As a result, the amino acid pool is maintained by increased protein breakdown[94]. Proteolysis is regulated in several pathways, among these the ubiquitin-proteasome pathway has been recognized to be important in critical illness[79]. Insulin

has been shown to be important in the regulation of protein homeostasis, both in healthy individuals as well as in critically ill patients. Insulin promotes a positive protein balance provided there is a sufficient amino acid availability[99, 100]. Administration of amino acids and insulin independently stimulate protein anabolism and a combination of both appears to be more beneficial than either stimulus alone[101]. The mechanism of insulin-stimulated protein synthesis is multifactorial and reflects gene transcription, translation initiation and activation of pre-activated enzymes[102]. Insulin has also been shown to inhibit protein breakdown and does this through reducing the ubiquitin-proteasome dependent pathway of proteolysis[102]. The effect of insulin on muscle protein synthesis in healthy individuals decreases with age[103-105]. There appears to be a different response to insulin-related protein synthesis in muscle between different disease conditions. Adult septic patients have a lesser response compared to adult burn patients[93, 106, 107]. Severe thermal injury induces a pro-inflammatory acute phase response for a prolonged period extending the catabolic period[74]. Additionally, in severely burned patients hyperglycemia exacerbates muscle protein catabolism[108, 109]. The ability of insulin therapy to stimulate muscle protein synthesis and increase wound healing in adult burn patients has been well established[109-112]. The effect of insulin therapy on muscle protein metabolism and lean body mass has not been investigated in burned children. However, high-carbohydrate feeding in severely burned children resulted in decreased muscle protein breakdown, probably due to endogenous insulin response[113]. Insulin therapy in burned children decreases prolonged hepatic acute phase protein levels, but did not have a positive effect on the hepatic constitutive proteins[114]. Jeschke et al. however did report increased synthesis of hepatic constitutive proteins (Table 3)[69]. Insulin also attenuates the inflammatory response in burned children decreasing the pro-inflammatory and increasing the anti-inflammatory response[69, 115].

In adult sepsis, muscle protein synthesis is profoundly decreased and exogenous insulin has little effect in reversing muscle catabolism[93, 106, 107]. The effect of sepsis and insulin on protein synthesis in neonates has been studied in greater detail in an elegant animal model with piglets[116-118]. These studies showed that, compared to adults, sepsis in neonatal pigs elicits only modest reduction in muscle protein synthesis[118-120]. This difference can in part be explained by a maintained sensitivity to insulin stimulation[121, 122]. Similar to adult models decreased muscle protein synthesis was accompanied by a reduced translation efficiency[96, 118-120, 123]. Endotoxemia lead to an increase in hepatic protein synthesis in these piglets despite repression of translation initiation. Furthermore, this response was not influenced by insulin levels[121]. In healthy piglets insulin also did not enhance protein synthesis in the liver[124]. In contrast, in endotoxemic rats insulin significantly altered hepatic protein synthesis, increasing albumin and decreasing c-reactive protein[125]. The effect of insulin on protein synthesis in neonatal sepsis may be explained by processes other than translation initiation.

Insulin-related protein homeostasis has been studied in two different conditions in critically ill children. In extreme low birth weight infants (ELBW) receiving no protein intake, insulin reduced protein breakdown but did not enhance protein synthesis[126]. In neonates on extracorporeal membrane oxygenation insulin did improve the net protein balance, mainly by reduction of protein breakdown[127, 128]. The effect of insulin therapy on protein metabolism and lean body mass remain to be investigated in septic children of different age groups. Whether the anabolic properties of insulin can, at least partially, explain the beneficial outcome of insulin therapy in critically ill patients remains to be established.

Non-metabolic properties of insulin

Improving inflammatory reactions

Tight glucose control with insulin therapy prevented serious infections and sepsis related multi organ failure and death in adults[11, 31]. Hyperglycemia impairs leukocyte function, most specifically the capacity of monocytes and neutrophils to phagocytose and to generate oxidative bursts and decreases complement function[129, 130]. Also certain distinctive pro-inflammatory alterations, such as an increase in early pro-inflammatory cytokine levels (eg. TNF-α, IL-1b, IL-6), activation of the three major pro-inflammatory transcription factors and elevation of complement products are seen during hyperglycemia[130-133].

Improving glucose levels would therefore improve inflammation in critically ill patients. However insulin also emerges as a molecule with strong direct immunomodulating properties, improving the innate immunity. Insulin has strong anti-inflammatory properties, suppressing a range of pro-inflammatory products and some major pro-inflammatory transcription factors (eg. NF-κB), while increasing anti-inflammatory cytokines (such as IL-10)[69, 115, 134-136].

This coincides with a suppression of the hepatic acute phase response, as shown by a reduced circulating C-reactive protein (CRP) seen in patients treated with insulin[69]. Whereas CRP is an acute phase protein used as an indicator for inflammation, mannose-binding lectin (MBL) is an acute phase protein related to innate immunity and host defense by recognizing and initiating opsonization. Low MBL levels may predict poor outcome and were increased significantly by intensive insulin therapy[137]. Whether this fully explains the reduction in bacteremia and sepsis related illness seen in these patients, remains to be established.

The effect of insulin therapy on immunomodulation has not been investigated in pediatric critical illness. One study reported a significant inversed relation between endogenous insulin and an inflammatory response in children with severe meningococcal disease[38]. Further investigations are needed to determine the effect of insulin therapy on immunomodulatory processes of pediatric critical illness.

Insulin improves vascular endothelial function and coagulopathy

Multi organ failure and death in critical illness is largely attributable to vascular endothelial dysfunction[138, 139]. The endothelium controls (micro-) vascular tone and blood flow and regulates permeability to nutrients and bioactive substrates[140]. Critical illness causes endothelial dysfunction via reactive oxygen species (ROS) and pro-inflammatory cytokines and growth factors, such as vascular endothelial growth factor (VEGF)[138, 139]. Endothelial dysfunction then leads to coagulopathy, decreased organ perfusion and cellular ischemia[138-141]. Nitric oxide (NO) plays an essential role in endothelial function[139]. Its function however is very delicate, as both low as well as high concentrations are detrimental. Under normal circumstances the endothelium generates low concentrations of NO through endothelial nitric oxide synthase (eNOS). Critical illness changes NO concentrations and effects through several mechanisms. First, it induces very high concentrations of NO through inducible nitric oxide synthase (iNOS). Uncontrolled high concentrations increase cellular and vascular injury through inflammation, ROS and VEGF[142, 143]. Secondly, asymmetric dimethylarginine (ADMA) concentrations are increased in critical ill and insulin resistant patients[144-147]. ADMA is produced by methylation of arginine parts of proteins and is released to the free amino acid pool during proteolysis. ADMA inhibits NOS, directly and non-specifically[144, 145, 147, 148]. High ADMA levels were recognized as an independent risk factor for ICU mortality[144]. The probable mechanism by which increased ADMA attributes to adverse outcome is suppression of endothelial NOS and interference with physiologic functions of NO[145].

Insulin therapy maintaining normoglycemia has been shown to protect the endothelium and thus contributed to prevention of multi organ failure and poor outcome in critically ill patients[143]. This favorable effect of insulin can substantially be ascribed to improvement of the delicate NO balance. Insulin induced a dose-dependent induction of eNOS in human aortic cells (and possibly arterial/endothelial cells)[149]. This was in contrast with another study where insulin reduced plasma NO concentrations by suppressing iNOS expression, while eNOS expression was not altered[143]. The latter study however could not exclude a beneficial effect on eNOS expression as tissue biopsies were only taken from non-survivors. Another effect of insulin on the NO balance is the reduction of ADMA levels in critically ill patients and this effect contributed to the beneficial effects of insulin therapy[150]. Insulin causes this modulation probably by reduced protein breakdown, preservation of the degrading enzyme dimethylarginine dimethylaminohydrolase (DDAH) and increased uptake. Finally, insulin also reduces ROS, such as superoxide which binds with NO to form peroxynitrite[151, 152]. All these effects are beneficial to the endothelial function and thus organ function and patient outcome.

Another consequence of endothelial damage in critical illness is an increased coagulopathy[141]. Hyperglycemia induces a pro-thrombotic state with increased vascular constriction, oxidative stress and elevated platelet aggregation[153]. Insulin improves

coagulopathy in critically ill patients. Insulin has anti-thrombotic and fibrinolytic effects by suppressing plasma tissue factor and plasminogen activator inhibitor-1[152, 154]. Endothelial dysfunction and vascular complications are seen in children with diabetes[155]. The detrimental effects of poor glycemic control on oxidative stress have been reported in pediatric studies. Markers for endothelial (intracellular cell adhesion molecule (ICAM), vascular endothelial growth factor (VEGF) as well as oxidative stress (Malondialdehyde, MDA) were increased in poorly controlled diabetic children[156-158]. Furthermore, anti-oxidant capacity and glutathione-peroxidase were significantly lower in diabetic children compared with healthy children[158]. However, the impact of insulin therapy on endothelial function, nitric oxide balance and coagulopathy in critically ill children has not been reported yet.

Conclusion

The beneficial effect of insulin administration on the outcome in critically ill patients has led to widespread implementation of this therapy in the ICU. Since then, our knowledge of several changes occurring in metabolic pathways during critical illness has increased significantly. However, these mechanistic and therapeutic approaches cannot be simply extrapolated to the pediatric population without validation. Most of the changes in metabolic pathways occurring in adult critical illness are likely to occur in critically ill children as well. However, some important differences are apparent for an attending pediatric intensivist in the treatment of a critically ill child and many questions remain to be answered. Hyperglycemia has been shown to be associated with increased morbidity and mortality in a diverse clinical spectrum of pediatric critical illness. Although a recent large randomized trial showed clear improvement of short-term outcome, the high frequency of (severe) hypoglycemia remains reason of concern. The results of other prospective studies in children on a tight glucose regimen are in anticipation to indicate whether the positive effects shown in adult patients will also count for the pediatric population. Moreover, the responsible pathways explaining the beneficial outcome of insulin treatment in critically ill patients need to be elucidated and compared with pediatric research data. Finally, the effects of various metabolic and non-metabolic properties of insulin in critically ill children of different age groups need to be studied. Although the effect of insulin on protein homeostasis and dyslipidemia in critical illness seems promising, the possible consequences for nutritional support need to be resolved. Furthermore, these metabolic properties are closely related to endothelial function and inflammation, both of which are affected by insulin as well. As pediatric critical illness involves changes in these pathways as well, these effects should also be addressed in future studies.

To achieve comparable results and answers, these studies should use and report glucose and energy requirements adapted to age and clinical condition. In anticipation of further evidence on thresholds of blood glucose levels, we propose to treat children in the ICU with insulin using a blood glucose threshold of 150 mg.dL^{-1} (8.3 mmol.L^{-1}) and are currently conducting studies with these thresholds.

References

1. Biolo G, Toigo G, Ciocchi B, et al. Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition 1997;13(9 Suppl):52S-7S.
2. Cogo PE, Carnielli VP, Rosso F, et al. Protein turnover, lipolysis, and endogenous hormonal secretion in critically ill children. Crit Care Med 2002;30(1):65-70.
3. Klein GW, Hojsak JM, Rapaport R. Hyperglycemia in the pediatric intensive care unit. Curr Opin Clin Nutr Metab Care 2007;10(2):187-92.
4. Mizock BA. Metabolic derangements in sepsis and septic shock. Crit Care Clin 2000;16(2):319-36, vii.
5. Mizock BA. Alterations in fuel metabolism in critical illness: hyperglycaemia. Best Pract Res Clin Endocrinol Metab 2001; 15(4):533-51.
6. Montori VM, Bistrian BR, McMahon MM. Hyperglycemia in acutely ill patients. Jama 2002;288(17):2167-9.
7. van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003; 31(5):1359-66.
8. Wolfe RR, Martini WZ. Changes in intermediary metabolism in severe surgical illness. World J Surg 2000;24(6):639-47.
9. Gore DC, Chinkes D, Heggers J, Herndon DN, Wolf SE, Desai M. Association of hyperglycemia with increased mortality after severe burn injury. J Trauma 2001; 51(3):540-4.
10. Preiser JC, Devos P, Van den Berghe G. Tight control of glycaemia in critically ill patients. Curr Opin Clin Nutr Metab Care 2002;5(5):533-7.
11. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001; 345(19):1359-67.
12. Branco RG, Garcia PC, Piva JP, Casartelli CH, Seibel V, Tasker RC. Glucose level and risk of mortality in pediatric septic shock. Pediatr Crit Care Med 2005;6(4): 470-2.
13. Cochran A, Scaife ER, Hansen KW, Downey EC. Hyperglycemia and outcomes from pediatric traumatic brain injury. J Trauma 2003;55(6):1035-8.
14. Faustino EV, Apkon M. Persistent hyperglycemia in critically ill children. J Pediatr 2005;146(1):30-4.
15. Srinivasan V, Spinella PC, Drott HR, Roth CL, Helfaer MA, Nadkarni V. 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.
16. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, Kache S, Wilson DM. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. Pediatrics 2006;118(1):173-9.
17. Yates AR, Dyke PC, 2nd, Taeed R, et al. Hyperglycemia is a marker for poor outcome in the postoperative pediatric

cardiac patient. Pediatr Crit Care Med 2006;7(4):351-5.
18. Alaedeen DI, Walsh MC, Chwals WJ. Total parenteral nutrition-associated hyperglycemia correlates with prolonged mechanical ventilation and hospital stay in septic infants. J Pediatr Surg 2006; 41(1):239-44; discussion -44.
19. Hall NJ, Peters M, Eaton S, Pierro A. Hyperglycemia is associated with increased morbidity and mortality rates in neonates with necrotizing enterocolitis. J Pediatr Surg 2004;39(6):898-901; discussion 898-901.
20. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373(9663):547-56.
21. Pham TN, Warren AJ, Phan HH, Molitor F, Greenhalgh DG, Palmieri TL. Impact of tight glycemic control in severely burned children. J Trauma 2005;59(5):1148-54.
22. Binder ND, Raschko PK, Benda GI, Reynolds JW. Insulin infusion with parenteral nutrition in extremely low birth weight infants with hyperglycemia. J Pediatr 1989;114(2):273-80.
23. Collins JW, Jr., Hoppe M, Brown K, Edidin DV, Padbury J, Ogata ES. A controlled trial of insulin infusion and parenteral nutrition in extremely low birth weight infants with glucose intolerance. J Pediatr 1991;118(6):921-7.
24. Meetze W, Bowsher R, Compton J, Moorehead H. Hyperglycemia in extremely- low-birth-weight infants. Biol Neonate 1998;74(3):214-21.
25. Ng SM, May JE, Emmerson AJ. Continuous insulin infusion in hyperglycaemic extremely-low- birth-weight neonates. Biol Neonate 2005;87(4):269-72.
26. Thabet F, Bourgeois J, Guy B, Putet G. Continuous insulin infusion in hyperglycaemic very-low-birth-weight infants receiving parenteral nutrition. Clin Nutr 2003;22(6):545-7.
27. Vaucher YE, Walson PD, Morrow G, 3rd. Continuous insulin infusion in hyperglycemic, very low birth weight infants. J Pediatr Gastroenterol Nutr 1982;1(2): 211-7.
28. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, et al. Early insulin therapy in very-low-birth-weight infants. N Engl J Med 2008;359(18):1873-84.
29. Soghier LM, Vega M, Aref K, et al. Diffuse basal ganglia or thalamus hyperechogenicity in preterm infants. J Perinatol 2006;26(4):230-6.
30. Finney SJ, Zekveld C, Elia A, Evans TW. Glucose control and mortality in critically ill patients. Jama 2003;290(15):2041-7.
31. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. N Engl J Med 2006;354(5): 449-61.
32. Van den Berghe G, Wilmer A, Milants I, et al. Intensive insulin therapy in mixed medical/surgical intensive care units: benefit versus harm. Diabetes 2006; 55(11):3151-9.
33. Vriesendorp TM, DeVries JH, van Santen S, et al. Evaluation of short-term consequences of hypoglycemia in an intensive care unit. Crit Care Med 2006;34(11): 2714-8.
34. Bagshaw SM, Egi M, George C, Bellomo R. Early blood glucose control and mortality in critically ill patients in Australia. Crit Care Med 2009;37(2):463-70.
35. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 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.
36. Verhoeven JJ, Brand JB, van de Polder MM, Joosten KF. Management of hyperglycemia in the pediatric intensive care unit; implementation of a glucose control protocol. Pediatr Crit Care Med 2009; 10(6):648-52.
37. Joosten KF, de Kleijn ED, Westerterp 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.
38. van Waardenburg DA, Jansen TC, Vos GD, Buurman WA. Hyperglycemia in children with meningococcal sepsis and septic shock: the relation between plasma levels of insulin and inflammatory mediators. J Clin Endocrinol Metab 2006;91(10):3916-21.
39. Sheridan RL, Yu YM, Prelack K, Young VR, Burke JF, Tompkins RG. Maximal parenteral glucose oxidation in hypermetabolic young children: a stable isotope study. JPEN J Parenter Enteral Nutr 1998;22(4):212-6.
40. Donmoyer CM, Chen SS, Lacy DB, et al. Infection impairs insulin-dependent hepatic glucose uptake during total parenteral nutrition. Am J Physiol Endocrinol Metab 2003;284(3):E574-82.
41. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004;114(9): 1187-95.
42. Andreelli F, Jacquier D, Troy S. Molecular aspects of insulin therapy in critically ill patients. Curr Opin Clin Nutr Metab Care 2006;9(2):124-30.
43. Mesotten D, Delhanty PJ, Vanderhoydonc F, et al. Regulation of insulin-like growth factor binding protein-1 during protracted critical illness. J Clin Endocrinol Metab 2002;87(12):5516-23.
44. Mesotten D, Swinnen JV, Vanderhoydonc F, Wouters PJ, Van den Berghe G. Contribution of circulating lipids to the improved outcome of critical illness by glycemic control with intensive insulin therapy. J Clin Endocrinol Metab 2004;89(1):219-26.
45. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet 2005; 365(9453):53-9.
46. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360(9328): 219-23.
47. Fram RY, Cree MG, Wolfe RR, et al. Intensive insulin therapy improves insulin sensitivity and mitochondrial function in severely burned children. Crit Care Med 2010;38(6):1475-83.
48. Ellger B, Debaveye Y, Vanhorebeek I, et al. Survival benefits of intensive insulin therapy in critical illness: impact of maintaining normoglycemia versus glycemia-independent actions of insulin. Diabetes 2006;55(4):1096-105.
49. Van den Berghe G, Wouters PJ, Bouillon R, et al. Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. Crit Care Med 2003;31(2):359-66.
50. Vanhorebeek I, Langouche L, Van den Berghe G. Glycemic and nonglycemic effects of insulin: how do they contribute to a better outcome of critical illness? Curr Opin Crit Care 2005;11(4):304-11.
51. Van den Berghe G, Schoonheydt K, Becx P, Bruyninckx F, Wouters PJ. Insulin therapy protects the central and peripheral nervous system of intensive care patients. Neurology 2005;64(8):1348-53.

52. Carcillo JA. What's new in pediatric intensive care. Crit Care Med 2006;34(9 Suppl):S183-90.
53. Goldhill DR, Sumner A. Outcome of intensive care patients in a group of British intensive care units. Crit Care Med 1998; 26(8):1337-45.
54. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
55. Ruttimann UE, Patel KM, Pollack MM. Length of stay and efficiency in pediatric intensive care units. J Pediatr 1998; 133(1):79-85.
56. Wong DT, Gomez M, McGuire GP, Kavanagh B. Utilization of intensive care unit days in a Canadian medical-surgical intensive care unit. Crit Care Med 1999; 27(7):1319-24.
57. Carpentier YA, Scruel O. Changes in the concentration and composition of plasma lipoproteins during the acute phase response. Curr Opin Clin Nutr Metab Care 2002;5(2):153-8.
58. Khovidhunkit W, Kim MS, Memon RA, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45(7):1169-96.
59. Khovidhunkit W, Memon RA, Feingold KR, Grunfeld C. Infection and inflammation-induced proatherogenic changes of lipoproteins. J Infect Dis 2000;181 Suppl 3:S462-72.
60. Krogh-Madsen R, Moller K, Dela F, Kronborg G, Jauffred S, Pedersen BK. Effect of hyperglycemia and hyperinsulinemia on the response of IL-6, TNF-alpha, and FFAs to low-dose endotoxemia in humans. Am J Physiol Endocrinol Metab 2004;286(5):E766-72.
61. Wolfe RR, Shaw JH, Durkot MJ. Energy metabolism in trauma and sepsis: the role of fat. Prog Clin Biol Res 1983;111: 89-109.
62. Gui D, Spada PL, De Gaetano A, Pacelli F. Hypocholesterolemia and risk of death in the critically ill surgical patient. Intensive Care Med 1996;22(8):790-4.
63. Chien JY, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. Crit Care Med 2005;33(8): 1688-93.
64. Giovannini I, Boldrini G, Chiarla C, Giuliante F, Vellone M, Nuzzo G. Pathophysiologic correlates of hypocholesterolemia in critically ill surgical patients. Intensive Care Med 1999;25(7):748-51.
65. Vermont CL, den Brinker M, Kakeci N, et al. Serum lipids and disease severity in children with severe meningococcal sepsis. Crit Care Med 2005;33(7): 1610-5.
66. Tulenko TN, Sumner AE. The physiology of lipoproteins. J Nucl Cardiol 2002;9(6): 638-49.
67. Gordon BR, Parker TS, Levine DM, et al. Low lipid concentrations in critical illness: implications for preventing and treating endotoxemia. Crit Care Med 1996;24(4): 584-9.
68. Lind L, Lithell H. Impaired glucose and lipid metabolism seen in intensive care patients is related to severity of illness and survival. Clin Intensive Care 1994; 5(3):100-5.
69. Jeschke MG, Klein D, Herndon DN. Insulin treatment improves the systemic inflammatory reaction to severe trauma. Ann Surg 2004;239(4):553-60.
70. Coss-Bu JA, Jefferson LS, Walding D, David Y, Smith EO, Klish WJ. Resting energy expenditure and nitrogen balance in critically ill pediatric patients on mechanical ventilation. Nutrition 1998;14(9):649-52.
71. Coss-Bu JA, Klish WJ, Walding D, Stein F, Smith EO, Jefferson LS. Energy metabolism, nitrogen balance, and substrate utilization in critically ill children. Am J Clin Nutr 2001;74(5):664-9.

72. Joosten KF, Verhoeven JJ, Hazelzet JA. Energy expenditure and substrate utilization in mechanically ventilated children. Nutrition 1999;15(6):444-8.
73. 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(6):1381-9.
74. Jeschke MG, Barrow RE, Herndon DN. Extended hypermetabolic response of the liver in severely burned pediatric patients. Arch Surg 2004;139(6):641-7.
75. Hulst J, Joosten K, Zimmermann L, et al. Malnutrition in critically ill children: from admission to 6 months after discharge. Clin Nutr 2004;23(2):223-32.
76. 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.
77. Pollack MM, Wiley JS, Holbrook PR. Early nutritional depletion in critically ill children. Crit Care Med 1981;9(8):580-3.
78. 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.
79. Hasselgren PO, Fischer JE. Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. Ann Surg 2001;233(1):9-17.
80. Oosterveld MJ, Van Der Kuip M, De Meer K, De Greef HJ, Gemke RJ. Energy expenditure and balance following pediatric intensive care unit admission: a longitudinal study of critically ill children. Pediatr Crit Care Med 2006;7(2):147-53.
81. Pollack MM, Wiley JS, Kanter R, Holbrook PR. Malnutrition in critically ill infants and children. JPEN J Parenter Enteral Nutr 1982;6(1):20-4.
82. Hoffer LJ. Protein and energy provision in critical illness. Am J Clin Nutr 2003;78(5):906-11.
83. Hadley JS, Hinds CJ. Anabolic strategies in critical illness. Curr Opin Pharmacol 2002;2(6):700-7.
84. Thissen JP. How proinflammatory cytokines may impair growth and cause muscle wasting. Horm Res 2007;67 Suppl 1:64-70.
85. Vary TC. Regulation of skeletal muscle protein turnover during sepsis. Curr Opin Clin Nutr Metab Care 1998;1(2):217-24.
86. de Groof F, Joosten KF, Janssen JA, 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.
87. Hulst JM, van Goudoever JB, Visser TJ, Tibboel D, Joosten KF. 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.
88. Jeschke MG, Barrow RE, Mlcak RP, Herndon DN. Endogenous anabolic hormones and hypermetabolism: effect of trauma and gender differences. Ann Surg 2005;241(5):759-67; discussion 67-8.
89. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab 1994;79(3):872-8.
90. Hermans G, Wilmer A, Meersseman W, et al. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. Am J Respir Crit Care Med 2007;175(5):480-9.
91. Mesotten D, Wouters PJ, Peeters RP, et al. Regulation of the somatotropic axis by intensive insulin therapy during protracted

critical illness. J Clin Endocrinol Metab 2004;89(7):3105-13.
92. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340(6): 448-54.
93. Wray CJ, Mammen JM, Hasselgren PO. Catabolic response to stress and potential benefits of nutrition support. Nutrition 2002;18(11-12):971-7.
94. Hasselgren PO, Pedersen P, Sax HC, Warner BW, Fischer JE. Current concepts of protein turnover and amino acid transport in liver and skeletal muscle during sepsis. Arch Surg 1988;123(8):992-9.
95. Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol 1992; 262(2 Pt 1):C445-52.
96. Vary TC, Deiter G, Lang CH. Cytokine-triggered decreases in levels of phosphorylated eukaryotic initiation factor 4G in skeletal muscle during sepsis. Shock 2006;26(6):631-6.
97. Vary TC, Deiter G, Kimball SR. Phosphorylation of eukaryotic initiation factor eIF2Bepsilon in skeletal muscle during sepsis. Am J Physiol Endocrinol Metab 2002;283(5):E1032-9.
98. Vary TC, Kimball SR. Effect of sepsis on eIE4E availability in skeletal muscle. Am J Physiol Endocrinol Metab 2000;279(5): E1178-84.
99. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab 2006;291(4):E745-54.
100. Bell JA, Fujita S, Volpi E, Cadenas JG, Rasmussen BB. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. Am J Physiol Endocrinol Metab 2005;289(6): E999-1006.
101. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78(2):250-8.
102. Wolfe RR. Effects of insulin on muscle tissue. Curr Opin Clin Nutr Metab Care 2000;3(1):67-71.
103. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. J Clin Endocrinol Metab 2000; 85(12):4481-90.
104. Wray-Cahen D, Beckett PR, Nguyen HV, Davis TA. Insulin-stimulated amino acid utilization during glucose and amino acid clamps decreases with development. Am J Physiol 1997;273(2 Pt 1):E305-14.
105. Wray-Cahen D, Nguyen HV, Burrin DG, et al. Response of skeletal muscle protein synthesis to insulin in suckling pigs decreases with development. Am J Physiol 1998;275(4 Pt 1):E602-9.
106. Vary TC, Jefferson LS, Kimball SR. Insulin fails to stimulate muscle protein synthesis in sepsis despite unimpaired signaling to 4E-BP1 and S6K1. Am J Physiol Endocrinol Metab 2001;281(5): E1045-53.
107. Hasselgren PO, Fischer JE. Regulation by insulin of muscle protein metabolism during sepsis and other catabolic conditions. Nutrition 1992;8(6):434-9.
108. Gore DC, Chinkes DL, Hart DW, Wolf SE, Herndon DN, Sanford AP. Hyperglycemia exacerbates muscle protein catabolism in burn-injured patients. Crit Care Med 2002;30(11):2438-42.
109. Gore DC, Wolf SE, Herndon DN, Wolfe RR. Relative influence of glucose and insulin on peripheral amino acid metabo-

lism in severely burned patients. JPEN J Parenter Enteral Nutr 2002;26(5):271-7.
110. Gore DC, Wolf SE, Sanford AP, Herndon DN, Wolfe RR. Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. Am J Physiol Endocrinol Metab 2004;286(4):E529-34.
111. Sakurai Y, Aarsland A, Herndon DN, et al. Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients. Ann Surg 1995;222(3): 283-94; 94-7.
112. Thomas SJ, Morimoto K, Herndon DN, et al. The effect of prolonged euglycemic hyperinsulinemia on lean body mass after severe burn. Surgery 2002;132(2): 341-7.
113. Hart DW, Wolf SE, Chinkes DL, et al. Effects of early excision and aggressive enteral feeding on hypermetabolism, catabolism, and sepsis after severe burn. J Trauma 2003;54(4):755-61; discussion 61-4.
114. Wu X, Thomas SJ, Herndon DN, Sanford AP, Wolf SE. Insulin decreases hepatic acute phase protein levels in severely burned children. Surgery 2004;135(2): 196-202.
115. Jeschke MG, Einspanier R, Klein D, Jauch KW. Insulin attenuates the systemic inflammatory response to thermal trauma. Mol Med 2002;8(8):443-50.
116. O'Connor PM, Bush JA, Suryawan A, Nguyen HV, Davis TA. Insulin and amino acids independently stimulate skeletal muscle protein synthesis in neonatal pigs. Am J Physiol Endocrinol Metab 2003;284(1):E110-9.
117. O'Connor PM, Kimball SR, Suryawan A, et al. Regulation of translation initiation by insulin and amino acids in skeletal muscle of neonatal pigs. Am J Physiol Endocrinol Metab 2003;285(1):E40-53.
118. Orellana RA, Kimball SR, Nguyen HV, et al. Regulation of muscle protein synthesis in neonatal pigs during prolonged endotoxemia. Pediatr Res 2004;55(3):442-9.
119. Orellana RA, O'Connor PM, Nguyen HV, et al. Endotoxemia reduces skeletal muscle protein synthesis in neonates. Am J Physiol Endocrinol Metab 2002; 283(5):E909-16.
120. Kimball SR, Orellana RA, O'Connor PM, et al. Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs. Am J Physiol Endocrinol Metab 2003;285(3):E637-44.
121. Orellana RA, Kimball SR, Suryawan A, et al. Insulin stimulates muscle protein synthesis in neonates during endotoxemia despite repression of translation initiation. Am J Physiol Endocrinol Metab 2007;292(2):E629-36.
122. Orellana RA, O'Connor PM, Bush JA, et al. Modulation of muscle protein synthesis by insulin is maintained during neonatal endotoxemia. Am J Physiol Endocrinol Metab 2006;291(1):E159-66.
123. Lang CH, Frost RA. Sepsis-induced suppression of skeletal muscle translation initiation mediated by tumor necrosis factor alpha. Metabolism 2007;56(1): 49-57.
124. O'Connor PM, Kimball SR, Suryawan A, et al. Regulation of neonatal liver protein synthesis by insulin and amino acids in pigs. Am J Physiol Endocrinol Metab 2004;286(6):E994-E1003.
125. Jeschke MG, Rensing H, Klein D, et al. Insulin prevents liver damage and preserves liver function in lipopolysaccharide-induced endotoxemic rats. J Hepatol 2005;42(6):870-9.
126. Poindexter BB, Karn CA, Denne SC. Exogenous insulin reduces proteolysis and protein synthesis in extremely low birth weight infants. J Pediatr 1998;132(6): 948-53.
127. Agus MS, Javid PJ, Piper HG, et al. The effect of insulin infusion upon protein

metabolism in neonates on extracorporeal life support. Ann Surg 2006;244(4): 536-44.
128. Agus MS, Javid PJ, Ryan DP, Jaksic T. Intravenous insulin decreases protein breakdown in infants on extracorporeal membrane oxygenation. J Pediatr Surg 2004;39(6):839-44; discussion -44.
129. Weekers F, Giulietti AP, Michalaki M, et al. Metabolic, endocrine, and immune effects of stress hyperglycemia in a rabbit model of prolonged critical illness. Endocrinology 2003;144(12):5329-38.
130. Turina M, Fry DE, Polk HC, Jr. Acute hyperglycemia and the innate immune system: clinical, cellular, and molecular aspects. Crit Care Med 2005;33(7): 1624-33.
131. Dandona P. The link between insulin resistance syndrome and inflammatory markers. Endocr Pract 2003;9 Suppl 2: 53-7.
132. Dandona P, Aljada A, Dhindsa S, Garg R. Insulin as an anti-inflammatory and antiatherosclerotic hormone. Clin Cornerstone 2003;Suppl 4:S13-20.
133. Turina M, Miller FN, Tucker C, Polk HC. Effects of hyperglycemia, hyperinsulinemia, and hyperosmolarity on neutrophil apoptosis. Surg Infect (Larchmt) 2006;7(2):111-21.
134. Su F, Wang Z, Bruhn A, Verdant C, Cai Y, Vincent JL. Euglycemic hyperinsulinemia in severe sepsis and septic shock. Eur Surg Res 2006;38(6):495-502.
135. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin attenuates the systemic inflammatory response in endotoxemic rats. Endocrinology 2004;145(9): 4084-93.
136. Soop M, Duxbury H, Agwunobi AO, et al. Euglycemic hyperinsulinemia augments the cytokine and endocrine responses to endotoxin in humans. Am J Physiol Endocrinol Metab 2002;282(6): E1276-85.
137. Hansen TK, Thiel S, Wouters PJ, Christiansen JS, Van den Berghe G. Intensive insulin therapy exerts antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. J Clin Endocrinol Metab 2003;88(3):1082-8.
138. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood 2003;101(10): 3765-77.
139. Vallet B. Bench-to-bedside review: endothelial cell dysfunction in severe sepsis: a role in organ dysfunction? Crit Care 2003;7(2):130-8.
140. Vallet B. Endothelial cell dysfunction and abnormal tissue perfusion. Crit Care Med 2002;30(5 Suppl):S229-34.
141. Vallet B, Wiel E. Endothelial cell dysfunction and coagulation. Crit Care Med 2001;29(7 Suppl):S36-41.
142. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. Diabetes Metab Res Rev 2006;22(6): 423-36.
143. Langouche L, Vanhorebeek I, Vlasselaers D, et al. Intensive insulin therapy protects the endothelium of critically ill patients. J Clin Invest 2005;115(8):2277-86.
144. Nijveldt RJ, Teerlink T, Van Der Hoven B, et al. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. Clin Nutr 2003;22(1):23-30.
145. Nijveldt RJ, Teerlink T, van Leeuwen PA. The asymmetrical dimethylarginine (ADMA)-multiple organ failure hypothesis. Clin Nutr 2003;22(1):99-104.
146. Stuhlinger MC, Abbasi F, Chu JW, et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. Jama 2002;287(11):1420-6.
147. Siroen MP, Teerlink T, Nijveldt RJ, Prins HA, Richir MC, van Leeuwen PA. The

clinical significance of asymmetric dimethylarginine. Annu Rev Nutr 2006;26: 203-28.
148. Nijveldt RJ, Teerlink T, Siroen MP, van Lambalgen AA, Rauwerda JA, van Leeuwen PA. The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). Clin Nutr 2003; 22(1):17-22.
149. Aljada A, Dandona P. Effect of insulin on human aortic endothelial nitric oxide synthase. Metabolism 2000;49(2):147-50.
150. Siroen MP, van Leeuwen PA, Nijveldt RJ, Teerlink T, Wouters PJ, Van den Berghe G. Modulation of asymmetric dimethylarginine in critically ill patients receiving intensive insulin treatment: a possible explanation of reduced morbidity and mortality? Crit Care Med 2005;33(3): 504-10.
151. Dandona P, Mohanty P, Chaudhuri A, Garg R, Aljada A. Insulin infusion in acute illness. J Clin Invest 2005;115(8): 2069-72.
152. Dandona P, Aljada A, Mohanty P, et al. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 2001;86(7): 3257-65.
153. Dandona P, Aljada A. Advances in diabetes for the millennium: diabetes and the endothelium. MedGenMed 2004;6(3 Suppl):6.
154. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. J Clin Endocrinol Metab 2002;87(3):1419-22.
155. Pomilio M, Mohn A, Verrotti A, Chiarelli F. Endothelial dysfunction in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2002;15(4):343-61.
156. Erciyas F, Taneli F, Arslan B, Uslu Y. Glycemic control, oxidative stress, and lipid profile in children with type 1 diabetes mellitus. Arch Med Res 2004;35(2): 134-40.
157. Seckin D, Ilhan N, Ilhan N, Ertugrul S. Glycaemic control, markers of endothelial cell activation and oxidative stress in children with type 1 diabetes mellitus. Diabetes Res Clin Pract 2006;73(2):191-7.
158. Varvarovska J, Racek J, Stetina R, et al. Aspects of oxidative stress in children with type 1 diabetes mellitus. Biomed Pharmacother 2004;58(10):539-45.

Chapter 3

The efficacy and safety of a tight glucose control protocol in critically ill term infants

Sascha CAT Verbruggen
Lonneke J Landzaat
Irwin KM Reiss
Johannes B van Goudoever
Koen FM Joosten

Submitted

Abstract

Objectives

Treating hyperglycemia in critically ill children improved outcome, despite an increased incidence of hypoglycemia, especially in infants. We evaluated the effectivity and safety of a tight glucose protocol in critically ill term infants.

Design

Term hyperglycemic infants (0 – 28 days) treated with a tight glucose protocol during a 3.5-year period in a tertiary PICU were retrospectively analyzed.

Participants

73 term hyperglycemic infants (age 0 days (0-6 d), 3.2 ± 0.8 kg, PRISM III 16 (11 – 20)) were included for analysis.

Main outcome measures

Protocol efficacy and incidence of hypoglycemia.

Results

The initial glucose level was 11.1 (9.6-15.2) mmol.L^{-1}, and normoglycemia (< 8 mmol.L^{-1}) was reached within 5.3h (range 1 – 25h) with an overall treatment duration of 27h (10 – 57h). Seven hypoglycemic incidents (5 times ≤ 2.2 mmol.L^{-1}, 2 times < 1.7 mmol.L^{-1}) occurred in five (6.7%) infants, with no effect on clinical outcome. Eighteen infants died (25%); one infant who had developed hypoglycemia before and 17 infants without hypoglycemia died. Three hypoglycemic incidents were directly explained due to a protocol violation. One hypoglycemic incident occurred in the onset of sepsis, while no apparent cause was identified for three hypoglycemic incidents.

Conclusions

Our glucose protocol was effective, but hypoglycemia occurred more frequently than in older children reported previously. Potential differences in glucose and insulin metabolism in term infants appear to justify additional safety approaches, while awaiting further studies assessing the benefits of tight glucose protocols in this population. Meanwhile, we decreased the initial insulin starting doses in our protocol.

Introduction

Hyperglycemia has been associated with increased morbidity and mortality in critically ill adults[1], children[2] and neonates[3]. A tight glucose regimen with insulin therapy improved outcome in critically ill children[4], despite the occurrence of hypoglycemia (≤ 2.2 mmol.L^{-1} (40 mg.dL^{-1})) and severe hypoglycemia (≤ 1.7 mmol.L^{-1} (31 mg.dL^{-1})) in 25% and 5% of the children, predominantly (>80%) in infants[4]. A study in hyperglycemic very low birth weight (VLBW) neonates was discontinued early because of an increased incidence of hypoglycemia (29% vs. 17%) and parenchymal abnormalities detected with cranial ultrasound in those treated with insulin[5].

Young age has been recognized for long as a risk factor for developing hypoglycemia[6, 7]. Furthermore, the young child's developing brain is more susceptible to hypoglycemia and may result in permanent damage[8-10].

Glucose control protocols designed to maintain normoglycemia while minimizing glucose variability and hypoglycemic incidents are therefore of the essence in this population. Simple model-based, computer-assisted or nurse driven protocols have been reported in critically ill adults[11-16] and children[17-19]. To date no studies have shown feasibility and safety of these protocols specifically in critically ill newborn infants. Therefore we evaluated the efficacy (time to achieve normoglycemia, duration of therapy) and safety (hypoglycemic incidents, protocol violations) of a tight glucose protocol in hyperglycemic term infants less than 28 days old.

Methods

Patients

The Pediatric Intensive Care Unit (PICU) at Erasmus MC-Sophia Children's hospital in Rotterdam, The Netherlands is a 34-bed medical and surgical ICU. Term infants less than 28 days old, admitted to our PICU from January 2006 to September 2009, and treated with our tight glucose protocol were retrospectively evaluated.

Insulin Protocol

The term infants received glucose according to our protocol (4-6 mg.kg^{-1}.min^{-1}) and were treated with a step-wise nurse driven glucose control protocol which was previously published[19]. Briefly, infants with sepsis, multiple organ failure, and/or receiving mechanical ventilation were treated after two consecutive blood glucose levels > 8 mmol.L^{-1} (>145 mg.dL^{-1}) within one hour. Depending on the blood glucose level at start of treatment, insulin was started at a dose ranging from 20 to 50 mIU.kg^{-1}.h^{-1}. Thereafter, the nurse was allowed to adjust the insulin rate according to the nurse-driven

protocol up to a rate of 200 mIU.kg^{-1}.h^{-1} after which the attending physician needed to be consulted. Blood glucose levels were checked hourly until the target range (4–8 mmol/L^{-1} (72–145 mg.dL^{-1})) was achieved for three consecutive measurements, after which measurements were performed every 3h. Insulin therapy was stopped at any time according to clearly defined criteria in the protocol[19].

Blood glucose analysis and definitions

Blood glucose measurements were obtained as soon as possible after admission from indwelling arterial catheters or from a capillary puncture and measured on a blood gas analyzer (ABL 625; Radiometer, Copenhagen, Denmark) or a point-of-care bedside system (HemoCue AB, Sweden).

Normoglycemia was defined as blood glucose levels between 4 and 8 mmol.L^{-1} (72–145 mg.dL^{-1}). Time to reach normoglycemia was defined as the time from start of insulin therapy until the first blood glucose level < 8 mmol.L^{-1} (< 145 mg.dL^{-1}). Hypoglycemia was defined as blood glucose ≤ 2.2 mmol.L^{-1} (≤ 40 mg.dL^{-1}) and severe hypoglycemia was defined as blood glucose < 1.7 mmol.L^{-1} (< 31 mg.dL^{-1})[4]. Symptoms of hypoglycemia were defined as mild (sweating, agitation, lethargy), severe (hemodynamic deterioration, neurological deteriorations (convulsions, coma)) or death. Measurements showing blood glucose levels < 2.6 mmol.L^{-1} (47 mg.dL^{-1}) or > 15 mmol.L^{-1} (272 mg.dL^{-1}) were repeated immediately on the blood gas analyzer. Infants with blood glucose levels < 2.6 (47 mg.dL^{-1}) were given a bolus of dextrose 10% 5 ml.kg^{-1} over 10 minutes.

Data collection

Patients were assessed by the Pediatric Risk of Mortality III (PRISM III) score[20], which is a validated measure of the severity of multiple organ dysfunction in PICU's. Hypoglycemic incidents were recorded and analyzed for protocol violations. Based on our previous evaluation of the glucose protocol in children[19], we focused on two types of protocol violations (incorrect insulin starting dose, inadequate insulin adjustment/discontinuation).

Statistical analysis

Data are presented as medians with interquartile ranges, unless specified otherwise. Statistical significance was considered at $p < 0.05$. Comparisons between infants with and without hypoglycemia were made with the Mann Whitney U test. Data were analyzed using a standard analysis software program (SPSS version 17.0 for Windows, SPSS, Chicago, IL.). The study was approved by the Medical Ethics Committee of Erasmus Medical Center, and was registered in the Dutch trial register (www.trialregister.nl) under registration number NTR2400.

Results

Patients

In the 44 month period 4919 children were admitted to our PICU, of which 799 (16.2%) were less than 28 days old. In total 383 children (7.8%; age 4.9 y; 0–18) were treated for hyperglycemia. Of the 799 infants, 73 (9.1%; 0 days (0–6 d), weight 3.2 ± 0.8 kg) were treated with the nurse-driven glucose control protocol. Their characteristics are shown in Table 1. Overall mortality was high (25%) in the hyperglycemic infants, diagnosed with various medical and surgical diagnoses (Table 2).

Intake

Overall glucose intake was 4.7 ± 2.3 mg.kg^{-1}.min^{-1} and not different in the infants who developed hypoglycemia (Table 1). At start of the insulin therapy, 5 infants received full continuous enteral feeding, 37 received full parenteral nutrition with amino acids (Primene; Baxter inc. Utrecht, The Netherlands) and lipids (Intralipid; Fresenius Kabi inc., Utrecht, The Netherlands), and 4 were receiving combined (par)enteral nutrition. Thirty-three patients received no nutrients other than parenteral glucose.

Table 1. Characteristics of infants (0 – 28 days) treated according to glucose control protocol*

	Normoglycemic (n = 68)	Hypoglycemic (n = 5)	All infants (n = 73)	*p* value‡
Male : Female	40 : 28	2 : 3	42 : 31	.41
Age on admission to PICU (days)	0 (0–5)	1 (0–21)	0 (0–6)	.38
Weight (kg; mean ± SD)	3.2±0.8	3.0±0.5	3.2±0.8	.31
PRISM III †	15 (12–22)	10 (5–12)	14 (11–20)	.02
Glucocorticoids n = (%)	40 (59%)	2 (40%)	42 (58%)	.41
Glucose intake (mg.kg^{-1}.min^{-1}; mean ± SD)	4.7±2.4	5.0±1.1	4.7±2.3	.37
Time to start insulin infusion after first hyperglycemic incident (h)	5.8 (2.5–11)	9.3 (2.3–25.4)	5.8 (2.5–11.0)	.53
Time to achieve normoglycemia (h)	5.3 (2.6–8.0)	6.1 (3.7–10.1)	5.3 (2.7–8.5)	.40
Length of stay (days)	15.5 (6.3–29.3)	7.0 (4.5–30.0)	15.0 (6.5–29.5)	.54
Glucose level at start of insulin therapy	11.2 (9.6–15.4)	10.8 (9.6–11.7)	11.1 (9.6–15.2)	.49
Initial insulin dose (mIU.kg^{-1}.h^{-1})	20 (20–30)	20 (15–20)	20 (20–30)	.13
Maximum insulin dose (mIU.kg^{-1}.h^{-1})	50 (30–88)	60 (45–75)	50 (30–80)	.49
Duration of insulin therapy (h)	26 (10–56)	33 (13–33)	27 (10–57)	.37
Mortality n = (%)	17 (27%)	1 (20%)	18 (25%)	.75

*Values are median and interquartile range unless depicted otherwise. † PRISM III; Pediatric Risk of Mortality III score [20], ‡ p values for differences between normoglycemic and hypoglycemic infants.

Table 2 Diagnoses of infants treated according to glucose control protocol

	N
Surgical	
Non-ECMO	
Congenital diaphragmatic hernia	18
Congenital heart defect	7
Congenital abdominal malformation	10
Congenital pulmonary malformation	2
Necrotizing enterocolitis	1
ECMO	
Congenital diaphragmatic hernia	9
Meconium aspiration	8
Persistent pulmonary hypertension	5
Congenital heart defect	1
Medical	
Infection	
Viral bronchiolitis	3
Meningitis	1
Sepsis	1
Meconium aspiration	1
Persistent pulmonary hypertension	3
Other	3

ECMO; Extracorporeal Membrane Oxygenation

Glucose control protocol

Insulin treatment was initiated 5.8h (1–38h) after the first episode of hyperglycemia (12.5 ± 4.1 mmol.L^{-1} (225 ± 74 mg.dL^{-1})). The initial insulin starting dose was 20 mIU.$kg^{-1}.h^{-1}$ (10–100 mIU.$kg^{-1}.h^{-1}$), the maximum dose reached was 50 mIU.$kg^{-1}.h^{-1}$ (20–450 mIU.$kg^{-1}.h^{-1}$). Normoglycemia was reached within 12h of initiating insulin therapy in 65 infants (90.3%), and the median time to reach normoglycemia was 5.3h (1–25h). One infant died before reaching the glucose target range. The overall duration of insulin therapy was 27h (1–308h), while 50 (68.5%) of the infants were treated less than 48h and only twelve (15.1%) infants received insulin for > 72h.

Hypoglycemic incidents and protocol violations

Episodes of moderate hypoglycemia occurred in three (4.1%) infants and severe hypoglycemia occurred in two (2.7%) infants, without any severe clinical symptoms. Four infants were recorded to be pale and lethargic. The intervention with a bolus of glucose was sufficient to treat the hypoglycemic incidents. One (20%) infant who developed hypoglycemia died, not directly related to the hypoglycemic incident. In the group who did not develop hypoglycemia 17 (27%) infants died. Blood glucose levels, age,

use of glucocorticoids, and clinical diagnoses at initiation of insulin therapy, dose and duration of insulin therapy, and time to achieve normoglycemia did not differ between hypoglycemic and non-hypoglycemic infants (Table 1). Hypoglycemia occurred twice in the two infants with severe hypoglycemia, although severe hypoglycemia occurred no more than once in these infants. Thus, a total of 5 hypoglycemic and 2 severe hypoglycemic incidents were recorded. The hypoglycemic incidents occurred at day 2 (1-5 d) of admission at the age of 4 days old (1-30 d), 8h (2-13 h) after initiation of insulin therapy.

Of these 7 hypoglycemic incidents, no apparent cause could be identified in three incidents. In retrospect, one hypoglycemic infant was diagnosed with sepsis within several hours after the hypoglycemic incident. Three protocol violations were identified which could have been responsible for the (severe) hypoglycemic incidents. In two infants insulin was decreased too late or not at all after plasma glucose levels dropped, whereas in one infant insulin was not discontinued according to the protocol.

In the 73 infants receiving insulin, 31 protocol violations were identified at the initiation of insulin therapy, which were not associated with the hypoglycemic incidents. The starting dose of insulin was below protocol recommendations in 26 (34.7%) infants and above protocol recommendations in three (4%) infants. In two patients the start of insulin should have been cancelled as the blood glucose was < 8 mmol.L^{-1} immediately prior to the start of the insulin treatment.

Discussion

This is the first evaluation of the efficacy and safety of a tight glucose protocol specifically in critically ill term infants. Consistent with previous evaluations of tight glucose protocols in older children[17-19], we showed that our nurse-driven glucose control protocol achieved normoglycemia within 5.3h and in 90% of the infants within 12h. Remarkably, the target ranges were achieved while a large proportion (34.7%) of the insulin starting doses was below protocol recommendation. Furthermore, the overall treatment duration was short, which makes it less likely that these infants could have benefited from the tight glucose regimen.

It is obvious that the infants described, with high mortality rates and a large proportion of infants treated on ECMO, are indeed one of the sickest populations on our PICU. This is consistent with a recent study showing that hyperglycemia was highly prevalent in children requiring mechanical ventilation, vasopressors, and support of continuous renal replacement therapy or ECMO[21]. Critical illness hyperglycemia, strongly associated with disease severity[22], is primarily caused by insulin resistance[23], induced by endogenous and exogenous triggers such as catacholamines, glucocorticoids and

inflammatory cytokines. While hyperglycemia is associated with a poor outcome in infants[3, 24, 25], outcome studies of tight glucose protocols have not focused specifically on this population. Additionally, no mechanistic studies have explored the effects of hyperglycemia or insulin therapy in infants. The principal cause of cellular and organ system failure in critical illness hyperglycemia is glucose overload resulting in excessive generation of oxygen radicals, which leads to mitochondrial dysfunction, increased generation of inflammatory cytokines and disturbed energy metabolism[26-28]. Insulin therapy has been shown to protect the function and structure of the mitochondrial compartment in adults and children[27, 29]. It is not likely that there will be substantial differences in the mechanistic effects of hyperglycemia or insulin therapy in infants. Therefore, despite the lack of beneficial results in outcome studies, potential benefits of insulin therapy in the infant population should not be ignored.

However, we recognize the 7 (severe) hypoglycemic incidents in 5 infants (6.7%). This was markedly lower than previously described by Vlasselaers and colleagues, who reported an incidence of hypoglycemia in 25% of children treated, predominantly (>80%) in infants[4]. Using a treatment algorithm in critically ill older children by us and others the reported incidence of hypoglycemia was 0-4%[17-19]. As the timeframe of the evaluation was similar for the older children without any hypoglycemic incidents[19] as for the infants in the present study, neither the protocol implementation, nor the experience working with the protocol by nursing staff and physicians explains this difference. So, it appears that infants are more susceptible to hypoglycemia following insulin administration.

Investigating the hypoglycemic incidents showed us that three of the hypoglycemic incidents were explained by protocol violations, all due to inadequate insulin dose adjustments, emphasizing the importance of adherence to the protocol. Notwithstanding the iatrogenic causes of these hypoglycemic incidents, we did find that insulin doses *above* recommendation occurred much less in our infants (4%), compared to our previous report in older children (21%)[19]. Remarkably, we found a disproportionate high incidence (34.7%) of insulin doses *below* the recommended dose. Although our study was not designed to show the grounds for protocol violations, a certain fear of iatrogenic hypoglycemia in these infants might be responsible for this latter violation. It has been shown recently that a disparity exists between physicians beliefs and actual practice habits regarding glycemic control in PICU's[30]. Although a clear causal relationship between hyperglycemia and poor outcome has become more evident, among pediatricians hypoglycemia is still considered more detrimental than hyperglycemia[30, 31]. Fear of hypoglycemia is a major barrier for the successful implementation of glycemic control in critically ill children[30].

Although three hypoglycemic incidents were explained by protocol violations, three hypoglycemic incidents occurred without apparent cause and one occurred in the onset

of sepsis. Furthermore, in two infants hypoglycemia occurred twice. These observations allow us to speculate that infants indeed are a specific and vulnerable age group on the PICU. Possibly the etiology in these infants is different from the "regular" insulin resistance induced critical illness hyperglycemia. For instance, in the pediatric population the initial phase of sepsis can cause insulin deficiency, rather than resistance[32]. Additionally, high parenteral glucose intake can, independent of insulin resistance, produce hyperglycemia. Under such circumstances, insulin infusion would decrease plasma glucose levels faster than under insulin resistant conditions. Furthermore, infants lack the precise control of glucose homeostasis as they undergo major changes in glucose and insulin metabolism. Glycogen stores are low and glucose homeostasis primarily depends on gluconeogenesis, partially explaining why young age itself is a risk factor for developing hypoglycemia[7]. Furthermore, a transformation in β-cell population, due to a transient wave of apoptosis and repopulation, causes a wide variation of insulin secretory capacities in newborn infants[33]. Moreover, hyperinsulinemic euglycemic clamp studies have shown that infants have greater peripheral glucose utilization[34] than older children[35] and adults[36]. Insulin receptors in infants are higher in number as well as affinity, partially explaining this increased sensitivity[37]. Additionally, it has recently been shown that there exists a developmental change in peripheral insulin signalling[38]. Although reduced contents of glucose transporters (GLUT) 1 and 4 were found, the proximal insulin signalling proteins (IR-β, IRS-1 and Akt) were increased in muscle of neonatal baboons[38]. These wide variations in the ability of glucose production, insulin secretory capacity, and insulin sensitivity might at least partially explain the differences from older children and adults. This suggests that when insulin therapy is considered in critically ill infants, increased awareness is needed. With the results of the present study we have adjusted the protocol on our PICU for infants less than 28 days old; we decreased the initial insulin starting doses by 10 mIU.kg^{-1}.h^{-1} and the minimum insulin dose as stop criteria (< 5 mIU.kg^{-1}.h^{-1}, or < 20 mIU.kg^{-1}.h^{-1} > 24h) (Figure 1). Our study has limitations. It is an observational study. It adds insight in the safety and effectivity of tight glucose protocols for critically ill infants, but it was not designed to show whether outcome improved with our tight glucose protocol. We further acknowledge that our glucose target range was higher than that of Vlasselaers and colleagues[4], which could at least partially explain the difference in hypoglycemic incidents. However, as the ideal target range of tight glucose regimens still needs to be established less strict protocols might be sensible.

Based on this study, we conclude that term infants can be treated with a tight glucose protocol, as target ranges were met, and overall treatment was short. However, hypoglycemia occurred more frequently in infants than in older children, alerting us that this is a vulnerable population where additional safety approaches are warranted. Future studies assessing the outcome of a tight glucose regimen in the infant popula-

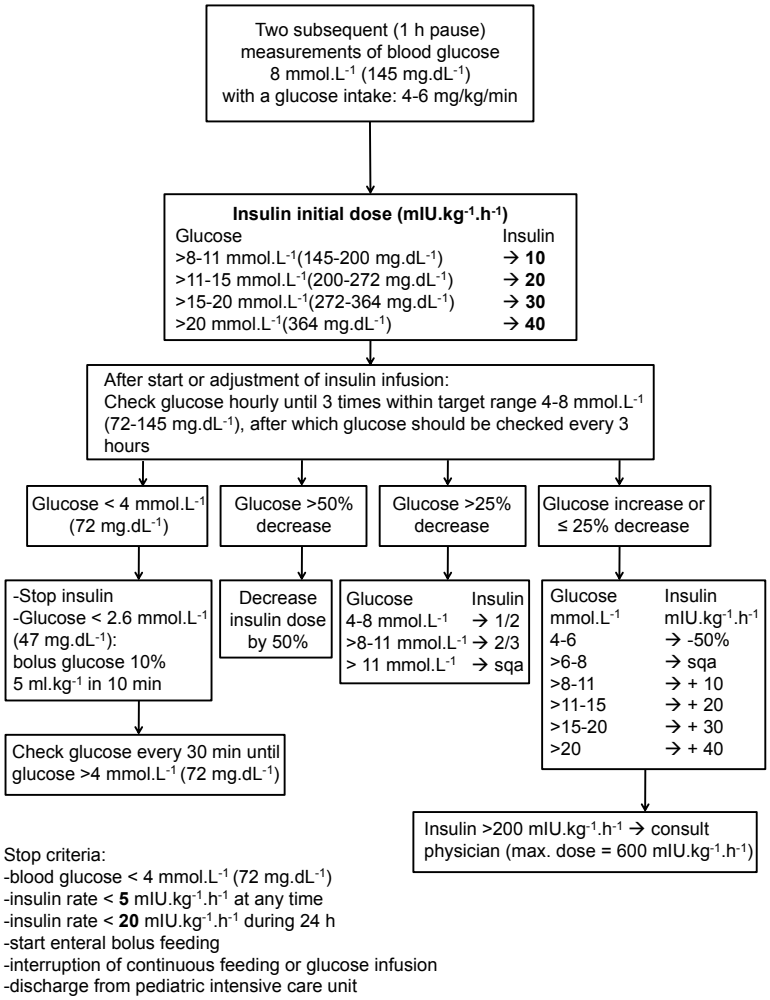


Figure 1. Adjusted step-wise nurse driven glucose control protocol for infants less than 28 days old. Glucose; blood glucose level in mmol.L^{-1} (mg.dL^{-1}), Insulin; Insulin dose in mIU.kg^{-1}.h^{-1}.

tion, and addressing approaches which will help prevent hypoglycemia are of utmost importance. Meanwhile, we will evaluate our adjusted protocol (Figure 1) and will have a long-term follow up of the infants.

Acknowledgements

We would like to thank Marjolein Augustus for her help with data collection.

References

1. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. Mayo Clin Proc 2003;78(12):1471-8.
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. Hays SP, Smith EO, Sunehag AL. Hyperglycemia is a risk factor for early death and morbidity in extremely low birth-weight infants. Pediatrics 2006;118(5):1811-8.
4. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373(9663):547-56.
5. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, et al. Early insulin therapy in very-low-birth-weight infants. N Engl J Med 2008;359(18):1873-84.
6. Solomon T, Felix JM, Samuel M, et al. Hypoglycaemia in paediatric admissions in Mozambique. Lancet 1994;343(8890): 149-50.
7. 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.
8. 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.
9. Vannucci RC, Vannucci SJ. Glucose metabolism in the developing brain. Semin Perinatol 2000;24(2):107-15.
10. Flykanaka-Gantenbein C. Hypoglycemia in childhood: long-term effects. Pediatr Endocrinol Rev 2004;1 Suppl 3:530-6.
11. Chase JG, Shaw GM, Lin J, et al. Targeted glycemic reduction in critical care using closed-loop control. Diabetes Technol Ther 2005;7(2):274-82.
12. Chase JG, Shaw GM, Lin J, et al. Adaptive bolus-based targeted glucose regulation of hyperglycaemia in critical care. Med Eng Phys 2005;27(1):1-11.
13. Chee F, Fernando T, van Heerden PV. Closed-loop control of blood glucose levels in critically ill patients. Anaesth Intensive Care 2002;30(3):295-307.
14. Lin J, Chase JG, Shaw GM, et al. Adaptive bolus-based set-point regulation of hyperglycemia in critical care. Conf Proc IEEE Eng Med Biol Soc 2004;5:3463-6.
15. Wong XW, Singh-Levett I, Hollingsworth LJ, et al. A novel, model-based insulin and nutrition delivery controller for glycemic regulation in critically ill patients. Diabetes Technol Ther 2006;8(2):174-90.
16. Vogelzang M, Loef BG, Regtien JG, et al. Computer-assisted glucose control in critically ill patients. Intensive Care Med 2008;34(8):1421-7.
17. Preissig CM, Hansen I, Roerig PL, Rigby MR. A protocolized approach to identify and manage hyperglycemia in a pediatric critical care unit. Pediatr Crit Care Med 2008;9(6):581-8.
18. Preissig CM, Rigby MR, Maher KO. Glycemic control for postoperative pediatric cardiac patients. Pediatr Cardiol 2009; 30(8):1098-104.
19. Verhoeven JJ, Brand JB, van de Polder MM, Joosten KF. Management of hyperglycemia in the pediatric intensive care unit; implementation of a glucose control protocol. Pediatr Crit Care Med 2009; 10(6):648-52.
20. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
21. Preissig CM, Rigby MR. Pediatric critical illness hyperglycemia: risk factors associated with development and severity of hyperglycemia in critically ill children.

J Pediatr 2009;155(5):734-9.
22. Klein GW, Hojsak JM, Schmeidler J, Rapaport R. Hyperglycemia and outcome in the pediatric intensive care unit. J Pediatr 2008;153(3):379-84.
23. Matsuda N, Yamamoto S, Yokoo H, Tobe K, Hattori Y. Nuclear factor-kappaB decoy oligodeoxynucleotides ameliorate impaired glucose tolerance and insulin resistance in mice with cecal ligation and puncture-induced sepsis. Crit Care Med 2009;37(10):2791-9.
24. Alaedeen DI, Walsh MC, Chwals WJ. Total parenteral nutrition-associated hyperglycemia correlates with prolonged mechanical ventilation and hospital stay in septic infants. J Pediatr Surg 2006; 41(1):239-44; discussion -44.
25. Hall NJ, Peters M, Eaton S, Pierro A. Hyperglycemia is associated with increased morbidity and mortality rates in neonates with necrotizing enterocolitis. J Pediatr Surg 2004;39(6):898-901; discussion 898-901.
26. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004;114(9): 1187-95.
27. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet 2005; 365(9453):53-9.
28. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360(9328):219-23.
29. Fram RY, Cree MG, Wolfe RR, et al. Intensive insulin therapy improves insulin sensitivity and mitochondrial function in severely burned children. Crit Care Med 2010;38(6):1475-83.
30. Preissig CM, Rigby MR. A disparity between physician attitudes and practice regarding hyperglycemia in pediatric intensive care units in the United States: a survey on actual practice habits. Crit Care 2010;14(1):R11.
31. Hirshberg E, Lacroix J, Sward K, Willson D, Morris AH. Blood glucose control in critically ill adults and children: a survey on stated practice. Chest 2008;133(6): 1328-35.
32. van Waardenburg DA, Jansen TC, Vos GD, Buurman WA. Hyperglycemia in children with meningococcal sepsis and septic shock: the relation between plasma levels of insulin and inflammatory mediators. J Clin Endocrinol Metab 2006;91(10):3916-21.
33. Lucas A, Bloom SR, Aynsley-Green A. Metabolic and endocrine events at the time of the first feed of human milk in preterm and term infants. Arch Dis Child 1978;53(9):731-6.
34. Farrag HM, Nawrath LM, Healey JE, et al. Persistent glucose production and greater peripheral sensitivity to insulin in the neonate vs. the adult. Am J Physiol 1997;272(1 Pt 1):E86-93.
35. Danadian K, Balasekaran G, Lewy V, Meza MP, Robertson R, Arslanian SA. Insulin sensitivity in African-American children with and without family history of type 2 diabetes. Diabetes Care 1999; 22(8):1325-9.
36. Wolfe RR, Allsop JR, Burke JF. Glucose metabolism in man: responses to intravenous glucose infusion. Metabolism 1979; 28(3):210-20.
37. Thorsson AV, Hintz RL. Insulin receptors in the newborn. Increase in receptor affinity and number. N Engl J Med 1977; 297(17):908-12.
38. Blanco CL, Liang H, Joya-Galeana J, DeFronzo RA, McCurnin D, Musi N. The ontogeny of insulin signaling in the preterm baboon model. Endocrinology 2010;151(5):1990-7.

Chapter 4

Reducing Glucose Intake Safely Prevents Hyperglycemia In Post-Surgical Children

Sascha CAT Verbruggen
Carlijn TI de Betue
Henk Schierbeek
Shaji Chacko
Leon NA van Adrichem
Jennifer J Verhoeven
Johannes B van Goudoever
Koen FM Joosten

Submitted

Abstract

Objective

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

Design

A single center, randomized crossover study.

Setting

A pediatric intensive care unit (PICU) in a tertiary university hospital.

Patients

Eight children (age 9.8 ± 1.9 months, weight 9.5 ± 1.1 kg) admitted after surgical correction for non-syndromal craniosynostosis.

Interventions

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 intake 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.

Measurements and Main Results

SG resulted in hyperglycemia (defined as > 110 $mg.dL^{-1}$), while during LG plasma glucose levels were normoglycemic (105 ± 10 vs. 133 ± 30 $mg.dL^{-1}$; LG vs. SG respectively, p = .02). Hypoglycemia did not occur during LG intake. Endogenous glucose production (EGP) was not fully suppressed during the hyperglycemic state under SG and increased with reduced glucose intake (2.6 ± 1.5 vs. 1.1 ± 1.4 $mg.kg^{-1}.min^{-1}$; LG vs. SG; p = .05). Leucine kinetics did not differ between glucose infusion rates. Phenylalanine hydroxylation was higher during LG (8.4 ± 1.7 vs. 7.4 ± 1.6 $\mu mol.kg^{-1}.h^{-1}$; LG vs. SG; p = .04). Whole body protein balance derived from with leucine and phenylalanine kinetics was slightly negative but not further affected with a decrease in glucose intake.

Conclusions

The current recommended glucose intake induces hyperglycemia in post-surgical children. A reduced glucose intake safely prevented hyperglycemia, while infants were capable to sustain normoglycemia with increased endogenous glucose production due to a combined increase in gluconeogenesis and glycogenolysis. The reduced glucose intake did not exacerbate the mild catabolic state in which the patients were.

Introduction

Plasma glucose levels are the resultant of a balance between 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]. A tight glucose regimen with insulin showed reduced morbidity and mortality in an adult Intensive Care Unit (ICU)[3, 4], although a number of subsequent studies were unable to replicate these results[5, 6]. Notwithstanding the widespread implementation of the tight glucose regimen[7], concerns regarding hypoglycemia have been raised[8]. 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 ($\leq$ 40 mg.dL^{-1} ~ $\leq$ 2.2 mmol.L^{-1}) and severe hypoglycemia ($\leq$ 31 mg.dL^{-1} ~ $\leq$ 1.7 mmol.L^{-1}) in 87 (25%) and 17 (5%) children, respectively[9].
The child's developing brain is more susceptible to hypoglycemia which can result in permanent damage[10-12]. Furthermore, young age is a risk factor for developing hypoglycemia, especially when the child is ill[13, 14]. It is therefore essential to prevent hypoglycemia, which led to a debate questioning the risks of insulin therapy in the pediatric population[15, 16].
An alternative to insulin therapy is to reduce the amount of glucose intake in critically ill young children. 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 intakes on glucose kinetics and amino acid metabolism in critically ill children.
We hypothesized that in post-surgical children, reduced glucose intake 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 intakes on glucose homeostasis and kinetics. Our second objective was to determine whether a low glucose intake 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 Pediatric Intensive Care Unit (PICU) of the Erasmus Medical Center - Sophia Children's Hospital. Written informed consent was obtained from the parents. Patients had infusing and drawing lines in place for clinical purposes. All were assessed by the Pediatric Logistic Organ Dysfunction (PELOD) score[17], Pediatric Index of Mortality (PIM2)[18, 19] and the Pediatric Risk of Mortality III (PRISM III) score[20], 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 1, consisted of a cross-over design, with a 4 h period of intravenous low glucose intake (LG; 2.5 $mg.kg^{-1}.min^{-1}$) versus a 4 h period of standard glucose intake (SG; 5.0 $mg.kg^{-1}.min^{-1}$)[21]. Patients were randomized for the order of glucose intake through a computer generated envelop. 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 intake as per standard care (4.0 – 6.0 $mg.kg^{-1}.min^{-1}$)[21] was stopped and the study glucose intake (SG or LG) started. Simultaneously, the patients received a primed, continuous, 8 hour intravenous tracer infusion (see below). Four hours after start of the tracer infusion (t=360) the glucose intake was switched.

Tracer Infusion studies

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

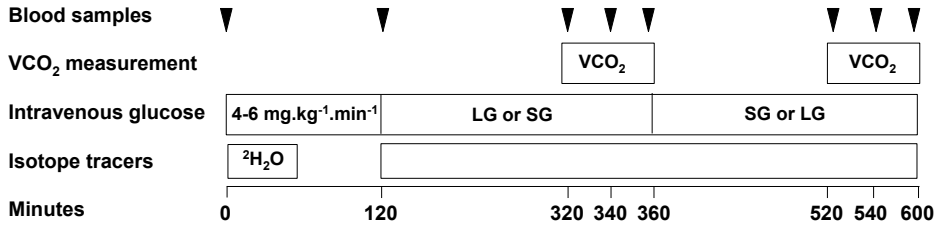


Figure 1. Schematic presentation of the tracer infusion study in eight post-surgical infants receiving either low (LG, 2.5 $mg.kg^{-1}.min^{-1}$) or standard (SG, 5.0 $mg.kg^{-1}.min^{-1}$) glucose intake. Black triangles indicate time points for plasma collection for laboratory parameters and isotopic enrichment measurements. Square boxes represent the time period in which carbon dioxide production (VCO_2) measurements took place.

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

Measurements and sample analysis

Blood samples were obtained at standard frequent intervals (Figure 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 (IRMS) (Delta+XP IRMS Thermo Fisher, Bremen, Germany) as an average of multiple measurements. 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 and analyzed in the positive chemical ionization scan mode of GC-MS as previously described[22, 23]. Plasma isotopic enrichment of [6,6-$^{2}H_2$]glucose (M+2) was determined by monitoring fragment ions at a mass-to-charge ratio (m/z) of 169 and 171. Selective ion monitoring of *m/z* 170/169 was performed to determine the M+1 enrichment of deuterium in the circulating glucose carbons (C-1,3,4,5,6,6)[23].

Leucine kinetics were calculated from plasma alpha-ketoisocaproate (α-KIC) enrichment, which is intracellularly produced from the leucine tracer infused. Plasma isotopic enrichment of [1-^{13}C]α-KIC were, after derivatization to butyldimethyl-silylquinoxalinol derivatives, determined by gas chromatography mass spectrometry (GCMS)[24]. Plasma isotopic enrichments of [ring-$^{2}H_5$]Phenylalanine, [ring-$^{2}H_4$]Tyrosine and [3,3-$^{2}H_2$]Tyrosine were determined by GCMS. Samples and calibration curves were analyzed with an Agilent 5975C GCMS (Agilent technologies, Amstelveen, The Netherlands) on a VF-17ms, 30m x 0.25mm ID capillary column (Varian Inc., Middelburg, The Netherlands) after using the *N*-ethoxycarbonylethylester derivative according to a modified method of Husek[25].

Carbon dioxide production (VCO_2), oxygen consumption (VO_2) and respiratory quotient (RQ), were obtained with a metabolic monitor in canopy mode (Deltatrac™ I MBM-200, Datex Division Instrumentarium Corp., Finland) during the last 40 minutes of study periods. 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 isotope ratio mass spectrometry (IRMS)[26, 27].

Plasma samples for glucose, insulin, cortisol, triglycerides, and free fatty acids were determined by standard in house protocols. Plasma glucose levels higher than > 110 $mg.dL^{-1}$ (> 6.1 $mmol.L^{-1}$) were considered hyperglycemic[4].

Calculations

Whole body kinetics of protein were calculated by conventional isotope dilution equations using a stochastic model during steady state enrichment[28] and glucose kinetics were estimated using the Steele equation[29]. At steady state plateau rate of appearance (Ra) is equal to rate of disappearance (Rd). The rate of appearance (Ra) of unlabeled substrate can be derived from the plasma isotope enrichment calculated by:

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

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

Glucose kinetics were calculated during the last 40 min of both study periods. Endogenous glucose production (EGP) rate is calculated by subtracting the glucose infusion rate from the steady state glucose appearance (Ra)

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

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

Fractional gluconeogenesis is calculated using the average deuterium enrichment method previously described[23]. Briefly, the average enrichment of ^2H on each glucose carbon is calculated with the following equation:

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

where $(M+1)d_{(m/z\ 169)}$ 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 (Frac GNG), the equation is

$$Frac\ GNG = average(M+1)d/E_{H2O} \quad (4)$$

where E_{H2O} is the deuterium enrichment in body water.
The absolute rate of appearance of gluconeogenic glucose in plasma (Ra_{GNG}) is then calculated by multiplying the rate of appearance of glucose in plasma by the fraction of gluconeogenesis:

$$\text{Gluconeogenesis} = Ra_{Gluc} \times \text{Frac GNG} \quad (5)$$

Glycogenolysis is than calculated by subtracting the gluconeogenesis from the EGP.

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

Leucine kinetics
Whole body leucine flux was calculated during the last 40 min of both study periods[30]. Leucine oxidation rates were calculated as follows;

$$\text{Leucine oxidation} = VCO_2 \times (E^{13}CO_2/69.18)/[^{13}C]\alpha - \text{KIC} \quad (7)$$

where 69.18 is the $^{13}CO_2$ refraction correction factor for critically ill children[31]. VCO_2 (carbon dioxide production) is measured in milliliters per minute and converted to millimoles per hour by multiplying by 60 min and dividing by 22.4, which is the number of 1 in 1 mole of an ideal gas at standard temperature and pressure to convert to milliliters per minute. Non-oxidative leucine disposal (NOLD; leucine converted into protein synthesis) is the leucine oxidation subtracted from the leucine rate of disappearance.

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

Phenylalanine and tyrosine kinetics
Whole body phenylalanine and tyrosine fluxes were calculated during the last 40 min of both study periods[32, 33].
Phenylalanine hydroxylation is the rate of phenylalanine conversion to tyrosine and is calculated as follows;

$$\text{Hydroxylation} = Ra_{tyr} \times (E_{[2H4]tyr}/E_{[2H5]Phe}) \times (Ra_{phe}/(i_{phe} + Ra_{phe}) \times 2.2 \quad (9)$$

where Ra_{phe} and Ra_{tyr} are the phenylalanine and tyrosine fluxes, calculated as described with *(1)* using [ring-$^2H_5$]phenylalanine and [3,3-$^2H_2$]tyrosine respectively; $E_{[2H4]tyr}$ and $E_{[2H5]Phe}$ are the plasma enrichments; and i_{phe} is the rate of infusion 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[34, 35]. Non-hydroxylation phenylalanine disposal (NHPD; phenylalanine converted into protein synthesis) is the phenylalanine hydroxylation subtracted from the phenylalanine rate of disappearance.

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

Whole body protein metabolism

Under the assumption that 1 gram of protein contains approximately 621 μmol of leucine[36] and 280 μmol of phenylalanine[37], 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[38]. Briefly, the model describes the following equation;

$$Ra = Rd = S + O = B + I. \quad (11)$$

Whole body protein synthesis (S) is equal to NOLD and NHPD subtracted by leucine oxidation and phenylalanine hydroxylation (O), respectively, converted into protein kinetics as described above. Protein breakdown (B) can be calculated by subtracting the leucine or phenylalanine intake (I) from the leucine or phenylalanine rate of appearance (Ra), respectively, and converted again into protein synthesis. In our study the intake is equal to the tracer infusion as our patients were fasted and did not receive any amino acids. Protein balance can then be calculated by subtracting whole body protein breakdown from whole body protein synthesis.

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. Comparisons between the two different glucose intakes at both infusion rates were 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 $p < 05$. 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

The patients' characteristics are shown in Table 1. A total of 8 children (9.8 ± 1.9 months) admitted to the PICU after surgical correction for non-syndromal craniosynostosis were included. All patients were hemodynamically stable without vasoactive drugs and breathing spontaneously with an inspiratory oxygen fraction of less than 0.6. They were receiving either opioids or acetaminophen as pain relief and were not sedated or receiving muscle relaxation at the time of study. Patients did not receive (par) enteral nutrition other than intravenous glucose infusion in the range of 4-6 $mg.kg^{-1}.min^{-1}$ as per standard care[21]. The resting energy expenditure and respiratory quotient did not change between glucose intake protocols (Table 1).

Table 1. Demographic and nutritional data of 8 post-surgical children*.

Age (months)	9.8 ± 1.9		
Gender (male:female)	6 : 2		
Weight (kg)	9.5 ± 1.1		
Height (cm)	74.3 ± 3.0		
PELOD†	10.1 ± 7.6		
PRISM III‡	7.4 ± 3.7		
PIM2^	14.2 ± 2.8		
	LG£	SG£	P value
Glucose intake ($mg.kg^{-1}.min^{-1}$)	2.6 ± 0.1	5.2 ± 0.1	< .0001
VO_2 ($mL.min^{-1}$)	69 ± 24	67 ± 17	.79
VCO_2 ($mL.min^{-1}$)	59 ± 11	55 ± 14	.29
Resting energy expenditure ($kcal.kg^{-1}.d^{-1}$)	49.8 ± 17.6	49.7 ± 14.9	.98
Respiratory quotient	0.88 ± 0.14	0.83 ± 0.10	.37
Caloric intake ($kcal.kg^{-1}.day^{-1}$)	12.7 ± 0.2	25.3 ± 0.5	< .0001
Caloric intake (%) #	24 ± 1	47 ± 2	< .0001
Glucose ($mg.dL^{-1}$)	105 ± 10	133 ± 30	.02
Triglycerides ($mmol.L^{-1}$)	0.38 ± 0.25	0.43 ± 0.20	.54
Free fatty acids ($mmol.L^{-1}$)	0.72 ± 0.20	0.63 ± 0.12	.34
C-reactive protein ($mg.dL^{-1}$)	24 ± 13	26 ± 16	.61
Cortisol ($nmol/L^{-1}$)	649 ± 160	681 ± 205	.77
Insulin ($\mu U.mL^{-1}$)	64 ± 48	90 ± 51	.16

*All values are mean ± SD. £LG = Low glucose, £SG = Standard glucose, †PELOD = Pediatric Logistic Organ Dysfunction [17], ‡PRISM III = Pediatric Risk of Mortality III [20], ^PIM2 = Pediatric index of Mortality [18, 19], # Caloric intake as percentage of requirements according to the Schofield equation [59].

Laboratory values and hormone concentrations

Patients were hyperglycemic during standard glucose intake (SG), while during LG plasma glucose levels were lower and normoglycemic (Figure 2, Table 1). LG did not cause hypoglycemia; the lowest plasma glucose was 91.8 mg.dL^{-1}. Insulin plasma concentrations did not differ significantly (Table 1).

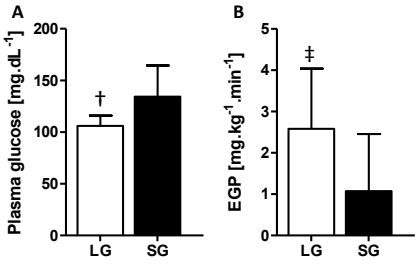


Figure 2. Glucose metabolism during low and standard glucose intake in eight post-surgical infants. LG = Low glucose, SG = Standard glucose, EGP = Endogenous glucose production. Panel A. Plasma glucose concentration, mg.dL^{-1}, mean ± SD, † p = .02, Panel B. Endogenous glucose production mg.kg^{-1}.min^{-1}, mean ± SD, ‡ p = .05.

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 rate of appearance (Ra) did not differ between glucose protocols (Table 2). Endogenous glucose production (EGP) increased during LG (Figure 2, Table 2). Fractional gluconeogenesis was higher during LG. Absolute gluconeogenesis and glycogenolysis were not significantly different (p =.08) and glycogenolysis was not significantly different from zero (Table 2).

Table 2. Glucose kinetics in eight post-surgical infants during two different glucose intakes.*

	LG£	SG£	p value
Glucose Ra^ (mg.kg^{-1}.min^{-1})	5.3 ± 1.5	6.4 ± 1.5	.14
Endogenous Glucose Production (mg.kg^{-1}.min^{-1})	2.6 ± 1.5	1.1 ± 1.4	.05
Fractional gluconeogenesis (% of Ra^)	43 ± 2	29 ± 7	< .01
Absolute Gluconeogenesis (mg.kg^{-1}.min^{-1})	2.3 ± 0.6	1.8 ± 0.4	.08
Glycogenolysis (mg.kg^{-1}.min^{-1})	0.3 ± 0.9	-0.7 ± 1.1	.08

*All values are depicted as mean ± SD), ^Ra; rate of appearance, £LG; Low glucose, SG; Standard glucose

Leucine flux and oxidation rates

Leucine Ra and Leucine oxidation were not significantly affected by the change in glucose intake. As a result, NOLD did not differ between glucose intake rates (Table 3).

Table 3. Leucine, phenylalanine and tyrosine kinetics in eight post-surgical infants during two different glucose intakes.*

	£LG	£SG	P value
Leucine Ra ^	160 ± 15	158 ± 16	.68
Leucine oxidation	33 (22-82)	29 (22-62)	.38
NOLD †	122 ± 14	124 ± 20	.76
Phenylalanine Ra	70 ± 6	68 ± 5	.25
Tyrosine Ra	39 ± 2	37 ± 2	.06
Phenylalanine hydroxylation	8.4 ± 1.7	7.4 ± 1.6	.04
NHPD ‡	61 ± 6	61 ± 6	1.0

*All values are measured in μmol.kg^{-1}.h^{-1} and depicted in mean ± SD, ^Ra; rate of appearance, £LG; Low glucose, £SG; Standard glucose, †NOLD; non-oxidative leucine disposal, ‡ NHPD; non-hydroxylation phenylalanine disposal

4

Phenylalanine and tyrosine flux and hydroxylation rates

Phenylalanine Ra and tyrosine Ra did not differ. Phenylalanine hydroxylation was significantly higher at the lower glucose intake rate (Table 3). However, non-hydroxylative phenylalanine disposal (NHPD) did not differ between glucose intake rates (Table 3). The phenylalanine hydroxylation fraction of the total phenylalanine Rd did not differ (12 ± 3% vs. 11 ± 3%; LG vs. SG, p = .07).

Protein metabolism

Whole body protein metabolism derived from with leucine kinetics was as follows (Figure 3). Whole body 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. The whole body protein balance was negative and did not differ between LG and SG (-1.2 ± 0.8 vs. -1.0 ± 0.6 g.kg^{-1}.d^{-1}, p = .38). Whole body protein metabolism corresponding with phenylalanine and tyrosine kinetics was as follows (Figure 3). Whole body 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 ±

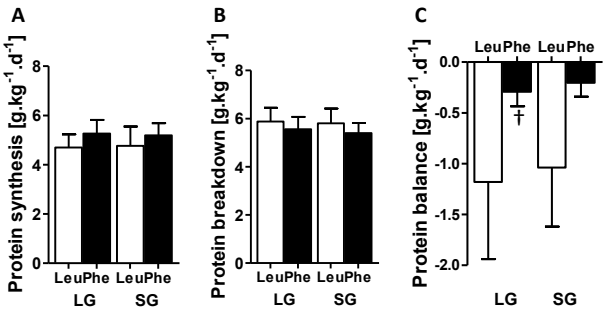


Figure 3. Whole body protein metabolism during low and standard glucose intake in eight post-surgical infants. LG = Low glucose, SG = Standard glucose, Leu = leucine, Phe = Phenylalanine. Panel A. protein synthesis, Panel B. protein breakdown, Panel C. protein balance; Values are g.kg^{-1}.d^{-1}, mean ± SD, † p = .04; LG vs. SG

0.4 g.kg^{-1}.d^{-1}) did not differ between LG and SG respectively. The whole body protein balance was negative and showed a statistically significant but not relevant difference (-0.3 ± 0.1 vs. -0.2 ± 0.1 g.kg^{-1}.d^{-1}; LG vs. SG, p =.04) (Figure 3).

Discussion

Tight glucose control improves morbidity and mortality in critically ill children, although hypoglycemia is a frequent and serious side effect of insulin therapy[9]. In this study we showed that in post-surgical children, normoglycemia could safely be achieved by a reduced glucose intake, without occurrence of hypoglycemia. The low glucose intake (LG; 2.5 mg.kg^{-1}.min^{-1}), half of what is considered standard practice for age[21], can be considered an alternative to insulin therapy in the initial phase of glycemic management. Additionally we observed that endogenous glucose production was not fully suppressed, despite high plasma glucose levels. Moreover, reducing the glucose intake induced an increase in endogenous glucose production due to an increase of both gluconeogenesis as well as glycogenolysis. Furthermore, a 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 intake 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 disappearance 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 intake. The current recommendations are based largely upon data for infants and children and 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[21]. Although conditions are different in post-surgical children, we did use 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 intake. This might suggest that a reduced glucose intake did not negatively impact cerebral glucose uptake or utilization.

Endogenous glucose production rates in our infants, receiving 2.5 mg.kg^{-1}.min^{-1}, were lower than those measured in healthy infants, fasted for 8 – 9 h (7.1 ± 0.3 mg.kg^{-1}.

min^{-1})[39]. So, although an increased EGP was observed during LG, their production was not at their full potential. During both glucose intakes glycogenolysis rates were futile. Glycogenolysis was probably suppressed by elevated insulin levels and suppressed glucagon levels during glucose infusion[40], although the latter could not be measured in our study due to blood draw limitations in our infants. 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.

Of interest, even during SG, despite supraphysiological glucose and insulin levels, EGP was not fully suppressed. During both glucose intakes, 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 intake. These observations might indicate hepatic insulin resistance in our patients. A clear association exists between increased contribution of gluconeogenesis and insulin resistance[41]. The expression of phosphoenolpyruvate carboxy kinase (PEPCK), the rate-limiting enzyme of gluconeogenesis, is increased and less sensitive to insulin during critical illness[42]. Therefore, we hypothesize that the increase in EGP and moreover gluconeogenesis during LG does not indicate that low glucose intake was below the threshold, but that it rather indicates a certain state of insulin resistance in these post-surgical infants.

We did not find relevant differences in amino acid or protein metabolism during low glucose intake. Although LG did not affect leucine kinetics and more specifically leucine oxidation, phenylalanine hydroxylation was slightly but statistically significant higher during LG. Increased availability of plasma amino acids, a known stimulant of hydroxylation[43-45], is not a likely cause for this increase as our patients did not receive amino acids and their proteolysis was not increased. One might hypothesize that the increased hydroxylation of phenylalanine is a physiological response to cope with a shortage of energy, providing energy by oxidation of the carbon skeleton of amino acids. However, this does not explain the indifference in leucine oxidation which would be expected to rise as well. 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[46]. This could explain the difference in catabolism between phenylalanine and leucine. Although not statistically different, we did observe a trend towards an increased gluconeogenesis during LG.

Whole body protein balance corresponding with leucine and phenylalanine was slightly negative during both study periods and consistent with those obtained from septic infants and children[36, 37]. In contrast with our data, normoglycemia, independent of plasma insulin levels, improved protein turnover after abdominal surgery in adults[47]. 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[48]. However, consistent with our data, it has been shown in neonates that different glucose intakes do not affect protein turnover[49]. Although under physiological conditions a clear relation exists between energy and protein metabolism, it has been recognized that a deficiency in energy supply is not solely responsible for protein catabolism during critical illness[50]. Proteolysis during critical illness is usually caused by activation of the ubiquitin-proteasome proteolytic pathway (UPP) in muscle initiated by activation of caspase 3[51, 52], and pathophysiological triggers include activation of lysosome-[53] and calpain-dependent pathways[54]. In addition, there appears to be a link between muscle wasting and insulin resistance[55-57].

There are some limitations to our study which need to be taken into account, 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[21, 48]. Furthermore, various diagnoses, such as trauma, burns or sepsis, as well as differences between single and multi organ failure yield a different response. We must also note that the negative glycogenolysis rates are physiological not possible. This is a consequence of a consistent underestimation of the EGP by the glucose tracer model, as part of the diluted tracer pool due to newly produced glucose that is taken up by the liver again[58].

Conclusion

Reduced glucose intake, half of what is considered standard practice for age, in the initial post-surgical phase safely prevented hyperglycemia in infants without occurrence of hypoglycemia. Additionally we observed that the post-surgical infants appeared insulin resistant, where 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 present but not exacerbated during the reduced glucose intake. A reduced glucose intake can be considered as an alternative to insulin therapy in the initial phase of glycemic management. Further studies on (long term) clinical implications are warranted.

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 the nursing staff,

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. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. N Engl J Med 2006;354(5): 449-61.
4. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001; 345(19):1359-67.
5. Finfer S, Chittock DR, Su SY, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med 2009;360(13):1283-97.
6. Preiser JC, Devos P, Ruiz-Santana S, et al. A prospective randomised multi-centre controlled trial on tight glucose control by intensive insulin therapy in adult intensive care units: the Glucontrol study. Intensive Care Med 2009;35(10):1738-48.
7. Wilson M, Weinreb J, Hoo GW. Intensive insulin therapy in critical care: a review of 12 protocols. Diabetes Care 2007;30(4): 1005-11.
8. 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.
9. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373(9663):547-56.
10. 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.
11. Vannucci RC, Vannucci SJ. Glucose metabolism in the developing brain. Semin Perinatol 2000;24(2):107-15.
12. Flykanaka-Gantenbein C. Hypoglycemia in childhood: long-term effects. Pediatr Endocrinol Rev 2004;1 Suppl 3:530-6.
13. Solomon T, Felix JM, Samuel M, et al. Hypoglycaemia in paediatric admissions in Mozambique. Lancet 1994;343(8890): 149-50.
14. 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.
15. Joosten K, Verbruggen SC, Verhoeven JJ. Glycaemic control in paediatric critical care. Lancet 2009;373(9673):1423-4; author reply 4.
16. Preissig C, Rigby M. Glycaemic control in paediatric critical care. Lancet 2009; 373(9673):1423; author reply 4.
17. Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003;362(9379):192-7.
18. 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.

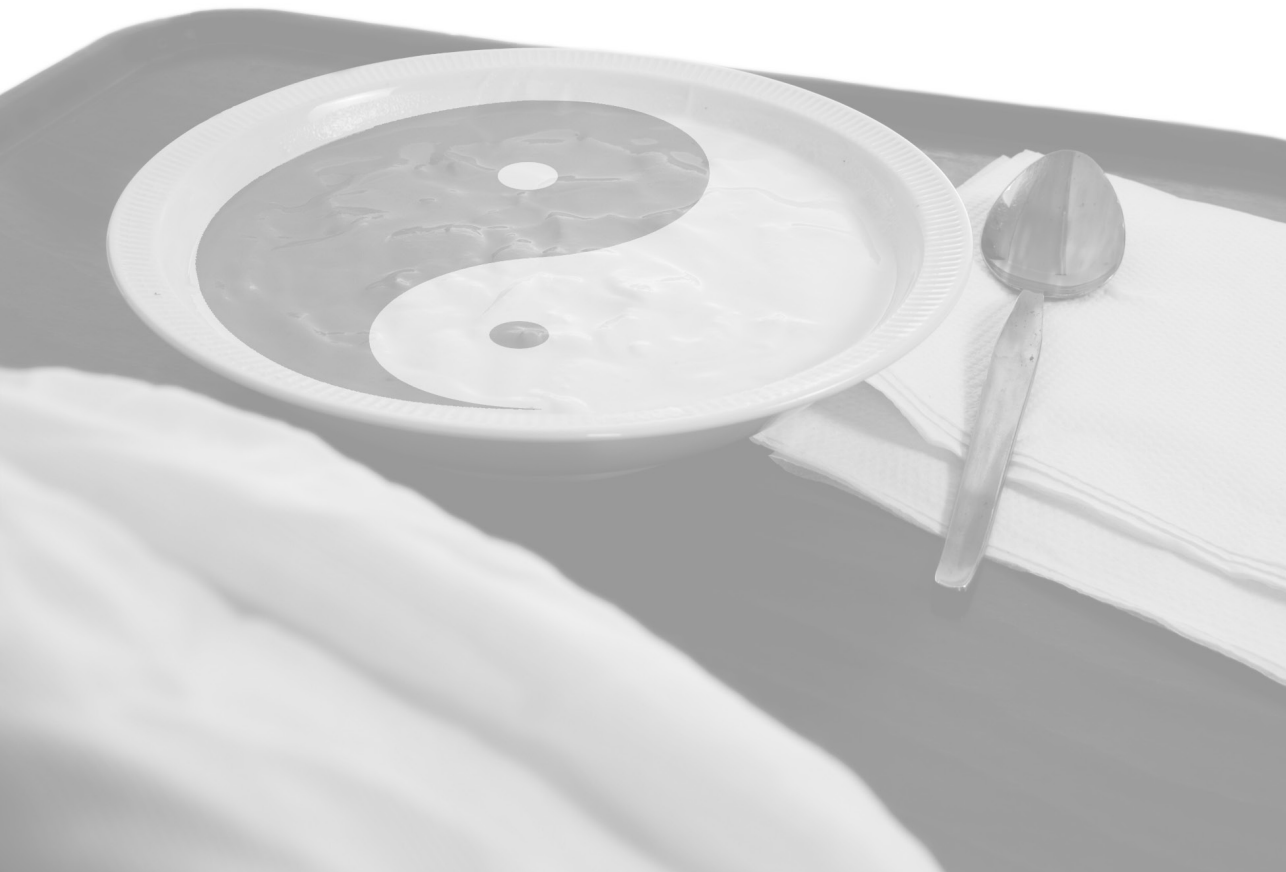
19. Slater A, Shann F, Pearson G. PIM2: a revised version of the Paediatric Index of Mortality. Intensive Care Med 2003;29(2): 278-85.
20. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
21. Kalhan SC, Kilic I. Carbohydrate as nutrient in the infant and child: range of acceptable intake. Eur J Clin Nutr 1999; 53 Suppl 1:S94-100.
22. 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.
23. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, Haymond MW. Measurement of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide. J Appl Physiol 2008;104(4):944-51.
24. Matthews DE, Schwarz HP, Yang RD, Motil KJ, Young VR, Bier DM. 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.
25. Husek P. Amino acid derivatization and analysis in five minutes. FEBS Lett 1991; 280(2):354-6.
26. 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.
27. Spear ML, Darmaun D, Sager BK, Parsons WR, Haymond MW. Use of [13C] bicarbonate infusion for measurement of CO2 production. Am J Physiol 1995; 268(6 Pt 1):E1123-7.
28. 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.
29. 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.
30. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C] leucine. Am J Physiol 1980;238(5): E473-9.
31. Sy J, Gourishankar A, Gordon WE, et al. Bicarbonate kinetics and predicted energy expenditure in critically ill children. Am J Clin Nutr 2008;88(2):340-7.
32. 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.
33. Thompson GN, Pacy PJ, Merritt H, 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.
34. Marchini JS, Castillo L, Chapman TE, Vogt JA, Ajami A, Young VR. 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.
35. Marchini JS, Cortiella J, Hiramatsu T, Castillo L, Chapman TE, Young VR. Phenylalanine and tyrosine kinetics for different patterns and indispensable amino acid intakes in adult humans. Am J Clin Nutr 1994;60(1):79-86.
36. Argaman Z, Young VR, Noviski N, et al. Arginine and nitric oxide metabolism in critically ill septic pediatric patients. Crit Care Med 2003;31(2):591-7.

37. Castillo L, Yu YM, Marchini JS, et al. Phenylalanine and tyrosine kinetics in critically ill children with sepsis. Pediatr Res 1994;35(5):580-8.
38. 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.
39. Bier DM, Leake RD, Haymond MW, et al. Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. Diabetes 1977; 26(11):1016-23.
40. Grasso S, Fallucca F, Mazzone D, Giangrande L, Romeo MG, Reitano G. Inhibition of glucagon secretion in the human newborn by glucose infusion. Diabetes 1983;32(6):489-92.
41. 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.
42. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004;114(9): 1187-95.
43. Cortiella J, Marchini JS, Branch S, Chapman TE, Young VR. 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.
44. 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.
45. 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.
46. Garrett RHCMG. Principles of Biochemistry with a Human Focus.: USA: Brooks/ Cole; 2002.
47. Biolo G, De Cicco M, Lorenzon S, et al. Treating hyperglycemia improves skeletal muscle protein metabolism in cancer patients after major surgery. Crit Care Med 2008;36(6):1768-75.
48. Verbruggen S, Sy J, Gordon WE, et al. Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am J Physiol Endocrinol Metab 2009(297):E1046-E55.
49. Hertz DE, Karn CA, Liu YM, Liechty EA, Denne SC. Intravenous glucose suppresses glucose production but not proteolysis in extremely premature newborns. J Clin Invest 1993;92(4):1752-8.
50. Jahoor F, Shangraw RE, Miyoshi H, Wallfish H, Herndon DN, Wolfe RR. 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.
51. 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-37S.
52. Voisin L, Breuille D, Combaret L, 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.
53. Deval C, Mordier S, Obled C, et al. Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting. Biochem J 2001;360(Pt 1):143-50.
54. Smith IJ, Lecker SH, Hasselgren PO. Calpain activity and muscle wasting in sepsis. Am J Physiol Endocrinol Metab 2008;295(4):E762-71.

55. Wang X, Hu Z, Hu J, Du J, Mitch WE. Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling. Endocrinology 2006;147(9):4160-8.
56. Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, Greenhaff PL. 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.
57. 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.
58. 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 2-3.

Part II

Amino Acid metabolism



Chapter 5

Ontogeny of methionine utilization and splanchnic uptake in critically ill children

Sascha CAT Verbruggen
Jama Sy
William E Gordon
Jean Hsu
Manhong Wu
Shaji Chacko
David Zurakowski
Douglas G Burrin
Leticia Castillo

Am J Physiol Endocrinol Metab. 2009;297:E1046-E1055

Abstract

To determine the rates of methionine splanchnic uptake and utilization in critically ill pediatric patients we used two kinetic models: the plasma methionine enrichment and the "intracellular" homocysteine enrichment. Twenty four patients, eight infants, eight children and eight adolescents were studied. They received simultaneous, primed, constant, intravenous infusions of L-[$^{2}H_{3}$] methyl methionine and enteral L-[1-^{13}C] methionine. The ratio of [^{13}C]homocysteine to [^{13}C]methionine enrichment (^{13}C Hcy/^{13}C meth) was 1.0±0.15, 0.80±0.20 and 0.66±0.10, respectively for the infants, children and adolescents, and different between the infants and adolescents ($p < 0.01$). Methionine splanchnic uptake was 63, 45 and 36% respectively in the infants, children and adolescents, and higher ($p < 0.01$) in the infants when compared to the adolescents. The infants utilized 73% of methionine flux for non-oxidative disposal (NOD), while 27% was used for transsulfuration ($p < 0.001$). Conversely, in the adolescents 40% was utilized for NOD while 60% was used for transsulfuration. There is ontogeny on the rates of methionine splanchnic uptake and on the fate of methionine utilization in critically ill children, with greater methionine utilization for synthesis of proteins and methionine-derived compounds ($p < 0.01$) and decreased transsulfuration rates in the infants ($p < 0.01$), while the opposite was observed in the adolescents. The plasma model underestimated methionine kinetics in children and adolescents, but not in the infants, suggesting lesser dilution and greater compartmentation of methionine metabolism in the infant population. All patients were in negative methionine balance, indicating that the current enteral nutritional support is inadequate in these patients.

Introduction

The portal-drained viscera involving the liver, stomach and intestines is profoundly deranged during critical illness. Altered splanchnic function, whether elicited by hypoperfusion, hypoxia or inflammation has been associated with systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS)[1, 2]. Under physiological conditions, the splanchnic tissues are important for whole body amino acid metabolism and selectively modify the pattern of absorbed amino acids that is subsequently presented to the peripheral circulation[3]. Thus, the pattern of amino acids in the diet is different from that in portal venous blood and does not reflect their availability to extraintestinal tissues. This concept has important implications for defining protein and amino acid requirements for nutritional and functional purposes. An extensive catabolism and/or utilization of dietary amino acids in the first pass by the small intestine results in decreased nutritional efficiency and will influence their requirements. The sulfur amino acids serve important protein and non-protein functions[4-7]. Methionine, an indispensable sulfur amino acid serves as the precursor in the synthesis of S-adenosylmethionine (AdoMet), which through the transmethylation pathway is the primary methyl group donor for methylation reactions involved in signal transduction, protein repair, chromatin regulation and gene silencing[4]. Methyl groups are required for biosynthesis of polyamines, choline, creatine, DNA and RNA intermediates[4]. Homocysteine, the demethylated product of methionine can be utilized through the transsulfuration pathway and serve as precursor for cystathionine and cysteine, which under physiological conditions is a limiting precursor in the synthesis of the tripeptide Glutathione (γ-glutamyl-cysteinyl-glycine;GSH), a major antioxidant with detoxifying and signaling properties that serves a key role in the control of apoptosis and inflammation[8]. Alternatively homocysteine can be remethylated to methionine with betaine or methyltetrahydrofolate as methyl donors[4-7, 9]. Therefore, the sulfur amino acids have major functional significance.

It is known that the splanchnic area is greatly affected during critical illness, but the uptake of methionine by the splanchnic area under these conditions has not been determined. Given the limited knowledge on the nutritional support provided to pediatric critically ill patients, and the nutritional and functional significance of methionine, it is important to examine the quantitative impact of the splanchnic area on methionine availability to the peripheral tissues under conditions of critical illness, and whether this is affected by age. Therefore, the objectives of these studies were: i) to determine the rates of methionine first-pass disappearance and oxidation (transsulfuration) by the splanchnic tissues of critically ill children by conducting simultaneous primed, continuous, enteral and intravenous tracer infusions of L-[^{13}C] and L-[$^{2}H_3$] methyl labels of methionine ii) To determine the difference between two kinetic models: The frequently

used plasma model which uses plasma methionine enrichment and the intracellular model using plasma homocysteine enrichment. Because plasma homocysteine and cystathionine arise from intracellular metabolism of methionine, enrichment of these substrates can be used to define intracellular methionine enrichment during an infusion of labeled methionine. Therefore plasma homocysteine enrichment serves as an indicator of "true" intracellular methionine enrichment[10] and iii) we aimed to ascertain whether the developmental stage from young infants to adolescents affects the rates of methionine splanchnic uptake and its utilization through different metabolic pathways.

Material and Methods

Subjects

These studies were conducted at the Pediatric Intensive Care Unit (PICU), Texas Children's Hospital, Baylor College of Medicine and at the Children's Nutrition Research Center, USDA. Twenty four consecutive critically ill children admitted to the PICU receiving enteral feedings, supplying complete protein and energy needs as per recommended standard of care, for at least 24 hours previous to the study were included. All patients were studied when hemodynamically stable. Three age groups were included: Infants 0-12 months, children >1-3 years and adolescents 13-18 years. In the infant group all patients had diagnosis of respiratory failure, five of them secondary to respiratory syncitial virus (RSV) bronchiolitis, and two patients had influenza A pneumonia. Another patient underwent a surgical procedure complicated by pneumonia. All infants had been receiving enteral feedings for an average of 8±5 days, and all had received complete feedings for at least 24 hours before the study. Six infants required mechanical ventilation, but only 2 patients required an inspiratory fraction of oxygen (FiO_2) greater than 0.6. One patient required vasopressin to maintain blood pressure. In the group of children >1 to 3 years the main diagnoses were respiratory failure secondary to Parainfluenza or influenza A pneumonia in 6 patients, septic shock due to methicillin resistant staphylococci aureus (MRSA) in one patient and acute lung injury secondary to aspiration pneumonia and viral sepsis in one patient. Six children received mechanical ventilation and two patients required non-invasive ventilatory support. One child required high frequency oscillatory ventilation and all others received conventional ventilation. The FiO_2 was 0.6 or below and none received vasopressors. They had been receiving feedings for an average of 9 ± 3 days, providing complete nutritional support as per current clinical standard for at least 24 hours previous to the study. In the adolescent group the main diagnoses were neurological conditions such as seizures and aspiration pneumonia, post-surgical patients from brain tumor resection and acute lung injury, post-tracheal reconstruction and pneu-

monia. Two previously healthy adolescents had MRSA sepsis. Six were mechanically ventilated on a conventional mode with less than 0.6 FiO_2. Two patients were breathing spontaneously. All adolescents were studied when hemodynamically stable and none were receiving vasopressors at the time of the study. The study was approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from parents or guardians. All patients had drawing and infusing intravascular lines and a post-pyloric feeding tube placed for clinical indication, and all had received full enteral feedings for at least 24 hours. All were assessed for severity of disease by the pediatric risk mortality score (PRISM III)[11], which predicts mortality rates in relation to acuity of disease. High scores indicate higher probability of mortality. Patients with metabolic diseases, diabetes mellitus, primary liver or renal failure, as well as those requiring renal replacement therapies were excluded. The demographic characteristics of the patients are shown in Table 1. Because of ethical constraints, studies in healthy infants and children were not conducted.

Table 1. Patient characteristics

	Infants	Children	Adolescents
Variable	n = 8	n = 8	n = 8
Sex (male : female)	5 : 3	3 : 5	5 : 3
Age, yr	0.5 ± 0.3	2.8 ± 1.3	15.0 ± 2.0
Weight, kg	5.5 ± 1.5	13.6 ± 3.1	63.3 ± 23
BMI, kg/m^2			27.9 ± 9.0
PRISM III	3.0 ± 3.0	8.0 ± 2.9	6.0 ± 5.0

All values are means ± SD. BMI, body mass index; PRISM III, pediatric risk mortality score

Diets

Patients received nutritional support according to current standard clinical practice. All patients were receiving complete enteral nutrition supplied through continuous 24 hour feedings, via a nasojejunal tube placed for clinical indication. The position of the tube was confirmed by x-ray. Protein and energy intake in all groups was directed by the attending physician(s) as per standard clinical care, in collaboration with the Nutrition Service. In the infant group, the formulas used were Enfamil (Meadjohnson, Atlanta, GA) in 4 patients, Prosobee (Meadjohnson, Atlanta, GA) in one patient, Goodstart (Nestle, Oakland CA) in one patient, Pregestamil (Meadjohnson, Atlanta, GA) in one patient and Portagen (Meadjohnson, Atlanta, GA) in one patient. In the children group, all patients received Pediasure (Abbott Laboratories, Grove city, OH). In the adolescent group 2 patients received Ensure (Abbott Laboratories, Grove city, OH), 4 patients received Jevity (Abbott Laboratories, Grove City, OH) and Osmolyte (Abbott Laboratories, Grove city, OH) was supplied to 2 patients. The protein and specific methionine and cysteine content of the formulas supplied were recorded and they are shown in Table 2. Average

Table 2. Enteral nutritional support

	Kcal.kg^{-1}.day^{-1}	Glucose, g.kg^{-1}.day^{-1}	Protein, g.kg^{-1}.day^{-1}	Lipids, g.kg^{-1}.day^{-1}	Methionine, mg.kg^{-1}.day^{-1}	Cysteine, mg.kg^{-1}.day^{-1}	Total Sulfur Amino Acid Intake, mg.kg^{-1}.day^{-1}
0-1 yr (n = 8)	87.6 ± 28.0	9.3 ± 3.4	2.3 ± 1.0	4.4 ± 1.5	51.1 ± 34.8	19.9 ± 12.4	70.9 ± 40.0
1-3 yr (n = 8)	66.1 ± 9.7	6.8 ± 2.0	2.0 ± 0.3	2.5 ± 0.4	55.2 ± 8.0	17.2 ± 2.6	72.3 ± 8.2
13-18 yr (n = 8)	37.1 ± 15.5	4.0 ± 1.8	1.4 ± 0.6	1.4 ± 1.0	39.2 ± 16.2	11.0 ± 3.9	50.1 ± 19.9

All values are mean ± SD.

protein intakes were 2.3 ± 1.0, 2.0 ± 0.3 and 1.4 ± 0.6 g.kg^{-1}.d^{-1} while energy intakes were 87.6 ± 28, 66.1 ± 9.7 and 37.1 ± 15.5 kcal.kg^{-1}.d^{-1} respectively, for the infants, children and adolescents. Sulfur amino acid intakes were variable in the infant group due to the variable content of sulfur amino acids in the formulas used, while similar formulas were used among children and adolescents, as shown in Table 2. The total sulfur amino acid (methionine and cysteine) intakes were 70.9 ± 40, 72.3 ± 8 and 50.1 ± 19.9 mg.kg^{-1}.d^{-1}, respectively for infants, children and adolescents and above the Institute of Medicine[12] recommended dietary intakes of 43, 28 and 22 mg.kg^{-1}.d^{-1} for infants, children and adolescents.

Materials

L-[^{13}C] methionine (99 atom %), L-[$^{2}H_3$ methyl] methionine (99 atom %) and $NaH^{13}CO_3$ were purchased from Cambridge isotopes (Andover, MA)

Tracer Study Protocol

The experimental design is shown in Figure 1. Twenty four patients distributed in three groups of 8 patients each, participated in this study. Each patient received one tracer infusion study after they had received complete, continuous, enteral feedings for at least 24 hours. On the day of the study priming doses of L-[^{13}C] methionine at

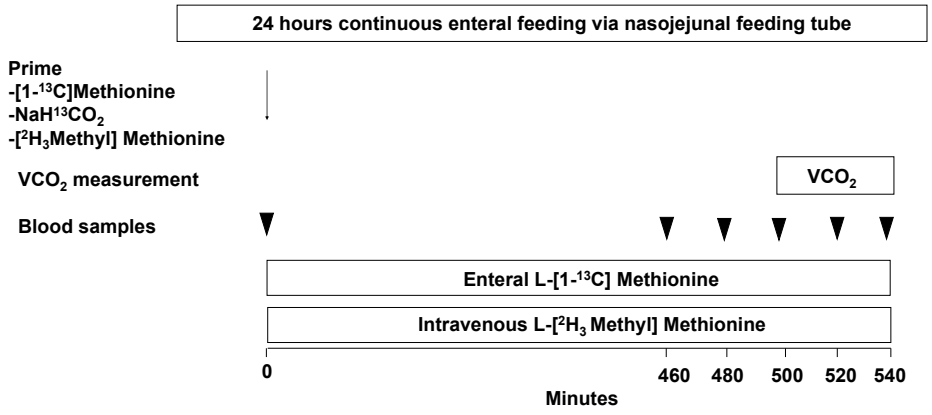


Figure 1. Experimental design. VCO_2, rate of CO_2 production

5 μmol.kg^{-1} and NaH$^{13}CO_3$ at 0.8 μmol.kg^{-1} were administered by the nasojejunal tube simultaneously with the enteral feedings. Concurrently L-[$^2H_3$ methyl] methionine was intravenously primed at 2.5 μmol.kg^{-1}, through a pre-existing indwelling intravenous catheter placed for clinical indication. These doses were immediately followed by a 9-hour, continuous nasojejunal infusion of the L-[^{13}C] methionine tracer at 5 μmol.kg^{-1}. hr^{-1} and intravenous infusion of L-[$^2H_3$ methyl] methionine at 2.5 μmol.kg^{-1}.hr^{-1}, infused by means of calibrated infusion pumps (Gemini PC-2TX infusion pump; Alaris Medical System, San Diego, California).

The tracers were prepared in sterile physiological saline solution by the Research Pharmacy at Texas Children's Hospital and filtered through a 0.22 μm-filter. Aliquotes of the infused solution were collected at the end of the tracer study for determination of infusate concentration. Exact tracer infusion doses were determined from analysis of the amino acid concentrations in the infusates. At times –30 and 0 min, two baseline blood samples were obtained, from a pre-existent intravascular catheter (arterial line) placed for clinical indication, followed by several samples at 500, 520 and 540 minutes for the infants, 480, 500, 520 and 540 minutes for the children and 460, 480, 500, 520 and 540 for the adolescents. For determination of blood $^{13}CO_2$ enrichment it was ensured that air entered neither the blood drawing nor the collecting tube during the blood transfer. Samples of 0.5 ml were transferred from the collection syringe into a 3 ml sodium-heparin-coated, capped, evacuated tube and maintained at room temperature. Samples were then processed as previously described[13]. For the plasma isotopic enrichment of the methionine tracers given and of derived homocysteine, blood samples were kept on ice immediately centrifuged, and plasma was then withdrawn and stored at -80°C until used for analysis.

Measurement of CO_2 production rates (VCO_2)

In the mechanically ventilated patients, the rates of VCO_2 (ml/min) were measured with a respiratory monitor (Cosmo, Respironics, Wallingworth, CT). The device was calibrated and directly connected to the endotracheal tube. Continuous measurements were obtained during the tracer study and the average value was recorded. This respiratory monitor is routinely used as per standard clinical practice in ventilated patients to adequately monitor pulmonary function. For the spontaneously breathing patients VCO_2 was determined by indirect calorimetry using a Vmax Encore (Vyassis Healthcare, Yorbalinda CA), calorimeter, connected to a plastic canopy, which was placed over the head and chest of the patients. None had air leaks through chest tubes.

Analytical Methods

Analyses of blood samples for V$^{13}CO_2$ enrichment were all conducted as previously described[13]. In brief, the carbon dioxide was liberated from the blood bicarbonate

by adding 2 mL of 85% (vol/vol) phosphoric acid into the evacuated tube and the contents vortexed. The evacuated tube was then backfilled with nitrogen to bring it to atmospheric pressure and let stand overnight. The liberated carbon dioxide was transferred to a plain, non-silicon coated 15 mL venoject tube, which was subsequently backfilled with nitrogen to bring it to atmospheric pressure. The $^{13}CO_2$ enrichment was then measured by isotope ratio mass spectrometry (ThermoQuest Finnigan Deltaplus XL Isotope Ratio Mass Spectrometer coupled with Gasbench-II, Bremen, Germany)[13]. The plasma enrichment of the methionine tracers given was determined as previously described[10]. In brief, the plasma methionine and homocysteine isotopic enrichments were measured by tandem liquid chromatography-mass spectrometry (LC-MS/MS). Plasma methionine and homocysteine were converted to their 5-(dimethylamino)-1-napthalene sulfonamide (DANS) derivatives and analyzed on a triple quadrupole mass spectrometer, TSQ Quantum Ultra (Thermo Fisher Scientific, San Jose, California), equipped with an electrospray ionization (ESI) source, a Survey pump (Thermo Fisher Scientific), and a HTC PAL autosampler (Leap Technologies Inc, Carrboro, NC, USA). The ions were then analyzed by selected reaction monitoring (SRM) mode. The transitions observed were precursor ions *m/z* 383, 384 and 386 to product ion m/z 170 for methionine and precursor ion *m/z* 426 and 427 to product ion m/z 170 for homocysteine. Instrumental control, data acquisition and analysis were done by XCalibur (version 2.0) software package (Thermo Fisher Scientific).

Calculations

Plasma Fluxes

We used two models to estimate plasma fluxes, splanchnic uptake and oxidation (transsulfuration) of methionine. In the first model, we used plasma methionine enrichment as the precursor. In the second model the plasma enrichment of [^{13}C] homocysteine intracellularly formed from transmethylation of the L-[^{13}C] methionine infused was used. This is the intracellular model. The methyl group of L-[$^{2}H_3$] methyl methionine is lost during transmethylation, therefore intracellular homocysteine enrichment derived from [$^{2}H_3$] methyl methionine tracer is estimated from the ratio of L-[^{13}C] homocysteine to L-[^{13}C] methionine (^{13}C hcy/^{13}C meth), as previously described[10]. Hence, all calculations were obtained with the plasma methionine and homocysteine enrichments (plasma and intracellular models). Plasma methionine fluxes obtained with the enteral L-[^{13}C] methionine or the intravenous [$^{2}H_3$] methyl methionine were calculated using the steady-state isotope dilution equation and a simplified single-pool model[14] as follows:

$$Ra\ (\mu mol.kg^{-1}.h^{-1}) = I \times [(E_i/E_p) - 1] \quad (1)$$

Where I is the rate of appearance, I is the rate of enteral [^{13}C] or intravenous [$^{2}H_3$] methylmethionine tracers infused (μmol.kg^{-1}.hr^{-1}), E_i is the enrichment of the labeled methionine tracer (99%) and E_p is the mean plasma isotopic enrichment of the [^{13}C] methionine for the plasma model, or [^{13}C] homocysteine for the intracellular model. For the intracellular model of the [$^{2}H_3$] methyl methionine tracer, the ^{13}Chcy/^{13}Cmeth ratio was applied, as previously described[10], while the plasma [$^{2}H_3$]methyl methionine enrichment was used for the plasma model.

Splanchnic uptake

The splanchnic uptake of dietary methionine was estimated, as previously described[3]. In brief, the plasma amino acid fluxes obtained with the enteral and intravenously administered tracers, and the fraction of labeled tracer taken up in a first-pass through the splanchnic area, before reaching the systemic circulation, can be determined as follows[3]:

$$Ra_{[2H3]\ methylmeth} = I_{IV} \times [(E_{iIV}/E_{PIV}) - 1] \quad (2)$$

Where the rate of appearance or plasma flux from the intravenous [$^{2}H_3$] methyl methionine tracer is obtained as in *equation 1*.

The rate of appearance or plasma flux from the enteral [^{13}C] methionine tracer ($Ra_{[13C]meth}$) is determined from the enteral tracer's plasma enrichment also as in equation 1:

$$Ra_{[13C]\ meth} = I_E \times [(E_{iE}/E_{PE}) - 1] \quad (3)$$

Because an unknown fraction (f) of the enterally infused tracer (IE) may be taken up by the first pass through the splanchnic tissues before reaching the plasma pool, the actual enteral tracer infusion rate into the plasma is IE x (1-f). Hence, IE x (1-f) is the actual rate of isotope tracer infusion into the sampled plasma compartment, and the true plasma methionine flux as estimated from the enteral infused tracer is:

$$Ra_{[13C]meth} = I_E \times (1 - f) \times [(E_{iE}/E_{PE}) - 1] \quad (4)$$

For estimates of the first pass disappearance of methionine within the splanchnic region, the following relationship stands:

$$I_{IV} \times [(E_{iIV}/E_{PIV}) - 1] = IE \times (1 - f) \times [(E_{iE}/E_{PE}) - 1] \quad (5)$$

Rearranging and solving for (1– f)

$$(1 - f) = (I_{IV} \times [(E_{iIV}/E_{PIV}) - 1]) / (IE \times [(E_{iE}/E_{PE}) - 1]) \quad (6)$$

Therefore, the fraction of labeled tracer taken up during the first pass through the splanchnic tissues, before reaching the systemic circulation, can be solved as the ratio of the intravenous and enteral plasma methionine fluxes.

$$(1 - f) = Ra_{\text{IV [2H3] methyl meth}} / Ra_{\text{E [13C] meth}} \quad (7)$$

And by rearrangement

$$f = 1 - (Ra_{\text{IV [2H3] methyl meth}} / Ra_{\text{E [13C] meth}}) \quad (8)$$

Where, the unknown fraction is 1 minus the ratio of plasma flux of the intravenous tracer over the plasma flux of the enteral administered tracer. The fraction of the tracer taken up in a first pass should be the same as the fraction of unlabeled dietary amino acid that is taken up in a first pass through splanchnic tissues. Therefore, the first pass disappearance of dietary methionine may be estimated by multiplying dietary methionine intake by the fraction of tracer taken up during the first pass.

Splanchnic Methionine Oxidation (Transsulfuration of homocysteine)

The rate of $^{13}CO_2$ appearance from methionine oxidation (Met ox μmol·kg^{-1}.hr^{-1}) corresponds to irreversible methionine loss through transsulfuration (TS). Hence, TS has been assumed to be equivalent to methionine oxidation[10, 15]. For determination of methionine oxidation in critically ill children using the enteral L-[1-^{13}C] methionine tracer, it was necessary to establish a factor to be used for the retention of $^{13}CO_2$ liberated from enterally supplied methionine, during its oxidative decarboxylation via α-ketobutyrate[7, 16]. For this purpose, we used previous data by our group obtained in enterally fed critically ill children receiving a primed, constant infusion of $NaH^{13}CO_3$ which showed bicarbonate recovery of 69.80%[13]. Although the bicarbonate tracer in the latter studies was provided by the parenteral route[13], there is no difference on bicarbonate recovery when the tracer is given through the enteral versus the parenteral routes[17]. Therefore the mean value of 69.80 was used to estimate methionine oxidation in the patients. Methionine oxidation (TS) was then calculated as follows:

$$\text{Meth ox} = TS = (VCO_2 \times {}^{13}CO_2) = [V^{13}CO_2/69.80)]/Ep \quad (9)$$

Where, methionine oxidation (TS) (μmol.kg^{-1}.hr^{-1}) is determined by the rate of CO_2 production (VCO_2) measured in mL/min, obtained by using a respiratory monitor or indirect calorimetry and converted to mmol/h by multiplying by 60 min and dividing by

22.4, which is the number of liters in one mole of an ideal gas at standard temperature and pressure (STP) to convert mL to mmol/min; $V^{13}CO_2$ is obtained by multiplying VCO_2 by blood $^{13}CO_2$ enrichment from the ^{13}C methionine tracer infused and corrected by 69.80, the bicarbonate retention factor obtained in critically ill children enterally fed[13], and Ep is the plasma enrichment of methionine for the plasma model or enrichment of homocysteine for the intracellular model.

Methionine balance

Methionine balance ($mg.kg^{-1}.d^{-1}$) was estimated as follows:

$$\text{Methionine Balance} = \text{Meth Intake} - \text{Meth ox} \quad (10)$$

Where intake is estimated from dietary methionine and cysteine over the 24h period and the intake of tracer infused. Cysteine is accounted for because it spares methionine[15, 16, 18, 19]. The methionine tracer contribution, although minimal was also estimated in the intake.

Statistical Analyses

Continuous variables were tested for normality using the Kolmogorov-Smirnov goodness-of-fit statistic and no significant departures were found, indicating that means and standard deviations are appropriate descriptive statistics. Two-way analysis of variance (ANOVA) was used to assess differences between the plasma and intracellular models for enrichment, fluxes, splanchnic uptake and oxidation within each age group and the ^{13}C homocysteine / ^{13}C methionine ratio, plasma fluxes, splanchnic uptake oxidative and non-oxidative disposal between groups (infants, children and adolescents). A mixed-model ANOVA approach was applied to maximize statistical power and to properly handle the within-subject correlation since each patient had measurements obtained from both plasma and intracellular models. Two-tailed $p < 0.05$ was considered statistically significant with Bonferroni correction to adjust for multiple comparisons and protect against false positive results due to multiple testing. Statistical analysis was performed using the SPSS software package (version 16.0, SPSS Inc., Chicago, IL).

Results

As shown in Table 3 the plasma isotopic enrichment in the critically ill infant group was 0.057 ± 0.01 for [^{13}C] methionine (plasma) and 0.056 ± 0.01 for [^{13}C] homocysteine (intracellular), and there was no difference (p= 0.77) between the plasma and

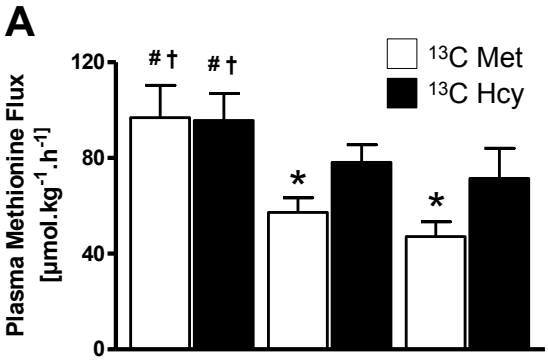
Table 3. Plasma isotopic enrichment for [^{13}C] and [$^{2}H_3$]methylmethionine, enrichment of $^{13}CO_2$ in expired air, VCO_2, $V^{13}CO_2$, rates of oxidation, and methionine intakes after enteral L-[^{13}C] methionine and intravenous [$^{2}H_3$]methyl tracer infusions in critically ill infants, children, and adolescents, obtained with the plasma and intracellular models

Parameter	Infants		Children		Adolescents	
	Plasma Methionine	Intracellular Homocysteine	Plasma Methionine	Intracellular Homocysteine	Plasma Methionine	Intracellular Homocysteine
[^{13}C] enrichment, molar fraction	0.057 ± 0.01‡	0.056 ± 0.01‡	0.087 ± 0.02*	0.067 ± 0.01	0.11 ± 0.01*	0.073 ± 0.01
[$^{2}H_3$] methyl enrichment, molar fraction	0.081 ± 0.02	0.080 ± 0.02	0.084 ± 0.02*	0.068 ± 0.02	0.092 ± 0.02*	0.060 ± 0.01
$^{13}CO_2$ enrichment, atom percent excess x 10^3	5.2 ± 1.2	5.2 ± 1.2	7.9 ± 0.1	7.9 ± 0.1	18.5 ± 0.7	18.5 ± 0.7
VCO_2, mmol.kg^{-1}.h^{-1}	15.5 ± 5.3	15.5 ± 5.3	14.8 ± 5.0	14.8 ± 5.0	8.1 ± 2.4	8.1 ± 2.4
$V^{13}CO_2$, µmol.kg^{-1}.h^{-1}	1.2 ± 0.3	1.2 ± 0.3	1.5 ± 0.0	1.5 ± 0.0	2.0 ± 0.9	2.0 ± 0.9
Methionine oxidation (transsulfuration), µmol.kg^{-1}.h^{-1}	23.5 ± 10.6	23.0 ± 9.4	19.5 ± 6.6 *	28.4 ± 11.9	22.1 ± 7.8†	39.4 ± 16.6
Dietary methionine intake, µmol.kg^{-1}.h^{-1}	14.3 ± 9.8	14.3 ± 9.8	15.4 ± 2.3	15.4 ± 2.3	11.0 ± 4.6	11.0 ± 4.6
Dietary splanchnic methionine uptake, µmol.kg^{-1}.h^{-1}	9.4 ± 7.4	9.4 ± 7.4	6.7 ± 3.0	6.8 ± 2.9	3.5 ± 1.6	3.6 ± 1.5

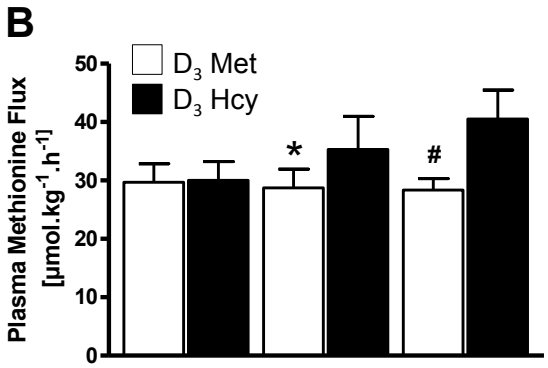
All values are means ± SD. VCO_2, carbon dioxide production; $V^{13}CO_2$, $^{13}CO_2$ output. * P < .05 plasma vs. intracellular model within a group; † P < .01 plasma vs. intracellular model adolescent group; ‡ P < .01 enrichments infants vs. adolescents

the intracellular models. In contrast, in the critically ill children there was a significant difference ($p < 0.05$) between [^{13}C] methionine and [^{13}C] homocysteine enrichment, with values of 0.087 ± 0.02 and 0.067 ± 0.01, respectively. Likewise, the plasma and intracellular enrichment models were different ($p < 0.01$) in the critically ill adolescent group with values of 0.11 ± 0.01 and 0.073 ± 0.01 respectively, for [^{13}C] methionine and [^{13}C] homocysteine.

Hence, the ratio of [^{13}C] homocysteine to [^{13}C] methionine (^{13}C Hcy/^{13}C meth) was 1.0 ± 0.15, 0.80 ± 0.20 and 0.66 ± 0.10, respectively for the infants, children and adolescents, and different between the infants and adolescents ($p < 0.01$), but not between the infants and children or the children and adolescents. The plasma isotopic enrichments for the intravenously infused L-[$^{2}H_3$] methyl methionine and blood $^{13}CO_2$ enrichments are also shown in Table 3. Plateau isotopic enrichment was achieved in the three patient groups, but a difference was observed again between the plasma and intracellular models in the children ($p < 0.05$) and adolescent ($p < 0.05$) groups. From these enrichments the rates of appearance (Ra) of methionine obtained with both tracers were estimated. The rates of appearance or plasma fluxes of the [^{13}C] and [$^{2}H_3$] methyl methionine obtained with the plasma and the intracellular models for the three patient groups are shown in Figure 2, panels A and B.



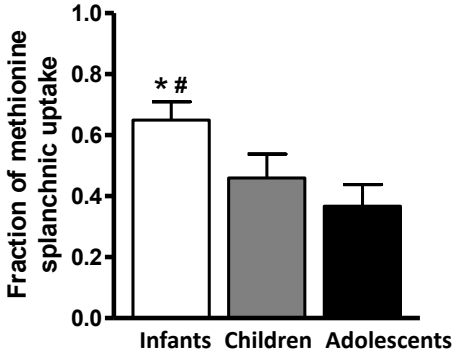
* p < .05 ^{13}C Methionine vs. ^{13}C Homocysteine within the same age group;
# p < .05 ^{13}C Methionine or ^{13}C Homocysteine infants vs. children;
† p < .001 ^{13}C Methionine or ^{13}C Homocysteine infants vs. adolescents



* p < .05 $^{2}H_3$ Methyl methionine vs. estimated $^{2}H_3$ Homocysteine children group;
# p < .001 $^{2}H_3$ Methyl methionine vs. estimated $^{2}H_3$ Homocysteine adolescent group;

Figure 2. A: Methionine fluxes estimated with the plasma [^{13}C] methionine (Met) and the intracellular [^{13}C]homocysteine (Hcy) models during enteral administration of L- [^{13}C]methionine tracer. B: Plasma methionine fluxes obtained with the intravenous [$^{2}H_3$]methylmethionine tracer (plasma model) and estimated homocysteine enrichment (intracellular model) in critically ill infants, children and adolescents.

In the infant group, there was no difference ($p > 0.5$) in the [^{13}C] carbon methionine fluxes between the plasma and the intracellular models, both yield rates of appearance of 85.4 ± 18 and 86.0 ± 14 µmol.kg^{-1}.hr^{-1} (mean ± SD), suggesting that there was no significant intracellular dilution of the ^{13}C methionine label. In contrast, in the children group the plasma ^{13}C methionine rate of appearance were 54.4 ± 12 and 69.7 ± 5 µmol.kg^{-1}.hr^{-1} respectively, and significantly different ($p< 0.05$) for the plasma and the intracellular models. Likewise in the adolescent group rates of appearance were 39.5 ± 3 vs. 63.7 ± 8 µmol.kg^{-1}.hr^{-1} respectively for the plasma and intracellular models, and significantly different ($p< 0.001$) among the models. The plasma fluxes obtained with homocysteine as the precursor were 22 and 38% higher respectively for children and adolescents, indicating a significant intracellular dilution of methionine enrichment



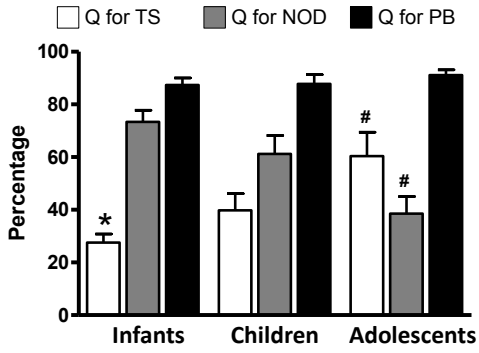
* p < .01 infants vs. children; # p < .001 infants vs. adolescents.

Figure 3. Methionine splanchnic uptake in critically ill infants, children and adolescents.

in these age groups. The plasma ^{13}C methionine fluxes obtained with either model were higher in the infants when compared to children ($p < 0.05$) and adolescents ($p < 0.001$). The methionine rate of appearance obtained with the intravenous [$^{2}H_3$]methyl methionine tracer were 29.7 ± 8.3 and 30.3 ± 8.2; 28.7 ± 8.5 and 38.7 ± 14; and 25.5 ± 6 and 40.5 ± 10 μmol.kg^{-1}.hr^{-1}, respectively for the plasma and intracellular models in the infants, children and adolescents. While in the infant group there was no difference in the methyl fluxes obtained with either model, in the children and adolescent groups the methyl methionine fluxes obtained with the intracellular model yield 25.6 ($p < 0.05$) and 37% ($p < 0.01$) higher values, respectively. We did not infuse intravenous ^{13}C methionine in these patients, thus for the intracellular model of the intravenously infused [$^{2}H_3$]methyl methionine, we used the ratio of ^{13}C hcy/^{13}C meth obtained with the enteral ^{13}C methionine tracer, as previously described[10]. We cannot determine if the ^{13}C hcy/^{13}C meth ratio is similar when the tracers are given by the intravenous[10] versus the enteral route in critically ill children. However, studies in the piglet model[20] suggest that the ^{13}C hcy/^{13}C meth ratio is 0.738 when the tracers are given intravenously and 0.795 when given intragastrically, and therefore they appear to be comparable. The methionine splanchnic uptake for the three groups using both models and estimated as described in the methods section, is shown in Figure 3. Both models yield the same fractional first-pass disappearance of methionine. Interestingly, the infant group had the highest fractional methionine splanchnic uptake of 0.63 ± 0.14 when compared to values of 0.45 ± 0.19 and 0.36 ± 0.13 for children ($p < 0.01$) and adolescents ($p < 0.001$), respectively. The absolute first-pass disappearance or dietary methionine splanchnic uptake in the infant group was 9.4 μmol.kg^{-1}.hr^{-1} and highly variable due to different methionine content in the infant formulas used, whereas in the children and adolescents the dietary methionine splanchnic uptake was about 6.7 and 3.6 μmol.kg^{-1}.hr^{-1}, respectively.

Table 3 also summarizes the results for methionine oxidation (transsulfuration), the blood $^{13}CO_2$ enrichment (APE x 10^3) and the rates of $^{13}CO_2$ production ($V^{13}CO_2$; μmol.kg^{-1}.hr^{-1}). In the infant group the rates of methionine oxidation (transsulfuration) were about 23.5 μmol.kg^{-1}.hr^{-1} with either the plasma or intracellular models, and not different from each other. In addition, the plasma model yielded comparable values of methionine oxidation among infants, children and adolescents. However, the plasma model in comparison to the intracellular model underestimated methionine oxidation rates by 32% ($p < 0.05$) in the children and by 44% ($p < 0.01$) in the adolescents. Hence, the model used had an impact on the children and adolescents, but not in the infant group. Furthermore, although there was not statistical difference in the rates of methionine oxidation obtained with the intracellular homocysteine model between the infants, children and adolescents ($p = 0.077$), biologically there was a difference in methionine oxidation rates of 16 μmol.kg^{-1}.hr^{-1} between the infants and adolescents.

The fraction of plasma methionine flux utilization for oxidative (TS) and non-oxidative disposal (NOD) and the proportion of plasma methionine flux originating from protein breakdown are shown in Figure 4. The pattern of enteral methionine utilization was different in the infants when compared to the adolescents. In the infants 26.7 ± 9.8% of plasma methionine flux was utilized for oxidative disposal (transsulfuration) and 73.3 ± 9.8% for NOD ($p < 0.01$). In contrast, in the adolescents 60.5 ± 21.2% was utilized for oxidative disposal and 39.5 ± 16.6% for NOD, and there were intermediate values of 40.7 ± 16.6% and 59.3 ± 16.6%, respectively, for the fraction of plasma methionine flux utilization for oxidative and NOD in the children. There was no difference in the pattern of methionine flux utilization among the children and adolescents, or children and infants. However, there was a significant difference in the pattern of



* p < .001 Infants Q for TS vs. Q for NOD.
# p < .001 Q for TS adolescents vs. Infants.
# p < .001 Q for NOD adolescents vs. infants.

Figure 4. Percentage of splanchnic methionine flux (Q) utilized for nonoxidative disposal (NOD), oxidative disposal (transsulfuration (TS)) and methionine flux derived from protein breakdown (PB) in critically ill infants, children and adolescents.

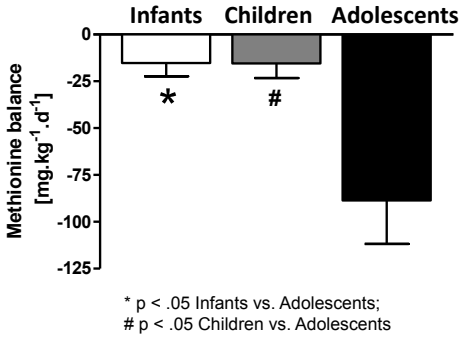


Figure 5. Methionine balance in critically ill infants, children and adolescents.

methionine utilization between infants and adolescents, with a greater proportion of methionine flux utilized for NOD in the infants when compared to adolescents ($p < 0.01$) and conversely, greater utilization for oxidative disposal in adolescents when compared to infants ($p < 0.01$). Most of the plasma methionine flux originated from protein breakdown in all age groups despite that they were receiving standard enteral nutritional intake. Methionine balance for the three age groups is shown in Figure 5. All groups were in negative balance with significant differences observed between infants and adolescents ($p<0.01$) and infants and children ($p < 0.01$).

Plasma amino acids and glutathione concentrations obtained in our patients and compared to reference values[21, 22] are shown in Table 4. Only the values that were

Table 4. Abnormal concentrations of plasma amino acids and plasma glutathione in critically ill enterally fed children

	Infants	Children	Adolescents
Essential			
Histidine	13.4 ± 3.2 (61-91)	19.4 ± 10.8 (61-91)	18.6 ± 7.0 (77-107)
Methionine	40.5 ± 7.8 (14-38)	47.5 ± 13.2 (13-22)	42.9 ± 9.2 (20-34)
Tryptophan	21.8 ± 10.5 (34-73)	23.0 ± 11.9 (35-73)	29.5 ± 13.3 (54-93)
Nonessential			
Cystine	18.0 ± 4.6 (21-53)	21.9 ± 9.3 (27-52)	26.3 ± 7.0 (36-61)
Cysteine	167 ± 52 (131 ± 40)*	159.7 ± 56.0 (209 ± 54)*	204.2 ± 34 (197 ± 56)*
Glutamine	622.5 ± 137.7 (474-747)	538.4 ± 159.3 (473-692)	513.1 ± 69.8 (551-797)
Glycine	172.1 ± 19.7 (138-276)	284.3 ± 148.2 (138-276)	162.7 ± 36.6 (183-322)
Serine	127.2 ± 37.9 (98-160)	100.4 ± 24.0 (97-154)	83.9 ± 23.9 (101-177)
Taurine	29.3 ± 15.6 (39-111)	30.7 ± 11.4 (39-80)	32.0 ± 16.6 (41-66)
Other			
Glutathione	2.21 ± 0.61 (12.5 ± 5.2)*	3.19 ± 0.94 (21.1 ± 8.9)*	2.94 ± 0.96 (12.2 ± 3.1)*
Homocysteine	3.37 ± 1.46 (5 ± 1.6)*	3.93 ± 1.26 (10)*	5.17 ± 2.25 (9)*

Values are means ± SD in micromoles. Nonreported amino acids were within normal range. Reference values are expressed as 10^{th}-90^{th} quantile from Reference *22*. *Values from Reference *28*.

abnormal or close to the abnormal range are reported. Among the essential amino acids, histidine and tryptophan were lower in all age groups and methionine appeared to be increased in the children and adolescents. For the nonessential amino acids, cystine appeared to be lower in all groups. Glutamine, glycine and serine appeared to be in the lower range only in the adolescent group. The children and adolescents appear to have lower taurine values. All other non-reported amino acid values were within normal range.

Plasma homocysteine appeared to be within normal range and plasma glutathione appeared to be lower in all groups when compared to reference values. However, plasma glutathione has limited value because the major pool of glutathione is intracellular.

Discussion

We have investigated *in vivo* aspects of methionine metabolism in three different age groups of critically ill children. We used the ^{13}C and $^{2}H_3$ methyl methionine tracers by the enteral and parenteral routes, respectively. We acknowledge that both tracers have different metabolic fates and given that they were not simultaneously administered by the same route, the splanchnic rates of transmethylation and remethylation could not be determined. It would have been difficult to obtain a second tracer study day using the alternate route of tracer administration (intravenous ^{13}C methionine and enteral $^{2}H_3$ methyl methionine) in these critically ill children. This limits the scope of our results but does not impact on our conclusions. Our data showed that the first pass disappearance or splanchnic uptake of methionine was about 63% and significantly higher in the critically ill infant population when compared to children and adolescents and almost double the value of ~33% estimated from various studies in healthy adults[15, 16, 18]. There are no comparable values on methionine splanchnic uptake in healthy children, infants or adolescents, but our observations suggest that in critically ill children there is a greater utilization of dietary methionine in the splanchnic tissues of sick infants when compared to sick adolescents and that the rates of splanchnic methionine utilization appear to decrease in the more mature individual. Greater utilization rates of methionine in the splanchnic tissues of infants may be due to increased demand for synthesis of methionine substrates in these critically ill patients. It has been demonstrated in the piglet model that methionine main metabolic fate in the gut is transmethylation to homocysteine and transsulfuration to cysteine[20]. Increased transmethylation rates will render methyl groups available for the synthesis of polyamines, creatine and DNA methylation and will increase production of homocysteine, which may be transsulfurated to cysteine with production of glutathione (γ-glutamyl-cysteinyl-glycine;GSH),

taurine and H_2S[6] or remethylated to methionine for further availability of methyl groups, with betaine or methyltetrahydrofolate as donors[5, 6].

The intestine is a highly active metabolic tissue and due to its high proliferation rate, the intestinal and colonic mucosa have a special demand for polyamines[23]. These low molecular weight cations have major regulatory roles on tissue growth and differentiation. Additionally, the use of broad spectrum antibiotics will result in changes in intestinal flora, which is an important contributor to intestinal polyamines. The lack or limited polyamines, creatine and choline content in the formulas supplied to critically ill infants may also impose a greater demand in the splanchnic tissues for the synthesis of these compounds[5, 24-26], and therefore the need for greater availability of methyl groups.

Interestingly, the elevated need for methionine precursor in the splanchnic tissues appears to decrease at later developmental stages, as demonstrated by the lower rates of methionine splanchnic uptake in adolescents. Hence, there appears to be ontogeny of methionine splanchnic uptake. The expression of amino acid transporter systems[27, 28] and spatiotemporal patterns of enzymatic expression may explain the ontogeny observed for amino acids and nutrients in the developing mammal[29, 30].

In the infant population there was no difference among the enrichments of ^{13}C methionine and ^{13}C homocysteine and therefore no difference in the plasma fluxes obtained with the plasma or the intracellular models, suggesting that there was minimal or no intracellular dilution of the ^{13}C methionine tracer enterally infused. In addition, there was no difference in the rates of transsulfuration (methionine oxidation) obtained with either model, and about 27% of the plasma methionine flux was used for transsulfuration to cysteine, while 73% was used for non-oxidative disposal, which involves the synthesis of protein and methionine derived compounds. However, the absolute rates of methionine transsulfuration of about 23 $\mu mol.kg^{-1}.hr^{-1}$ were significantly higher than the dietary methionine splanchnic uptake of 9.4 $\mu mol.kg^{-1}.hr^{-1}$ indicating that dietary methionine is insufficient to maintain transsulfuration rates, and therefore plasma methionine from protein breakdown has to be utilized under these conditions. This is confirmed by the fact that about 89% of the ^{13}C methionine flux originates from protein breakdown and that these critically ill infants are in a negative methionine balance of about 11.4 $mg.kg^{-1}.d^{-1}$. The fact that the enrichments obtained with the intracellular homocysteine and the plasma methionine models do not differ in the infant population, despite significant protein breakdown and expected intracellular dilution, suggests that there is major compartmentation of methionine pools in the splanchnic tissues in this age group. The mechanism(s) whereby this compartmentation occurs is beyond the scope of this investigation. However, nuclear magnetic spectroscopy of subcellular fractions in splanchnic tissues may help to better understand the underlying mechanisms[31].

In contrast, in the children and adolescent patients there were clear differences in methionine enrichments between the plasma and the intracellular models, and the

ratio of ^{13}C Hcy /^{13}C meth of 0.80 in the children and 0.66 in the adolescents were closer to values of 0.80 reported in healthy adult women[32] and 0.58 reported in healthy men[10] suggesting that there was significant intracellular dilution of labeled methionine. Therefore, there was a marked difference in plasma ^{13}C methionine fluxes in children and adolescents when obtained with the intracellular versus the plasma model.

Likewise, the rates of methionine oxidation (transsulfuration) obtained with the oral ^{13}C methionine tracer were 31% and 43% higher, respectively in children and adolescents when the intracellular model was used. Therefore the plasma model underestimated *in vivo*, whole body oxidation rates of methionine when the tracer was given by the enteral route.

Irrespective of the model used, the rates of methionine transsulfuration (oxidation) in the infants, children and adolescents were higher than the dietary sulfur (methionine and cysteine) amino acid intake. In addition, the fraction of methionine flux utilized for methionine transsulfuration, and therefore irreversible carbon losses, significantly increased with age, from 27% in the infants to 61% in the adolescents. Conversely, the infants utilized a higher proportion of methionine for non-oxidative disposal.

Taken together, these data suggest that the metabolic fate and utilization of methionine differ in the critically ill pediatric population depending on age, and that there is a greater contribution of plasma methionine for transsulfuration in the adolescents, which may reflect a greater need in this age group for transsulfuration substrates, such as cysteine, glutathione, taurine, and H_2S, while there is a lesser utilization for methylated substrates. Conversely, in the young infants the opposite holds true and the group of children had intermediate values. Our infant population was 6 months of age in average, fluctuating from 1 to 11 months, but provided that the same physiological processes take place in the younger newborn and premature infant populations, the decreased methionine transsulfuration and therefore limited glutathione availability in young infants may contribute to make young infants more susceptible to oxidative injury and necrotizing enterocolitis, which is seldom observed in older children.

It is known from animal data that nutrition and age influence enzymatic capacity and therefore metabolic pathways. Finkelstein[9] found in the rat model that the hepatic content of the methionine catabolizing enzymes methionine adenosyl transferase III (EC 2.5.1.6), cystathionine β-synthase (EC4.2.1.22;CBA) and γ-cystathionase (EC 4.4.1.1) increase with age and in response to higher dietary protein or methionine, or both.

It is also known that transsulfuration flux is increased by oxidative stress, whereas antioxidants decrease it[6]. However, there was no difference in severity of disease or in the acid base status among our patients, although this could have been limited by our sample size. The redox regulation of the transsulfuration pathway may occur at the level of cystathionine β-synthase, which contains a heme group that may serve as a sensor of the oxidative environment[33, 34]. There is a tissue-specific metabolic

distribution of methionine cycle enzymes[5, 9] and the liver, kidney, small intestine, and pancreas contain both cystathionine β-synthase and γ-cystathionase, key enzymes in the transsulfuration pathway, which produces glutathione. Hence, the splanchnic tissues in mature animals, in addition to the kidney, have the most rapid turnover of glutathione[35]. Furthermore, It has been shown that hypoxia may affect remethylation by altering methionine adenosyl transferase expression[36], and that these changes are comparable to those of a methyl-deficient diet[37]. However, none of our patients were hypoxemic during the study or in the immediate preceding days.

Regardless of the rates and fate of methionine utilization, these critically ill patients were in significantly negative balance despite receiving complete enteral feedings as per current clinical standards, demonstrating that our understanding of enteral nutritional support in critically ill patients is rather limited.

The oxidative irreversible loss of carbon moiety during transsulfuration is consistent with a lesser efficiency in methionine utilization of critically ill children and adolescents and probably a greater dietary need for sulfur amino acids. On the contrary, in young infants there is a greater utilization of methionine for non-oxidative disposal, and therefore synthesis of protein and methylated compounds, which may impose a greater need for neomethylgenesis, and therefore folate, betaine and vitamin B 12. These observations are supported by the significantly negative methionine balance observed in the adolescents when compared to the infants and children. Specific measurements of transmethylation and remethylation in the splanchnic tissues of critically ill children remain to be determined.

Jahoor and coworkers[38] estimated methionine kinetics in malnourished, edematous and non-edematous children, slightly older than our infant population, using intravenous tracers. Although the malnourished population is physiologically different from the critically ill population, these authors found that methionine transsulfuration was maintained despite slower methionine turnover and as a consequence, less methionine was available for protein synthesis and synthesis of methionine-derived compounds.

It is important to underscore that methionine metabolism occurs at the intracellular level and therefore the homocysteine intracellular model is more accurate, while the plasma model underestimates methionine kinetics in populations other than young infants.

In summary, we investigated for the first time methionine splanchnic uptake in critically ill infants, children and adolescents using the plasma and intracellular kinetic models, and found that there is ontogeny on splanchnic uptake and methionine utilization in critically ill children, characterized by a significant compartmentation of methionine metabolism in young infants, with a greater utilization of methionine for non-oxidative disposal, while in the adolescents methionine splanchnic uptake was lower and there was greater methionine utilization for transsulfuration. Methionine balance was nega-

tive in all age groups, indicating that current sulfur amino acid intakes provided to critically ill pediatric patients as per current standard care are insufficient to maintain sulfur amino acid balance and probably functional needs. The plasma methionine enrichment model underestimated methionine kinetics in children and adolescents.

References

1. Chang JX, Chen S, Ma LP, et al. Functional and morphological changes of the gut barrier during the restitution process after hemorrhagic shock. World J Gastroenterol 2005;11(35):5485-91.
2. Pastores SM, Katz DP, Kvetan V. Splanchnic ischemia and gut mucosal injury in sepsis and the multiple organ dysfunction syndrome. Am J Gastroenterol 1996;91(9):1697-710.
3. Castillo L, Chapman TE, Yu YM, Ajami A, Burke JF, Young VR. Dietary arginine uptake by the splanchnic region in adult humans. Am J Physiol 1993;265(4 Pt 1): E532-9.
4. Fenech M. Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs). Food Chem Toxicol 2002;40(8):1113-7.
5. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. J Biol Chem 1984;259(15): 9508-13.
6. Stipanuk MH, Dominy JE, Jr., Lee JI, Coloso RM. Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. J Nutr 2006;136(6 Suppl):1652S-9S.
7. Stipanuk MH, Ueki I, Dominy JE, Jr., Simmons CR, Hirschberger LL. Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. Amino Acids 2009;37(1):55-63.
8. Deneke SM, Fanburg BL. Regulation of cellular glutathione. Am J Physiol 1989; 257(4 Pt 1):L163-73.
9. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost 2000;26(3): 219-25.
10. MacCoss MJ, Fukagawa NK, Matthews DE. Measurement of intracellular sulfur amino acid metabolism in humans. Am J Physiol Endocrinol Metab 2001;280(6): E947-55.
11. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
12. Food and Nutrition Board Institute of Medicine. Panel on Macronutrients. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty acids, Cholesterol, Proteins and Amino Acids. Washington DC: The National Press; 2005.
13. Sy J, Gourishankar A, Gordon WE, et al. Bicarbonate kinetics and predicted energy expenditure in critically ill children. Am J Clin Nutr 2008;88(2):340-7.
14. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C] leucine. Am J Physiol 1980;238(5): E473-9.
15. Raguso CA, Ajami AM, Gleason R, Young VR. Effect of cystine intake on methionine kinetics and oxidation determined with oral tracers of methionine and cysteine in

healthy adults. Am J Clin Nutr 1997;66(2): 283-92.
16. Hiramatsu T, Fukagawa NK, Marchini JS, et al. Methionine and cysteine kinetics at different intakes of cystine in healthy adult men. Am J Clin Nutr 1994;60(4): 525-33.
17. Riedijk MA, Voortman G, van Goudoever JB. Use of [13C]bicarbonate for metabolic studies in preterm infants: intragastric versus intravenous administration. Pediatr Res 2005;58(5):861-4.
18. Burrin DG, Davis TA. Proteins and amino acids in enteral nutrition. Curr Opin Clin Nutr Metab Care 2004;7(1):79-87.
19. Di Buono M, Wykes LJ, Ball RO, Pencharz PB. Dietary cysteine reduces the methionine requirement in men. Am J Clin Nutr 2001;74(6):761-6.
20. Riedijk MA, Stoll B, Chacko S, et al. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. Proc Natl Acad Sci U S A 2007; 104(9):3408-13.
21. Lepage N, McDonald N, Dallaire L, Lambert M. Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. Clin Chem 1997;43(12):2397-402.
22. Pastore A, Massoud R, Motti C, et al. Fully automated assay for total homocysteine, cysteine, cysteinylglycine, glutathione, cysteamine, and 2-mercaptopropionylglycine in plasma and urine. Clin Chem 1998;44(4):825-32.
23. Loser C, Eisel A, Harms D, Folsch UR. Dietary polyamines are essential luminal growth factors for small intestinal and colonic mucosal growth and development. Gut 1999;44(1):12-6.
24. Brosnan JT, Brosnan ME. Creatine: endogenous metabolite, dietary, and therapeutic supplement. Annu Rev Nutr 2007;27:241-61.
25. Buts JP, De Keyser N, De Raedemaeker L, Collette E, Sokal EM. Polyamine profiles in human milk, infant artificial formulas, and semi-elemental diets. J Pediatr Gastroenterol Nutr 1995;21(1):44-9.
26. Li Z, Vance DE. Phosphatidylcholine and choline homeostasis. J Lipid Res 2008; 49(6):1187-94.
27. Buddington RK, Diamond JM. Ontogenetic development of intestinal nutrient transporters. Annu Rev Physiol 1989;51: 601-19.
28. Buddington RK, Diamond JM. Ontogenetic development of monosaccharide and amino acid transporters in rabbit intestine. Am J Physiol 1990;259(4 Pt 1): G544-55.
29. Reisenauer AM, Castillo RO. Regulation of the ontogenic and regional expression of intestinal aminooligopeptidase. J Pediatr Gastroenterol Nutr 1994;18(1):1-8.
30. Shenoy V, Roig JC, Chakrabarti R, Kubilis P, Neu J. Ontogeny of glutamine synthetase in rat small intestine. Pediatr Res 1996;39(4 Pt 1):643-8.
31. Lapidot A, Gopher A. Cerebral metabolic compartmentation. Estimation of glucose flux via pyruvate carboxylase/pyruvate dehydrogenase by 13C NMR isotopomer analysis of D-[U-13C]glucose metabolites. J Biol Chem 1994;269(44):27198-208.
32. Davis SR, Stacpoole PW, Williamson J, et al. Tracer-derived total and folate-dependent homocysteine remethylation and synthesis rates in humans indicate that serine is the main one-carbon donor. Am J Physiol Endocrinol Metab 2004; 286(2):E272-9.
33. Banerjee R, Zou CG. Redox regulation and reaction mechanism of human cystathionine-beta-synthase: a PLP-dependent hemesensor protein. Arch Biochem Biophys 2005;433(1):144-56.
34. Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. J Nutr 2006;136(6 Suppl):1636S-40S.

35. Martensson J, Jain A, Meister A. Glutathione is required for intestinal function. Proc Natl Acad Sci U S A 1990;87(5): 1715-9.
36. Blaise SA, Alberto JM, Audonnet-Blaise S, Gueant JL, Daval JL. Influence of preconditioning-like hypoxia on the liver of developing methyl-deficient rats. Am J Physiol Endocrinol Metab 2007;293(6): E1492-502.
37. Chawla RK, Jones DP. Abnormal metabolism of S-adenosyl-L-methionine in hypoxic rat liver. Similarities to its abnormal metabolism in alcoholic cirrhosis. Biochim Biophys Acta 1994;1199(1): 45-51.
38. Jahoor F, Badaloo A, Reid M, Forrester T. Sulfur amino acid metabolism in children with severe childhood undernutrition: methionine kinetics. Am J Clin Nutr 2006; 84(6):1400-5.

Chapter 6

Parenteral amino acid intakes in critically ill children: A matter of convenience

Sascha CAT Verbruggen
Jama Sy
Ana Arrivillaga
Koen FM Joosten
Johannes B van Goudoever
Leticia Castillo

J Parenter Enteral Nutr. 2010;34(3):329-40.

Abstract

Background

Parenteral and enteral amino acid requirements for nutritional balance and function have not been defined in critically ill children or adults. In addition to their role in protein synthesis, amino acids trigger signaling cascades that regulate various aspects of fuel and energy metabolism and serve as precursors for important substrates. Amino acids can also be toxic. We assessed parenteral intakes of essential (EAA) and non-essential amino acids (NEAA) supplied to critically ill children as an initial step for further studies aimed at establishing parenteral amino acid requirements.

Methods

We conducted a retrospective review of parenteral amino acid intakes provided to 116 critically ill children and compared them to recommended EAA intakes by the Institute of Medicine. There are no recommended intakes for NEAA. Hence, we compared NEAA intakes to mixed muscle protein content in the older children, and breast milk amino acid content in the infants.

Results

Parenteral EAA are provided several fold in excess of recommended intakes for healthy children except for phenylalanine and methionine, which although excessive, were given in less generous amounts. NEEA were supplied in lower or higher amounts than the content of mixed muscle proteins or breast milk. Parenteral amino acid formulas are limited in taurine, glutamine and asparagine despite that inflammatory/immune proteins are rich in these amino acids.

Conclusions

Amino acid composition of parenteral formulas is variable and lacks scientific support. Parenteral amino acid intakes should be based on measured requirements to maintain nutritional and functional balance and on knowledge of toxicity.

Introduction

Amino acids are precursors for the biosynthesis of proteins and other substrates such as nitric oxide, polyamines, collagen etc[1]. However, it is known that amino acids also serve as signaling molecules in functionally diverse signal transduction pathways[2]. Amino acids trigger signaling cascades that regulate various aspects of fuel and energy metabolism[3, 4] and control the growth, proliferation, and survival of cells[5]. Given their essential role, if they are not provided in sufficient amounts, they will be obtained through protein breakdown, a process that in critically ill septic patients was described as septic auto-cannibalism[6]. However, excessive administration of amino acids may result in toxicity[7-9].

The dietary amino acid requirements of parenterally or enterally fed critically ill children for nutritional balance or functional purpose have not been determined[10]. Knowledge on amino acid toxicity is also limited[11, 12]. Current parenteral and enteral amino acid formulas provided to critically ill children are based on sparse data despite that nutritional support is an essential component in the management of these patients. Moreover, critically ill patients present metabolic alterations of energy and protein homeostasis. They undergo increased proteolysis unresponsive to the administration of protein and energy intake, and there is increased hepatic synthesis of immune/inflammatory proteins, at the expense of protein accretion, and decreased muscle protein synthesis in fast-twitch fibers[10, 13-15]. Hence, although whole body protein turnover is increased, protein balance is often negative. In children, this situation has a major impact because in this population growth is an important function, which is suspended during critical illness and results in the need to catch-up during convalescence. This may have a developmental impact. In addition, critically ill patients present altered energy metabolism manifested by glucose and lipid intolerance[16] and changes in substrate utilization[16, 17]. These alterations have also an impact on protein homeostasis because protein and energy metabolism are closely interrelated.

Given the limited knowledge on the nutritional support of critically ill children, and the significant role of amino acids, it is important to examine the specific parenteral amino acid intakes that these patients receive. Therefore, the objective of this study was to determine the parenteral amino acid intakes of essential (EAA) and non-essential amino acids (NEAA) that critically ill children admitted to a Pediatric Intensive Care Unit at a tertiary Hospital receive as per standard clinical care. We further contrasted essential and non-essential amino acid intakes with the composition of representative inflammatory/immune proteins, because these and not muscle proteins are the major source of amino acid utilization for protein synthesis during critical illness. Therefore protein synthesis composition may change the quality and quantity of specific amino acid requirements during critical illness.

Material and Methods

Data Collection

This study was conducted at the Pediatric Intensive Care Unit (PICU), Texas Children's Hospital, Baylor College of Medicine. A retrospective chart and pharmacy records review was performed in all patients that received parenteral nutritional support in the PICU over a 12 month period from January 1st 2006 through December 31st 2006. Only patients that received total parenteral nutritional support (TPN) for at least 24 hours providing 100% of their daily fluid requirements were included. The nutritional support and the type of commercially available parenteral amino acid formulas used were determined as clinically indicated by the attending(s) physicians and the nutrition team. A total of 116 patients met these criteria. The study was exempt from full Institutional Review Board review because it involved the use of non-identifiable existing records. Demographic information, including age, height and weight, as well as the type, concentration and amount of parenteral amino acid formulas received, and length of administration were recorded. The patient population was stratified within the following five age groups: One month to 1 year, greater than one to three, four to eight, nine to thirteen and fourteen to eighteen years. We used these age groups because they are used by the IOM recommendations for dietary amino acid intakes, which served as reference for established dietary intakes[18]. We compared parenteral EAA intakes received by these patients with the Food and Nutrition Board, Institute of Medicine (IOM), National Academy of Sciences recommendations for enteral EAA intakes for age matched healthy children[18].

There are no available recommended dietary intakes from the IOM, the World Health Organization or any other official organization for NEAA intakes in the pediatric or adult populations. Hence, the parenteral NEAA intakes supplied to critically ill children and adolescents were compared with the composition of mixed muscle proteins, and in the critically ill infant population the parenteral NEAA intakes supplied where compared with the NEAA composition of breast milk.

Calculations

Three different parenteral nutritional formulas were used in the PICU during the study period: Aminosyn (Hospira Inc. Lake Forest, IL) and Trophamine (Braun Medical Inc. Bethlehem, PA), which are commercially available as a 10% amino acid solution, and Clinisol (Baxter Inc. Deerfield, IL), which is commercially available as a 15% amino acid solution. Their amino acid content (mg/100mL) is shown in Table 1. From these solutions, parenteral amino acid concentrations of 1.5, 2.5 or 3.5% are routinely prepared and used by the Hospital Pharmacists, depending on the amount of protein and fluid intake that patients receive.

Table 1. Amino Acid Composition of Formulas Administered to Critically Ill Children

	TrophAmine	Aminosyn	Clinisol
Essential			
Histidine	480	300	894
Isoleucine	820	660	749
Leucine	1400	1000	1040
Lysine	820	1050	1180
Methionine	340	172	749
Phenylalanine	480	298	1040
Threonine	420	400	749
Tryptophan	200	200	250
Valine	780	500	960
Non-essential			
Arginine	1200	1018	1470
Alanine	540	993	2170
Asparagine	0	0	0
Aspartate	320	700	434
Cysteine	16	0	0
Glutamine	0	0	0
Glutamate	500	738	749
Glycine	360	500	1040
Proline	680	722	894
Serine	380	530	592
Tyrosine	240	270	39
Taurine	25	0	0
Total	10001	10051	14999

All values are mg/100mL. Composition provided by TrophAmine (Braun Medical, Bethlehem, PA), Aminosyn (Hospira, Lake Forest, IL), and Clinisol (Baxter, Deerfield, IL).

The total parenteral amino acid intake of each patient over a 24 hour period (mL.day^{-1}) was determined from the chart review. The specific parenteral EAA and NEAA intakes (mg.kg^{-1}.d^{-1}) for each patient were derived from the known concentration of the parenteral amino acid formulas provided to the patient (1.5, 2.5 or 3.5%) and the total volume of parenteral amino acid formula infused over the 24 hour period (mL.d^{-1}). The EAA intakes were then compared with the recommended enteral dietary allowances (RDA) established by the IOM for age matched healthy children. However, the IOM does not provide recommendations on NEAA intakes. Therefore, in the children and adolescents studied, the parenteral NEAA intakes were compared with data on the free amino acid content in muscle (mg.kg^{-1} wet tissue) of children obtained from the literature[19, 20], and as previously described in the adult population[21, 22]. In the infant population we compared the parenteral NEAA intakes with the average NEAA composition of human breast milk[23-25], assuming an average intake of 100 ml.kg^{-1}.d^{-1}.

Statistical analysis

Comparisons between parenteral EAA intakes and the IOM recommendations for EAA, as well as NEAA intakes and NEAA content in mixed muscle proteins and breast milk were analyzed using a one sample student t-test. Data were analyzed using standard computer statistical software (Minitab 14, Minitab Inc. State College, PA).

Results

Demographics and Nutritional Support

A total of 116 children admitted to the multidisciplinary PICU at the Texas Children's Hospital, and receiving parenteral nutrition providing complete nutritional support for more than 24 hours were included in the study. The major diagnoses of the patient population requiring parenteral nutrition were sepsis (n=58), malignancies (n=21), congenital anomalies (n=5), solid organ transplant (liver) (n=8) and chronic lung disease (n=24). The demographic characteristics of the subjects, the parenteral amino acid formulas received and the length of administration are shown in Table 2. The infants 0-12 months of age and children >1-3 years predominantly received Trophamine (100% and 75%, respectively), while older children (4-8 and 9-13 yrs) and adolescents (> 14 yrs)

Table 2. Demographic Characteristics of the Patients, Formulas Used, and Days of Parenteral Nutrition (PN) Administration

Age, y	n	Diagnoses	Age, y	Sex, F:M	BMI, $kg.m^{-2}$	Aminosyn, n (%)	Trophamine, n (%)	Clinisol, n (%)	Days on PN
0 – 1 yr	9	Sepsis (n = 3); congenital anomalies (n = 3); solid organ transplant (n = 2); CLD (n = 1)	0.8 ± 0.2	3:6	17.9 ± 5.0	0 (0)	9 (100)	0 (0)	42.8 ± 40.7
1 – 3 yrs	48	Sepsis (n = 21); congenital anomalies (n = 2); malignancies (n = 8); CLD (n = 17)	1.6 ± 0.8	20:28	21.8 ± 7.0	11 (23)	36 (75)	1 (2)	30.4 ± 37.9
4 – 8 yrs	12	Sepsis (n = 8); solid organ transplant (n = 1); CLD (n = 3)	6.3 ± 1.7	5:7	19.0 ± 4.8	12 (100)	0 (0)	0 (0)	24.1 ± 28.2
9 –13 yrs	27	Sepsis (n = 17); malignancies (n = 8); solid organ transplant (n = 2)	11.0 ± 1.5	11:16	19.0 ± 5.2	26 (96)	1 (4)	0 (0)	36.8 ± 40.3
14 –18 yrs	20	Sepsis (n = 9); malignancies (n = 5); solid organ transplant (n = 3); CLD (n = 3)	15.8 ± 1.2	12:8	24.8 ± 5.0	16 (80)	1 (5)	3 (15)	13.6 ± 11.8

BMI, body mass index; CLD, chronic lung disease; SD, standard deviation. Data are given as mean ± SD, except where noted otherwise

Table 3. Parenteral nutrition support received by critically ill children

	n =	Kcal.kg^{-1}.d^{-1}	Calorie distribution, % Carbohydrate:Protein:Fat	Glucose mg.kg^{-1}.min^{-1}	Protein g.kg^{-1}.d^{-1}	Lipids g.kg^{-1}.d^{-1}
0 – 1 yr	9	77.5 ± 17.6	75.1%:10.6%:14.3%	11.3 ± 4.9	2.2 ± 0.4	1.4 ± 1.0
1 – 3 yrs	48	68.1 ± 28.0	62.9%:15.4%:21.8%	8.5 ± 3.1	2.5 ± 1.0	1.6 ± 1.5
4 – 8 yrs	12	51.8 ± 22.9	64.6%:16.0%:19.4%	7.0 ± 4.0	2.1 ± 0.9	0.9 ± 0.6
9 –13 yrs	27	48.2 ± 21.5	62.2%:14.5%:23.2%	6.1 ± 3.0	1.8 ± 0.8	1.2 ± 0.8
14 – 18yrs	20	35.2 ± 18.6	63.4%:16.2%:20.4%	4.6 ± 2.7	1.3 ± 0.6	0.7 ± 0.6

Data are given as mean ± SD, except where noted otherwise

predominantly received Aminosyn (100%, 96% and 80%, respectively). Trophamine was used occasionally in older children receiving long-term parenteral nutritional support based on the fact that it contains small amounts of cysteine and taurine. All available commercial amino acid formulas are devoid of glutamine and asparagine.

The specific protein and energy intakes received are shown in Table 3. and they were within current recommendations for critically ill children[26]. Parenteral glucose administration is also shown in Table 3. The infant group of 0-12 months received higher glucose infusion rates of about 11 mg.kg^{-1}.min^{-1} while the older children received between 6 and 8.5 mg.kg^{-1}.min^{-1} and the adolescents received about 4.6 mg.kg^{-1}.min^{-1}. Fat intake was variable within the groups due to hypertriglyceridemia frequently observed in these patients, but it was supplied between 0.93 and 1.62 g.kg^{-1}.d^{-1} for infants, children and adolescents.

Parenteral Amino Acid Intakes

In the infants 0-12 months of age (Table 4), all parenteral essential amino acids were provided in amounts one to four fold in excess ($p < 0.0001$) of the IOM recommended enteral dietary intakes for healthy infants[18], except for phenylalanine which was only 120% ($p = 0.045$) of recommended enteral intakes. Parenteral intake of NEAA was variable when compared to the content of NEAA in breast milk[23, 24], but overall Trophamine provided an excess of 6 fold the amount of arginine ($p < 0.001$), 2.6 fold of cysteine ($p < 0.005$), 1.8 fold of proline ($p < 0.001$) and 1.5 fold of tyrosine ($p < 0.005$), while the following amino acids were provided at deficient amounts when compared to those supplied by breast milk: aspartate at 50% ($p < 0.0001$), serine at 71% ($p < 0.0001$), taurine at 2% ($p < 0.0001$) and glutamate at 6% ($p < 0.0001$). Alanine and glycine were provided in similar amounts to those of breast milk (p=NS).

Table 5 shows data on the 48 children >1 to 3 years of age. All the EAA were provided in amounts of about 2 to 7 fold in excess ($p < 0.0001$) of the recommended DRI's by the IOM[18], but phenylalanine and methionine were provided at a lower range than the other EAA. The parenteral NEAA intakes were variable, but in general arginine was provided at 5.7 ($p < 0.0001$), cysteine at 21.7 ($p < 0.0001$), glycine at 1.6 ($p < 0.0001$),

Table 4. Parenteral essential (EAA) and non-essential amino acid (NEAA) intakes provided to 9 critically ill infants 0-12 months of age and enteral dietary recommended intakes (DRI) for EAAs by the Institute of Medicine.

EAA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Enteral DRI, † $mg.kg^{-1}.d^{-1}$	Parenteral Intake as a percentage of DRI	Excess or deficiency	p value
Histidine	100.6	32	314	↑	< 0.0001
Isoleucine	171.8	43	400	↑	< 0.0001
Leucine	293.4	93	315	↑	< 0.0001
Lysine	171.8	89	193	↑	< 0.0001
Methionine	71.3	43	166	↑	< 0.0001
Phenylalanine	100.6	84	120	↑	0.045
Threonine	88.0	49	180	↑	< 0.0001
Tryptophan	41.9	13	322	↑	< 0.0001
Valine	163.5	58	282	↑	< 0.0001
NEEA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Breast milk NEAA intake, ‡ $mg.kg^{-1}.d^{-1}$	Parenteral intake as a percentage of breast milk amino acid intake	Excess or deficiency	p value
Arginine	251.5	41.3	609	↑	< 0.0001
Alanine	113.2	98.3	115	↔	0.095
Aspartate	67.1	133.1	50	↓	< 0.0001
Cysteine	60.9	22.9	266	↑	< 0.005
Glutamine	0.0	227.2	0	↓	< 0.0001
Glutamate	104.8	1632.4	6	↓	< 0.0001
Glycine	75.4	71.1	106	↔	0.43
Proline	142.5	77.6	184	↑	< 0.0001
Serine	79.6	111.7	71	↓	< 0.0001
Tyrosine	50.3	32.1	157	↑	< 0.005
Taurine	5.2	334.7	2	↓	< 0.0001

NEEAs are compared with breast milk amino acid content assuming intakes of $mL^{-1}.kg^{-1}.d^{-1}$

† Reference *18*

‡ References *23-25*, assuming 100 $mL.kg^{-1}.d^{-1}$ intake

proline at 3.4 (p< 0.0001), serine at 1.4 (p< 0.0001) and tyrosine at 84.8 (p< 0.0001) fold in excess of the content of these amino acids in mixed muscle proteins[19-21]. In contrast, taurine (p< 0.0001), glutamate (p< 0.0001) and aspartic acid (p< 0.02) were given at lower amounts than those found in mixed muscle proteins. Alanine (p=0.96) was given in comparable amounts to the amino acid composition of mixed muscle proteins and glutamine and asparagine were not supplied.

Over the study period twelve children age 4-8 years received TPN (Table 6). In this group again, all the essential amino acids were provided between 3.4 and 6.8 fold higher (p< 0.0001) than recommended DRI's[18], except for methionine and phenylalanine which were provided at 1.6 (p<0.013) and 1.5 fold higher (p<0.026) than DRI's recommendations[18]. While parenteral arginine (p< 0.0001), proline (p< 0.0001) and tyrosine were

Table 5. Parenteral essential (EAA) and non-essential amino acid (NEAA) intakes provided to 48 critically ill children 1 – 3 years of age and enteral dietary recommended intakes (DRI) for EAAs by the Institute of Medicine.

EAA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Enteral DRI, † $mg.kg^{-1}.d^{-1}$	Parenteral Intake as a percentage of DRI	Excess or deficiency	p value
Histidine	111.5	21	531	↑	< 0.0001
Isoleucine	195.4	28	698	↑	< 0.0001
Leucine	323.5	63	513	↑	< 0.0001
Lysine	224.5	58	387	↑	< 0.0001
Methionine	72.7	28	260	↑	< 0.0001
Phenylalanine	112.2	54	208	↑	< 0.0001
Threonine	106.6	32	333	↑	< 0.0001
Tryptophan	50.7	8	634	↑	< 0.0001
Valine	178.8	37	483	↑	< 0.0001
NEAA	**Parenteral intake, $mg.kg^{-1}.d^{-1}$**	**Mixed muscle protein amino acid content, ‡ $mg.kg^{-1}$ wet weight**	**Parenteral intake as a percentage of mixed muscle amino acid intake**	**Excess or deficiency**	**p value**
Arginine	291.9	51.2	570	↑	< 0.0001
Alanine	177.1	177.9	100	↔	0.96
Asparagine	0.0	41.8	0	↓	< 0.0001
Aspartate	107.0	132.6	81	↓	0.02
Cysteine	53.0	2.4	2177	↑	< 0.0001
Glutamine	0.0	953.2	0	↓	< 0.0001
Glutamate	143.7	278.8	52	↓	< 0.0001
Glycine	104.5	64.9	161	↑	< 0.0001
Proline	175.6	51.4	341	↑	< 0.0001
Serine	107.3	73.1	147	↑	< 0.0001
Tyrosine	61.2	7.2	848	↑	< 0.0001
Taurine	4.5	1040.6	0	↓	< 0.0001

NEEAs are compared with mixed muscle protein content
† Reference *18*
‡ References *19* and *20*

provided in amounts between 2.9 and 7 fold higher than the content of mixed muscle proteins, serine was provided at about 1.5 fold higher ($p< 0.03$). Cysteine, taurine, asparagine and glutamine are not supplied by Aminosyn. Glutamate was given 55% lower ($p<0.0001$) and alanine and aspartic acid were given in comparable amounts ($p>0.1$) to those of the amino acid composition of mixed muscle proteins.

Twenty seven patients received TPN in the age group of 9-13 years (Table 7). All EAA were given between one and 6 fold higher than DRI's, except again for methionine and phenylalanine which were provided only at 1.48 ($p< 0.005$) and 1.39 fold higher ($p=0.005$), respectively. In regard to the NEAA, arginine was given at 3.6 ($p< 0.0001$), proline at 2.5 ($p< 0.0001$) tyrosine at 6.7 ($p< 0.0001$) and serine at 1.2 ($p=0.01$) fold

Table 6. Parenteral essential (EAA) and non-essential amino acid (NEAA) intakes provided to 12 critically ill children 4 - 8 years of age and enteral dietary recommended intakes (DRI) for EAAs by the Institute of Medicine.

EAA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Enteral DRI, † $mg.kg^{-1}.d^{-1}$	Parenteral Intake as a percentage of DRI	Excess or deficiency	p value
Histidine	61.9	16	387	↑	< 0.0001
Isoleucine	136.1	22	619	↑	< 0.0001
Leucine	206.2	49	421	↑	< 0.0001
Lysine	216.5	46	471	↑	< 0.0001
Methionine	35.5	22	161	↑	0.013
Phenylalanine	61.5	41	150	↑	0.026
Threonine	82.5	24	344	↑	< 0.0001
Tryptophan	41.2	6	687	↑	< 0.0001
Valine	103.1	28	368	↑	< 0.0001
NEEA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Mixed muscle protein amino acid content, ‡ $mg.kg^{-1}$ wet weight	Parenteral intake as a percentage of mixed muscle amino acid intake	Excess or deficiency	p value
Arginine	209.9	51.2	410	↑	< 0.0001
Alanine	204.8	178.0	115	↔	0.33
Asparagine	0.0	41.8	0	↓	< 0.0001
Aspartate	144.3	132.6	109	↔	0.54
Cysteine	0.0	2.4	0	↓	< 0.0001
Glutamine	0.0	953.2	0	↓	< 0.0001
Glutamate	152.2	278.8	55	↓	< 0.0001
Glycine	103.1	64.9	159	↑	0.02
Proline	148.9	51.4	289	↑	< 0.0001
Serine	109.3	73.1	150	↑	0.03
Tyrosine	55.7	7.2	771	↑	< 0.0001
Taurine	0.0	1040.6	0	↓	< 0.0001

NEEAs are compared with mixed muscle protein content
† Reference *18*
‡ References *19* and *20*

higher than the amounts found in mixed muscle proteins, while alanine and aspartate were no different ($p>0.1$). Asparagine, cysteine, glutamine and taurine are not supplied in Aminosyn. One patient received Trophamine which provides small amounts of cysteine and taurine although in significantly ($p< 0.0001$) lower amounts than those found in mixed muscle proteins.

Twenty children age 14-18 years received TPN during the study period (Table 8). Eighty percent of these received Aminosyn and 5 and 15% received Trophamine and Clinisol, respectively. Hence, negligible amounts of taurine and cysteine were given as a group, but indeed those children receiving Aminosyn and Clinisol who were the majority, received formulas devoid of cysteine and taurine, and all were devoid of glutamine and

Table 7. Parenteral essential (EAA) and non-essential amino acid (NEAA) intakes provided to 27 critically ill children 9-13 years of age and enteral dietary recommended intakes (DRI) for EAAs by the Institute of Medicine.

EAA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Enteral DRI, † $mg.kg^{-1}.d^{-1}$	Parenteral Intake as a percentage of DRI	Excess or deficiency	p value
Histidine	55.8	16	349	↑	< 0.0001
Isoleucine	120.5	22	548	↑	< 0.0001
Leucine	184.0	48	383	↑	< 0.0001
Lysine	187.5	45	417	↑	< 0.0001
Methionine	32.5	22	148	↑	< 0.005
Phenylalanine	55.4	40	139	↑	0.005
Threonine	72.4	23	315	↑	< 0.0001
Tryptophan	36.1	6	602	↑	< 0.0001
Valine	92.7	28	331	↑	< 0.0001
NEEA	**Parenteral intake, $mg.kg^{-1}.d^{-1}$**	**Mixed muscle protein amino acid content, ‡ $mg.kg^{-1}$ wet weight**	**Parenteral intake as a percentage of mixed muscle amino acid intake**	**Excess or deficiency**	**p value**
Arginine	185.3	51.2	362	↑	< 0.0001
Alanine	175.3	177.9	99	↑	0.859
Asparagine	0.0	41.8	0	↓	< 0.0001
Aspartate	123.0	132.6	93	↔	0.374
Cysteine	0.1	2.4	6	↓	< 0.0001
Glutamine	0.0	953.2	0	↓	< 0.0001
Glutamate	131.1	278.8	47	↓	< 0.0001
Glycine	89.0	64.9	137	↑	< 0.005
Proline	129.9	51.4	253	↑	< 0.0001
Serine	94.3	73.1	129	↑	0.01
Tyrosine	48.5	7.2	672	↑	< 0.0001
Taurine	0.2	1040.6	0	↓	< 0.0001

NEEAs are compared with mixed muscle protein content
† References *18*
‡ References *19* and *20*

asparagine. As shown in Table 8, EAA were given between 2 and 4 fold in excess of DRI's[18]. However, methionine and phenylalanine intakes were provided at 1.9 ($p>0.06$) and 1.4 ($p>0.11$) fold higher than DRI's but not statistically significant. NEAA were given in variable amounts, with an excess of arginine and proline of about 2.7 ($p< 0.0001$) and tyrosine 4.1 fold ($p< 0.0001$), while alanine ($p=0.12$), glycine ($p=0.96$) and serine ($p=0.94$) were given in comparable amounts to mixed muscle proteins. Aspartate ($p<0.0001$) and cysteine ($p<0.0001$) were supplied in lower amounts when compared to mixed muscle proteins. Glutamine and asparagine were not provided and taurine was negligible.

Table 8. Parenteral essential (EAA) and non-essential amino acid (NEAA) intakes provided to 20 critically ill children 14-18 years of age and enteral dietary recommended intakes (DRI) for EAAs by the Institute of Medicine.

EAA	Parenteral intake, $mg.kg^{-1}.d^{-1}$	Enteral DRI, † $mg.kg^{-1}.d^{-1}$	Parenteral Intake as a percentage of DRI	Excess or deficiency	p value
Histidine	49.3	15	329	↑	< 0.0001
Isoleucine	85.5	20	428	↑	< 0.0001
Leucine	130.0	46	283	↑	< 0.0001
Lysine	129.6	42	309	↑	< 0.0001
Methionine	38.6	20	193	↔	0.061
Phenylalanine	51.5	37	139	↔	0.115
Threonine	55.7	22	253	↑	< 0.0001
Tryptophan	25.7	6	428	↑	< 0.0001
Valine	73.2	26	282	↑	< 0.0001
NEEA	**Parenteral intake, $mg.kg^{-1}.d^{-1}$**	**Mixed muscle protein amino acid content, ‡ $mg.kg^{-1}$ wet weight**	**Parenteral intake as a percentage of mixed muscle amino acid intake**	**Excess or deficiency**	**p value**
Arginine	136.2	51.2	266	↑	< 0.0001
Alanine	136.8	177.9	77	↔	0.12
Asparagine	0.0	41.8	0	↓	< 0.0001
Aspartate	77.7	132.6	59	↓	< 0.0001
Cysteine	0.2	2.4	9	↓	< 0.0001
Glutamine	0.0	953.2	0	↓	< 0.0001
Glutamate	88.8	278.8	32	↓	< 0.0001
Glycine	69.1	64.9	106	↔	0.96
Proline	92.0	51.4	179	↑	< 0.0001
Serine	64.9	73.1	89	↔	0.94
Tyrosine	29.4	7.2	408	↑	< 0.0001
Taurine	0.3	1040.6	0	↓	< 0.0001

NEEAs are compared with mixed muscle protein content
† Reference *18*
‡ References *19* and *20*

Discussion

Amino acids have active physiological roles not only in protein synthesis, but as signaling molecules in various signal transduction pathways[3] and regulate various aspects of fuel and energy metabolism[27]. They also serve as precursors for important compounds such as glutathione[28, 29], creatine[30], nitric oxide[31], polyamines[32], etc. Nutritional support, whether it is provided through the enteral or parenteral route, remains an essential component of the care of critically ill patients. There is consensus on the total amount of protein that critically ill children should receive, although based on limited evidence[26]. However, the parenteral or enteral specific amino acid requirements of critically ill

children have not been defined, and current parenteral amino acid administration is rather variable depending on the amino acid formula used.
Except for Trophamine, which was designed to maintain the plasma amino acid pattern of healthy, breast fed infants[25] and therefore contains small amounts of cysteine and taurine, the amino acid composition of commercial parenteral formulas provided to critically ill children is based on the convenience of adequate solubility, stability, and lack of side effects, such as hyperammonemia or metabolic acidosis, rather than on detailed data on specific amino acid requirements, dose response and safe upper limits of intake. Hence, cysteine, taurine and glutamine are not provided in most parenteral amino acid formulas due to its lower solubility and/or poor stability. Trophamine is mainly utilized in young infants and it contains small amounts of cysteine and taurine, while aminosyn and clinisol were given to older children, and are devoid of these amino acids.
Our data showed that most EAA are provided several fold in excess of recommended intakes for enterally fed children[18], except for phenylalanine and methionine, which although excessive, were given in less generous amounts. NEAA are supplied in excess or in deficient amounts when compared to breast milk or the content or mixed muscle proteins. Taurine and cysteine are provided in general in minimal amounts, particularly in the older children while other important amino acids such as glutamine and asparagine, which are important components of inflammatory/immune proteins, are lacking in all age groups.
Several observations can be derived from these data and various issues need to be taken into consideration. First, we acknowledge that it is difficult to compare enteral amino acid intakes recommended for healthy children with parenteral amino acid intakes received by critically ill children, but in the absence of quantitative estimates of parenteral amino acid requirements in critically ill children or adults, our comparisons are a reasonable start point. Breast milk is the “gold” standard for adequate nutrition in the infant population[18, 24, 25] and muscle mass accretion and therefore growth, is an important source of amino acid utilization in healthy children. Therefore, comparing NEAA from breast milk and muscle tissue composition with the NEAA intakes received by the patients, although not ideal, it is reasonable as an initial comparison, particularly in light that there are no official recommendations for the intakes of NEAA, which although considered non-essential, many are indeed conditionally essential during stress.
Second, parenteral amino acid utilization and therefore requirements are different when provided by the enteral versus the parenteral route. When the enteral route is used, there is a first-pass disappearance of amino acids by the splanchnic area or splanchnic uptake. The splanchnic area comprises the liver, stomach, intestines, pancreas, and spleen. Except for the liver, all other organs constitute the portal-drained viscera (PDV). Once extracted by the PDV, the first-pass disappearance or

splanchnic uptake of amino acids is variable depending on its metabolic utilization by the liver and PDV. For instance, in healthy humans 64% of dietary glutamate[33], and 96% of glutamine[34, 35] undergo a first-pass disappearance indicating their active metabolic role in the splanchnic area as a fuel for enterocytes[36] and also as precursors of citrulline, polyamines[37], etc. In contrast, leucine, phenylalanine and arginine have a splanchnic disappearance of 21, 29 and 38%[38, 39], respectively, indicating that a large proportion of these amino acids are utilized outside the splanchnic region, while the splanchnic uptake of methionine and cysteine is about 33 and 44% respectively[38, 40]. In recent studies by our group, we observed that in critically ill infants, children and adolescents, methionine splanchnic uptake was 63, 43 and 36%, respectively, and it was higher ($p < 0.05$) in the infant group when compared to critically ill adolescents[41]. Furthermore, the children and adolescents had higher methionine splanchnic uptake when compared to values of about 33% estimated in healthy adults[40]. These findings suggest that developmental stage and critical illness will influence the metabolic needs of the splanchnic area. Whether there is a higher requirement for substrates such as polyamines, creatine, glutathione or methyl groups in the splanchnic area of critically ill patients remains to be determined, but it is clear that specific amino acid needs are different in critically ill patients and are influenced by age.

Leucine and phenylalanine splanchnic uptake is increased under conditions of endotoxemia in healthy subjects[42]; and in healthy adults subjected to a short, experimental course of steroids the splanchnic uptake of glutamine was increased by 50%[43]. Hence, conditions that are frequently seen in the ICU, such as sepsis and steroid therapy, will influence amino acid requirements and utilization. However, it is not standard practice to modify the nutritional support provided to critically ill children according to clinical stage, medications received, etc.

The enteral or parenteral route of amino acid administration will significantly impact on amino acid utilization and requirements, and the extraction and use of dietary amino acids by the splanchnic area will have a major influence on their systemic availability and requirements. Parenteral nutrition bypasses the splanchnic area and nutrients are presented to the liver through the hepatic arterial circulation, instead of the portal venous circulation[44]. Studies in piglets suggest that after enteral feeding, portal rather than arterial phenylalanine is preferentially used for the synthesis of constitutive and secretory hepatic proteins[44]. Spatial cellular localization will also influence amino acid metabolism. It has been shown that in periportal hepatocytes excess portal ammonia is scavenged by ureagenesis, whereas perivenous hepatocytes scavenge ammonia for glutamine synthesis[45]. Hence, nitrogen metabolism will be directed to different pathways depending on the anatomical presentation.

In the healthy piglet model, parenteral requirements of threonine, lysine, phenylalanine and branch chain amino acids are lower than the enteral requirements[46-49], and clearly

the difference depends on the rates of the splanchnic uptake of these amino acids. Thus, because the splanchnic metabolic needs are by-passed during parenteral nutrition, the parenteral amino acid requirements will be greatly modified by its degree of splanchnic uptake. Hence, the route of nutrient administration will have a profound impact on amino acid requirements and their utilization.

As shown in Table 9, the composition of acute, inflammatory/immune proteins[21] is different from that of mixed muscle proteins[21, 22] or from breast milk[24, 50]. Taurine is the major component of polymorphonuclear cells, and except for Trophamine, the other parenteral amino acid formulas used in children are devoid of taurine. NEAA and specifically glutamine are found in higher concentrations in inflammatory/immune proteins, and this may explain the "conditional" essentiality of some amino acids traditionally considered non-essential. Furthermore, synthesis of inflammatory/immune proteins and not muscle accretion is a major source of amino acid utilization during critical illness[21, 51]. Indeed, the majority of protein loss originates from muscle[51]. Therefore the quality and quantity of protein synthesized during pediatric critical illness will have a major impact on the adequacy of the parenteral or enteral amino acid formulas provided to these patients. Reeds and coworkers[21] compared the amino acid composition of inflammatory proteins in relation to mixed muscle proteins, to estimate the amino acid demand of a typical inflammatory response. They observed that various amino acids among them phenylalanine, tryptophan, serine, cysteine and tyrosine were limiting. Indeed, it would be difficult to determine the amino acid composition of the inflammatory proteome *in vivo*, in critically ill children, given that many different inflammatory proteins are expressed in different organs at the intracellular level or in organelles, and they may not be detectable in plasma. However, the plasma inflammatory proteins selected by Reeds[21, 52, 53] are expressed in measurable concentrations and are grossly representative of a clinical inflammatory response.

In our previous studies in critically ill infants[54] we observed that greater parenteral intakes of phenylalanine were associated with a lesser negative protein balance and decreased rates of phenylalanine catabolism through hydroxylation to tyrosine. In the present study, the EAA were provided several fold in excess, but phenylalanine and methionine although excessive when compared to enteral recommendations, were provided at lower rates. Furthermore, some NEAA were not provided at all, while others were deficient and yet others were supplied in excess.

These observations raise the question of amino acid balance. For protein synthesis to occur, all the necessary amino acids need to be available. If one amino acid is limiting, protein catabolism will be maintained in order to support synthesis of proteins essential for survival. Hence, if an EAA or a NEAA that becomes "conditionally" essential during stress is limiting, protein catabolism will persist, despite adequate protein intake, as defined by our current standards. Hence, a balanced composition

Table 9. Amino acid composition of 7 major acute phase proteins, polymorphonuclear lymphocytes (PMNs), breast milk, and mixed muscle proteins

	C-Reactive Protein,† mg/100 mg	Fibrinogen, † mg/100 mg	Haptoglobin, † mg/100 mg	Amyloid A, † mg/100 mg	α1-anti-trypsin, † mg/100 mg	Complement C3, † mg/100 mg	α 1-glycoprotein, † mg/100 mg	PMN, ‡ mg/10^9 cells	Breast milk, ^ mg.kg^{-1}.day^{-1}	Skeletal muscle proteins,† mg/100 mg
EAA										
Histidine	1.4	2.8	3.8	3.5	3.7	2.4	1.7	0.023	22.0	5.1
Isoleucine	5.2	3.7	4.7	2.9	4.9	5.8	4.8	0.006	49.9	4.8
Leucine	8.2	6.7	8.2	2.9	12.4	11.2	10.1	0.010	92.1	8.1
Lysine	7.3	8.3	9.2	3.3	9.2	9.6	7.5	0.015	68.9	9.8
Methionine	1.4	3.1	1.6	2.2	2.8	2.9	1.1	0.004	15.1	2.5
Phenylalanine	9.6	4.7	3.0	10.3	8.3	5.3	6.4	0.006	39.4	4.0
Threonine	6.0	6.5	5.4	3.0	6.6	6.8	7.4	0.083	71.5	4.7
Tryptophan	3.2	4.2	3.2	4.5	1.1	1.8	3.0	0	20.5	1.3
Valine	7.8	4.8	8.4	1.8	5.9	9.1	4.6	0.008	66.1	5.4
NEAA										
Arginine	3.9	8.1	2.8	11.6	2.3	7.5	5.2	0.013	41.3	6.9
Alanine	3.1	3.4	5.4	10.6	4.3	4.6	3.6	0.023	98.3	5.9
ASPX	8.2	12.8	11.3	12.8	10.6	6.5	10.2	0.091	133.1	9.2
Cysteine	1.1	1.1	2.4	0	0.6	1.8	1.8	0	22.9	1.3
GLUX	11.7	13.6	11.5	8.7	13.6	9.6	17.3	0.142	1859.6	14.5
Glycine	4.6	6.2	4.4	6.1	3.3	4.0	1.9	0.028	71.1	4.5
Proline	4.6	5.1	4.4	3.4	4.1	5.0	3.4	0	77.6	4.8
Serine	8.6	8.6	4.0	4.7	4.9	6.2	3.1	0.028	111.7	4.1
Tyrosine	5.5	4.7	7.0	6.7	2.7	3.6	7.4	0.008	32.1	3.6
Taurine								1.409	334.7	

ASPX, aspartate plus asparagine; GLUX, glutamine plus glutamate; EAA, essential amino acid; NEAA, nonessential amino acid.

†Reference *21*

‡Reference *52*

^Reference *24*, assuming 100 ml.kg^{-1} per day intake

of the amino acid formula tailored after the amino acid needs during the inflammatory response will be critical to improving protein (nitrogen) homeostasis. It is not known if a more appropriate supply of amino acids patterned after the patient's specific needs would have an impact on ameliorating the catabolic response, but it is evident that the degree of stress response and interventions such as steroid therapy, will impose a further demand on specific amino acid needs.

Courtney-Martin and coworkers[55] estimated total sulfur amino acid requirements provided as methionine of about 47.4 $mg.kg^{-1}.d^{-1}$ and a safe level of intake of 56 $mg.kg^{-1}.d^{-1}$ in surgical neonates. We found in a previous study in critically ill infants 1-3 years of age, that total parenteral sulfur amino acid requirements were 56 $mg.kg^{-1}.d^{-1}$ provided as methionine and cysteine[56]. These values are about two fold higher than recommended enteral intakes by the IOM, but lower than the total parenteral sulfur amino acid (TSAA: methionine, cysteine, taurine) intakes of 137.4 $mg.kg^{-1}.d^{-1}$ provided to the infants in the current study. Hence, although it is expected that critically ill children will require higher parenteral amino acid intakes, given their increased demands, it is concerning that intakes of several fold above requirements are provided to these patients. These data raise questions about the adequacy of amino acids intakes provided to critically ill children and the potential for toxicity.

Finally, whether the excessive amounts of amino acids provided to critically ill children result in toxic effects cannot be established from this study, but it is well established that amino acids can have toxic effects[7] and that long-term use of parenteral nutrition results on cholestasis and eventual liver failure[57]. Moss and coworkers[58] demonstrated that methionine is hepatotoxic in a rabbit model receiving large amounts of parenteral methionine with or without enteral feedings[58], and it induced changes similar to those observed in TPN cholestasis. Methionine toxicity appears to be related to 3-methylthiopropionic acid (3-MTP), a known methionine transamination metabolite[59]. L-arginine, through L-ornithine production is known to induce necrotizing pancreatitis in a rat model[60] and L-Lysine at large doses induces renal failure in dogs[61].

The knowledge on safe levels of enteral amino acid intakes is sparse, and there is further limitation on the knowledge on human parenteral amino acid toxicity, beyond hyperammonemia, acidosis and seizures. Finally, in the original limited studies on the development of parenteral amino acid formulas, it was decided that if the parenteral amino acid formulas did not induce the latter complications, it was safe to use. This limited evidence was accepted and has remained unchallenged for several decades. It is clear that parenteral amino acid administration needs to be based on better knowledge on the nutritional and functional requirements of individual amino acids under specific pathophysiological conditions, as well as on a better understanding of toxicity and safe upper limits of intake.

Conclusions

We observed that the amino acid composition of commercially available parenteral amino acid formulas provided to critically ill children are rather variable and unbalanced to support inflammatory/immune protein synthesis, resulting on excessive or deficient supply of amino acids when compared to IOM recommendations, breast milk and mixed muscle proteins. These formulas are based on the convenience of solubility and stability and the lack of obvious, acute side effects such as acidosis and hyperammonemia. Some NEAA, the major component of inflammatory-immune proteins, were not provided while other NEAA were provided in excess. Parenteral or enteral amino acid intakes in critically ill children should be based on established requirements, not only to maintain nutritional balance but also to maintain non-nutritional amino acid functions. Dose-response, upper intake limits and surveillance of toxicity need also to be defined in patients receiving parenteral amino acid solutions. These data have also important implications for chronically ill children receiving long-term parenteral nutrition, who may have increased amino acid needs for longer periods of time.

References

1. Morris SM, Jr. Arginine: beyond protein. Am J Clin Nutr 2006;83(2):508S-12S.
2. Gulati P, Thomas G. Nutrient sensing in the mTOR/S6K1 signalling pathway. Biochem Soc Trans 2007;35(Pt 2):236-8.
3. Tokunaga C, Yoshino K, Yonezawa K. mTOR integrates amino acid- and energy-sensing pathways. Biochem Biophys Res Commun 2004;313(2):443-6.
4. Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr 2007; 27:293-310.
5. Marshall S. Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. Sci STKE 2006;2006(346):re7.
6. Cerra FB, Siegel JH, Coleman B, Border JR, McMenamy RR. Septic autocannibalism. A failure of exogenous nutritional support. Ann Surg 1980;192(4):570-80.
7. Baker DH. Comparative species utilization and toxicity of sulfur amino acids. J Nutr 2006;136(6 Suppl):1670S-5S.
8. Garlick PJ. Assessment of the safety of glutamine and other amino acids. J Nutr 2001;131(9 Suppl):2556S-61S.
9. Munro IC, Renwick AG. The 5th workshop on the assessment of adequate intake of dietary amino acids: general discussion 2. J Nutr 2006;136(6 Suppl):1755S-7S.
10. Curley MA, Castillo L. Nutrition and shock in pediatric patients. New Horiz 1998;6(2):212-25.
11. Bier DM. Amino acid pharmacokinetics and safety assessment. J Nutr 2003; 133(6 Suppl 1):2034S-9S.
12. Renwick AG, Walker R. An analysis of the risk of exceeding the acceptable or tolerable daily intake. Regul Toxicol Pharmacol 1993;18(3):463-80.

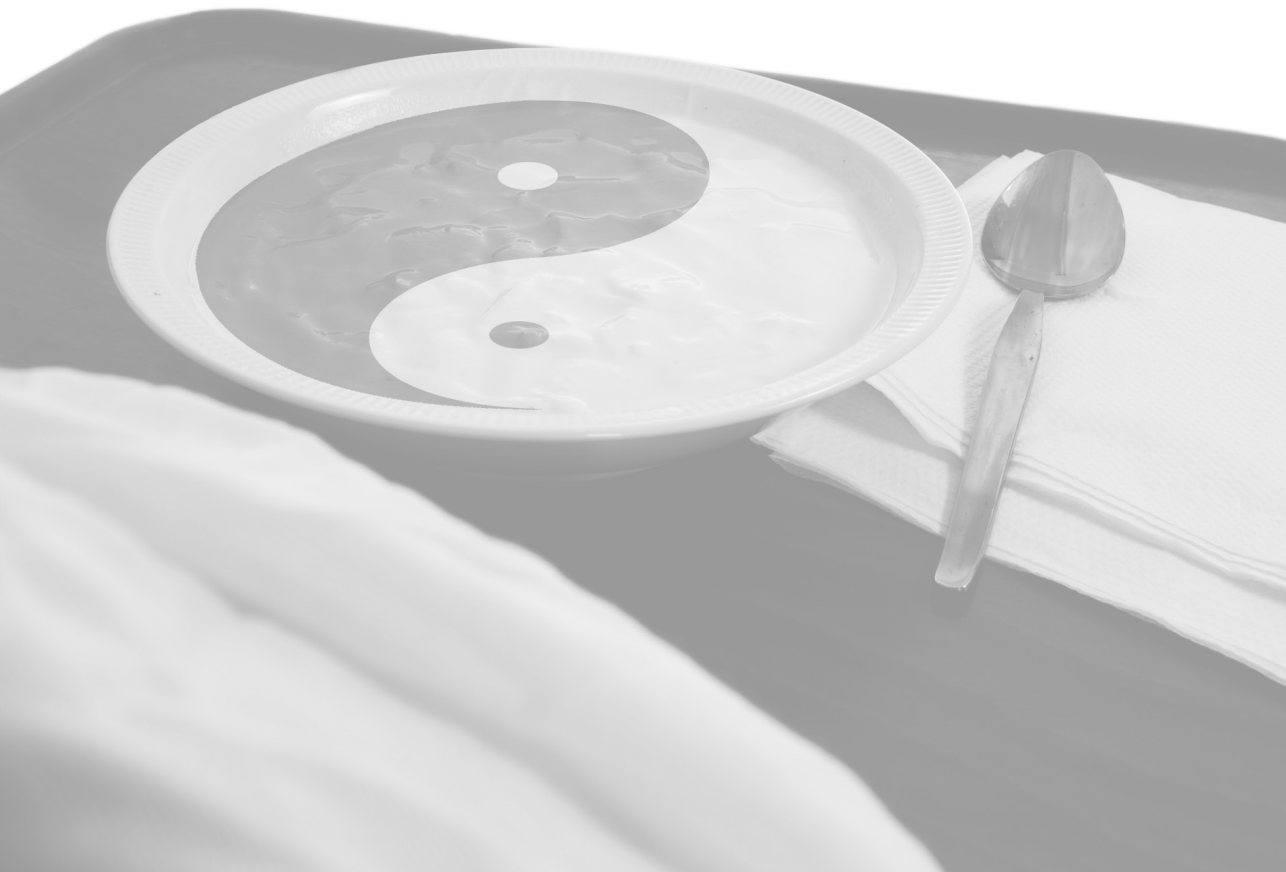
13. Vary TC. Inter-organ protein and carbohydrate metabolic relationships during sepsis: necessary evils or uncanny coincidences? Curr Opin Clin Nutr Metab Care 1999;2(3):235-42.
14. Vary TC, Hazen S. Sepsis alters pyruvate dehydrogenase kinase activity in skeletal muscle. Mol Cell Biochem 1999;198(1-2): 113-8.
15. Vary TC, Jefferson LS, Kimball SR. Insulin fails to stimulate muscle protein synthesis in sepsis despite unimpaired signaling to 4E-BP1 and S6K1. Am J Physiol Endocrinol Metab 2001;281(5):E1045-53.
16. Chiolero R, Revelly JP, Tappy L. Energy metabolism in sepsis and injury. Nutrition 1997;13(9 Suppl):45S-51S.
17. Tappy L, Chiolero R. Substrate utilization in sepsis and multiple organ failure. Crit Care Med 2007;35(9 Suppl):S531-4.
18. Food and Nutrition Board Institute of Medicine. Panel on Macronutrients. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty acids, Cholesterol, Proteins and Amino Acids. Washington DC: The National Press; 2005.
19. Broyer M, Jean G, Dartois AM, Kleinknecht C. Plasma and muscle free amino acids in children at the early stages of renal failure. Am J Clin Nutr 1980;33(7):1396-401.
20. Delaporte C, Jean G, Broyer M. Free plasma and muscle amino acids in uremic children. Am J Clin Nutr 1978;31(9): 1647-51.
21. 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.
22. Young VR, el-Khoury AE. Can amino acid requirements for nutritional maintenance in adult humans be approximated from the amino acid composition of body mixed proteins? Proc Natl Acad Sci U S A 1995;92(1):300-4.
23. Agostoni C, Carratu B, Boniglia C, Riva E, Sanzini E. Free amino acid content in standard infant formulas: comparison with human milk. J Am Coll Nutr 2000; 19(4):434-8.
24. Chuang CK, Lin SP, Lee HC, et al. Free amino acids in full-term and pre-term human milk and infant formula. J Pediatr Gastroenterol Nutr 2005;40(4):496-500.
25. Heird WC, Dell RB, Helms RA, et al. Amino acid mixture designed to maintain normal plasma amino acid patterns in infants and children requiring parenteral nutrition. Pediatrics 1987;80(3):401-8.
26. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 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.
27. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J Nutr Biochem 2006;17(9):571-88.
28. Lyons J, Rauh-Pfeiffer A, Ming-Yu Y, et al. Cysteine metabolism and whole blood glutathione synthesis in septic pediatric patients. Crit Care Med 2001;29(4): 870-7.
29. Lyons J, Rauh-Pfeiffer A, Yu YM, et al. Blood glutathione synthesis rates in healthy adults receiving a sulfur amino acid-free diet. Proc Natl Acad Sci U S A 2000;97(10):5071-6.
30. Brosnan JT, Brosnan ME. Creatine: endogenous metabolite, dietary, and

therapeutic supplement. Annu Rev Nutr 2007;27:241-61.
31. 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.
32. Montanez R, Sanchez-Jimenez F, Aldana-Montes JF, Medina MA. Polyamines: metabolism to systems biology and beyond. Amino Acids 2007;33(2): 283-9.
33. Battezzati A, Brillon DJ, Matthews DE. Oxidation of glutamic acid by the splanchnic bed in humans. Am J Physiol 1995;269(2 Pt 1):E269-76.
34. Haisch M, Fukagawa NK, Matthews DE. Oxidation of glutamine by the splanchnic bed in humans. Am J Physiol Endocrinol Metab 2000;278(4):E593-602.
35. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of glutamine and glutamic acid in humans. Am J Physiol 1993;264(6 Pt 1):E848-54.
36. Wang J, Chen L, Li P, et al. Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J Nutr 2008;138(6):1025-32.
37. Morris SM, Jr. Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 2002;22:87-105.
38. Burrin DG, Davis TA. Proteins and amino acids in enteral nutrition. Curr Opin Clin Nutr Metab Care 2004;7(1):79-87.
39. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of leucine and phenylalanine in humans. Am J Physiol 1993;264(1 Pt 1):E109-18.
40. Raguso CA, Ajami AM, Gleason R, Young VR. Effect of cystine intake on methionine kinetics and oxidation determined with oral tracers of methionine and cysteine in healthy adults. Am J Clin Nutr 1997;66(2): 283-92.
41. Verbruggen S, Sy J, Gordon WE, et al. Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am J Physiol Endocrinol Metab 2009(297):E1046-E55.
42. Fong Y, Matthews DE, He W, Marano MA, Moldawer LL, Lowry SF. Whole body and splanchnic leucine, phenylalanine, and glucose kinetics during endotoxemia in humans. Am J Physiol 1994;266(2 Pt 2): R419-25.
43. Thibault R, Welch S, Mauras N, et al. Corticosteroids increase glutamine utilization in human splanchnic bed. Am J Physiol Gastrointest Liver Physiol 2008; 294(2):G548-53.
44. Stoll B, Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 2006; 84 Suppl:E60-72.
45. Haussinger D, Steeb R, Kaiser S, Wettstein M, Stoll B, Gerok W. Nitrogen metabolism in normal and cirrhotic liver. Adv Exp Med Biol 1990;272:47-64.
46. Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. J Nutr 1998;128(10):1752-9.
47. Brunton JA, Ball RO, Pencharz PB. Current total parenteral nutrition solutions for the neonate are inadequate. Curr Opin Clin Nutr Metab Care 2000;3(4):299-304.
48. Elango R, Goonewardene LA, Pencharz PB, Ball RO. Parenteral and enteral routes of feeding in neonatal piglets require different ratios of branched-chain amino acids. J Nutr 2004;134(1):72-8.
49. Elango R, Pencharz PB, Ball RO. The branched-chain amino acid requirement of parenterally fed neonatal piglets is less than the enteral requirement. J Nutr 2002;132(10):3123-9.
50. Agostoni C, Carratu B, Boniglia C, Lammardo AM, Riva E, Sanzini E. Free glutamine and glutamic acid increase in human milk through a three-month lacta-

tion period. J Pediatr Gastroenterol Nutr 2000;31(5):508-12.
51. Mansoor O, Breuille D, Bechereau F, et al. Effect of an enteral diet supplemented with a specific blend of amino acid on plasma and muscle protein synthesis in ICU patients. Clin Nutr 2007;26(1):30-40.
52. Fukuda K, Nishi Y, Usui T. Free amino acid concentrations in plasma, erythrocytes, granulocytes, and lymphocytes in umbilical cord blood, children, and adults. J Pediatr Gastroenterol Nutr 1984;3(3):432-9.
53. Gotschlich EC, Edelman GM. C-reactive protein: a molecule composed of subunits. Proc Natl Acad Sci U S A 1965; 54(2):558-66.
54. Castillo L, Yu YM, Marchini JS, et al. Phenylalanine and tyrosine kinetics in critically ill children with sepsis. Pediatr Res 1994;35(5):580-8.
55. Courtney-Martin G, Chapman KP, Moore AM, Kim JH, Ball RO, Pencharz PB. Total sulfur amino acid requirement and metabolism in parenterally fed postsurgical human neonates. Am J Clin Nutr 2008; 88(1):115-24.
56. Sy J, Gordon W, Griffin D, et al. Parenteral sulfur amino acid requirements in septic infants. FASEB J 2007;21(6):A1072-c-.
57. Kelly DA. Intestinal failure-associated liver disease: what do we know today? Gastroenterology 2006;130(2 Suppl 1):S70-7.
58. Moss RL, Haynes AL, Pastuszyn A, Glew RH. Methionine infusion reproduces liver injury of parenteral nutrition cholestasis. Pediatr Res 1999;45(5 Pt 1):664-8.
59. Dever JT, Elfarra AA. L-methionine toxicity in freshly isolated mouse hepatocytes is gender-dependent and mediated in part by transamination. J Pharmacol Exp Ther 2008;326(3):809-17.
60. Rakonczay Z, Jr., Hegyi P, Dosa S, et al. A new severe acute necrotizing pancreatitis model induced by L-ornithine in rats. Crit Care Med 2008;36(7):2117-27.
61. Asanuma K, Adachi K, Sugimoto T, Chiba S. Effects of lysine-induced acute renal failure in dogs. J Toxicol Sci 2006;31(2): 87-98.

Part III

Protein and Energy interactions

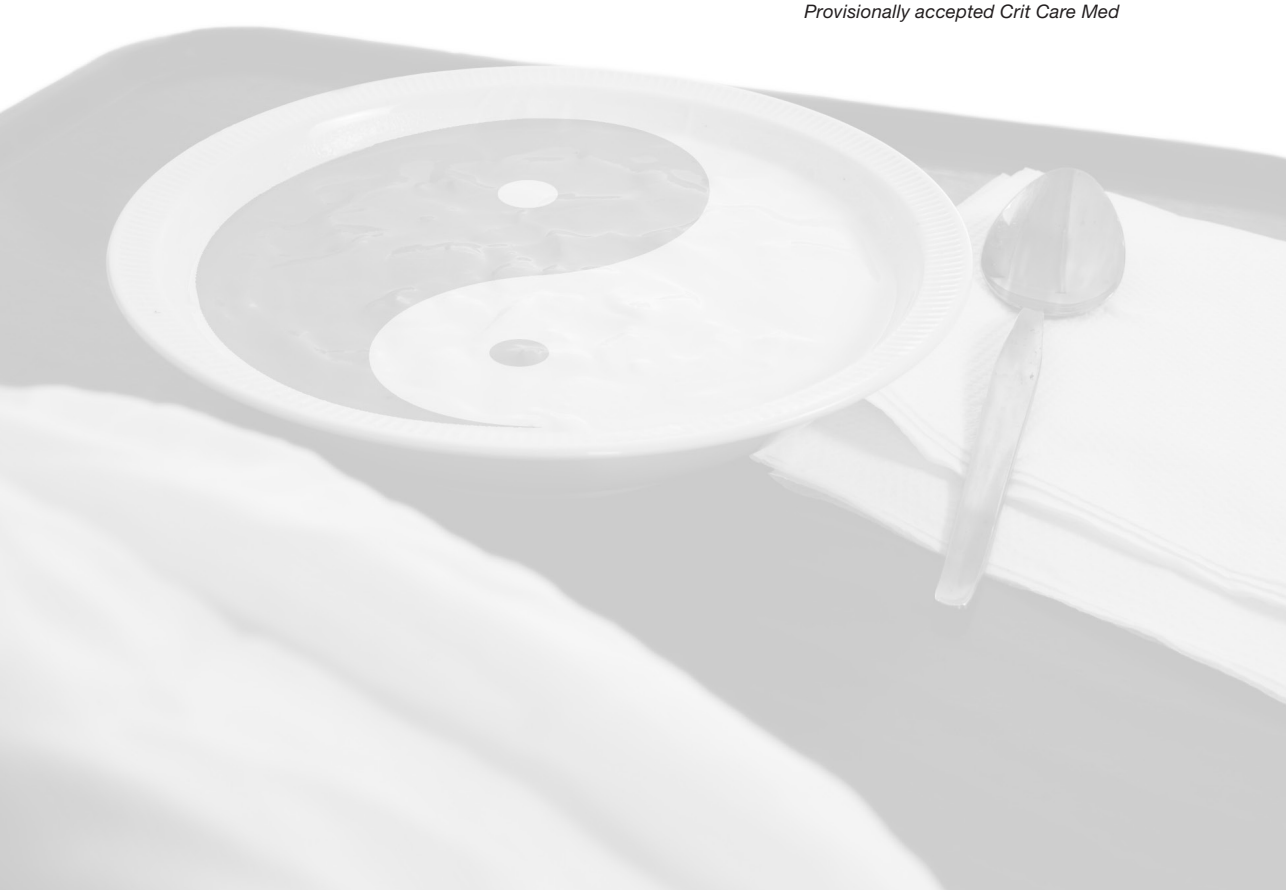


Chapter 7

Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin resistant adolescents with tight glucose control

Sascha CAT Verbruggen
Jorge Coss-Bu
Manhong Wu
Henk Schierbeek
Koen FM Joosten
Johannes B van Goudoever
Leticia Castillo

Provisionally accepted Crit Care Med



Abstract

Objective

To investigate the effects of insulin infusion and increased parenteral amino acids intakes on whole body protein balance, glucose kinetics, and lipolysis in critically ill insulin resistant septic adolescents.

Design

A single center, randomized, crossover study.

Setting

A medico-surgical intensive care unit in a tertiary university hospital.

Patients

Nine critically ill, septic adolescents (age 15.0 ± 1.2 years, BMI 20 ± 4 $kg.m^{-2}$) receiving total parenteral nutrition.

Interventions

Patients receive total parenteral nutrition with standard (SAA; 1.5 $gr.kg^{-1}.day^{-1}$) and high (HAA; 3.0 $gr.kg^{-1}.day^{-1}$) amino acid intake in a two day crossover setting, randomized to the order in which they received it. On both study days, we conducted a primed, constant, 7 h. stable isotope tracer infusion with [1-^{13}C]Leucine, [6,6-$^{2}H_2$]Glucose and [1,1,2,3,3-$^{2}H_5$]Glycerol, in combination with a Hyperinsulinemic Euglycemic Clamp (HEC) during the last 3 hours.

Measurements and Main Results

Insulin decreased protein synthesis at SAA and HAA intakes ($p < .01$), while protein breakdown decreased with insulin at SAA ($p < .05$), but not with the HAA intake. HAA intake improved protein balance ($p < .05$), but insulin did not have an additive effect. There was significant insulin resistance with an M-value of ~3 $mg.kg^{-1}.min^{-1}/\mu U.mL^{-1}$, which was 30% of reported normal values. At HAA intake endogenous glucose production was not suppressed by insulin and lipolysis rates increased.

Conclusion

The current recommended parenteral amino acid intakes are insufficient to maintain protein balance in insulin resistant patients during tight glucose control. During sepsis insulin decreases protein synthesis and breakdown, and while HAA intake improves protein balance, its beneficial effects may be offset by enhanced endogenous glucose production and lipolysis, raising concerns that insulin resistance may have been exac-

erbated and that gluconeogenesis may have been favored by high amino acid intakes. Dose response studies on the effect of the level of amino acid intakes on protein an energy metabolism are needed.

Introduction

During sepsis and inflammation, cytokine release through Nuclear Factor-Kappa B activation induces suppression of insulin receptor signaling via reduced tyrosine phosphorylation of IRS-1 and IRS-2, as well as decreased- activation of phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (Akt), resulting in insulin resistance[1]. Insulin has pleiotropic effects, and insulin resistance affects both, protein and energy metabolism, in addition to multiple other processes[2].

The inflammatory response elicited by sepsis induces altered whole body protein turnover with increased hepatic synthesis of inflammatory/immune proteins and decreased synthesis of myofibrillar and sarcoplasmic muscle proteins[3-5], due to changes in translation initiation and reduction of translation efficiency[6-8]. Changes on protein turnover during sepsis are organ specific and influenced by age[9, 10].

Sepsis also triggers a profound catabolic response characterized by increased muscle protein breakdown. Loss of lean body mass is caused by activation of the ubiquitin-proteasome proteolytic pathway (UPP) in muscle initiated by activation of caspase 3[11, 12]. Furthermore, there appears to be a link between muscle wasting and insulin resistance[13-15].

Most critically ill septic patients present with insulin resistance and receive insulin therapy to maintain plasma glucose concentrations within acceptable range[16]. Nevertheless, protein and energy supply is rather variable and constrained by fluid restriction, feeding intolerance and lack of evidence on appropriate nutrient requirements under these conditions[17].

There is a close interrelationship between protein (nitrogen) and energy metabolism. Protein accretion will not occur without sufficient energy supply, and sufficient energy supply will not support anabolism in the absence of adequate protein (nitrogen) intake. We have shown that the parenteral protein requirements of critically ill children have been based on limited data[17] and that at currently recommended enteral protein intakes, critically ill children remain in significantly negative protein balance[18].

Based on the knowledge that amino acid availability is a key component in the drive for protein synthesis[19, 20], we hypothesized that critically ill, insulin resistant, septic adolescents would require higher parenteral amino acid intakes to maintain protein balance in the presence of insulin administration. For this purpose we conducted a two day, prospective, randomized, crossover study to compare the effects of a high

parenteral amino acid intake of 3 g.kg^{-1}.d^{-1} versus standard recommended amino acid intake[21] of 1.5 g.kg^{-1}.d^{-1} on the rates of protein turnover, glucose and lipid kinetics in critically ill insulin resistant septic adolescents, under basal conditions and while receiving a hyperinsulinemic euglycemic clamp (HEC), aiming to maintain plasma glucose concentrations at rates equivalent to a tight glucose control regimen (90-110 mg.dL^{-1}; 5.0 – 6.1 mM)[22].

Material and Methods

Patients

The study was approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from parents. Studies were conducted at the Pediatric Intensive Care Unit at Texas Children's Hospital. Hyperglycemic (> 120 mg.dL^{-1}; > 6.7 mM) adolescents (13-18 yrs of age) with a diagnosis of severe sepsis, septic shock or Systemic Inflammatory Response Syndrome (SIRS)[23], and receiving TPN. Only adolescents age 13-18 years were included in the study as we have previously observed that metabolic processes greatly varies with age[18]. All patients had drawing and infusing intravascular lines and had received complete parenteral feedings for at least 24 h before the study. All were assessed for severity of disease by the Pediatric Logistic Organ Dysfunction (PELOD) score[24] and the Pediatric Risk of Mortality III (PRISM III) score[25], and Tanner classification[26, 27]. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded.

A total of thirteen consecutive, hemodynamically stable, critically ill adolescents admitted to the Pediatric Intensive Care Unit were included. From the thirteen adolescents, nine patients (age 15.0 ± 1.2 years, BMI 20 ± 4 kg.m^{-2}, PRISM 11 ± 4) were enrolled in the actual two-day study and randomized to receive first, either the standard (SAA) or high (HAA) amino acid intake, and the alternate level of parenteral amino acid intake was supplied over the next 24h (Figure 1), and four patients (age 16.5 ± 3.6 years,

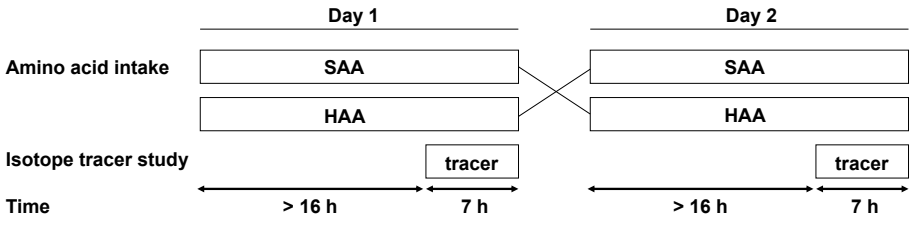


Figure 1. Schematic presentation of the study protocol in nine critically ill septic adolescents randomized to either standard or high parenteral amino acid intake in a cross-over design. SAA = standard amino acid intake (1.5 g.kg^{-1}.d^{-1}), HAA = high amino acid intake (3.0 g.kg^{-1}.d^{-1}).

BMI 25 ± 9 kg.m^{-2}, PRISM 6 ± 3) with similar conditions participated in a one-day pilot study to determine the contribution of *^{13}C* carbon from the dextrose infusion to the recovery of $^{13}CO_2$ from *^{13}C* labeled leucine oxidation[28]. The pilot patients received a hyperinsulinemic euglycemic clamp *without* additional infusion of stable isotope tracers (see below).

The characteristics of the patients are described in Tables 1 and 2. One patient randomized to start first with the standard amino acid intake group (SAA) died the next day after the first study (SAA) was completed, therefore the high amino acid intake (HAA) arm of the study could not be conducted. In a second patient a technical error occurred during the insulin infusion protocol on the study day with SAA intake. Therefore, complete two-day study data were available on 7 patients. Data available from the two incomplete studies were included according to intention-to-treat principle. In three patients with severe insulin resistance, plasma glucose levels did not decrease within the first hour of the HEC. Insulin infusion rates were increased until plasma

Table 1. Demographic and nutritional data in nine critically ill septic adolescents*

Age (years)	15.0 ± 1.2		
Gender (male:female)	3 : 6		
BMI (kg.m^{-2})	20 ± 4		
Tanner score	4.0 ± 0.9		
REE^ according to Schofield (*69*)Preschool (kcal.kg^{-1}.day^{-1})	29.3 ± 6.0		
	SAA$^{£}$	HAA$^{£}$	p-value
PICU LOS† (days)	5.9 ± 3.6	7.0 ± 3.6	.53
PELOD‡	9 ± 11	6 ± 7	.61
PRISM$^{\#}$	10 ± 4	8 ± 4	.65
C-Reactive Protein (mg.dL^{-1})	16.5 ± 9.4	15.1 ± 12.0	.61
Highest Glucose prior study (mg.dL^{-1})	182 ± 36	186 ± 81	.64
Catecholamines (n=)	1	0	
Glucocorticoids (n=)	4	3	
Protein intake (gr.kg^{-1}.day^{-1})	1.5 ± 0.2	2.8 ± 0.4	< .001
Caloric intake (kcal.kg^{-1}.day^{-1})	32.7 ± 10.0	37.8 ± 9.9	.36
Protein calories (kcal.kg^{-1}.day^{-1})	5.8 ± 0.8	11.2 ± 1.4	< .001
Carbohydrate calories (kcal.kg^{-1}.day^{-1})	22.5 ± 9.5	23.8 ± 10.3	.20
Lipid calories (kcal.kg^{-1}.day^{-1})	5.7 ± 3.9	4.7 ± 3.5	.19
Glucose prior to HEC (mg.dL^{-1})	168 ± 58	172 ± 62	.75
Glucose during HEC (mg.dL^{-1})	98 ± 6	101 ± 15	.78
Insulin plasma levels baseline (μU.mL^{-1})	32 (17 – 122)	51 (29 – 153)	.16
Insulin plasma levels during HEC (μU.mL^{-1})	144 (94 – 2385)	168 (93 – 2239)	1.0

*All values are mean ± SD. ^REE = Resting energy expenditure, $^{£}$SAA = Standard amino acid intake, $^{£}$HAA = High amino acid intake, †PICU LOS = Length of stay on the PICU at start of study, ‡PELOD = Pediatric Logistic Organ Dysfunction [24], $^{\#}$PRISM III = Pediatric Risk of Mortality III [25]

Table 2. Characteristics of nine critically ill septic adolescents included in the two day study.

Patient	Diagnosis	Tanner	BMI* ($kg.m^{-2}$)	Gender	Mechanical Ventilation	Steroids	Age (years)	Pressors
1	MRSA† myositis	3	15.8	Male	Yes	Yes	17.0	Yes
2	acute lymphatic leukemia, viral sepsis	5	24.2	Female	Yes	Yes	16.3	No
3	peritonitis due to small bowel perforation	4	20.7	Female	Yes	No	13.6	No
4	sepsis post lung and kidney transplantation	3	14.8	Male	Yes	Yes	15.9	No
5	sepsis post spinal surgery	5	29.3	Female	Yes	No	14.4	No
6	MSSA‡ pneumonia	4	21.4	Female	Yes	Yes	15.8	No
7	peritonitis due to small bowel perforation	3	19.6	Male	Yes	No	13.9	No
8	MRSA† myositis	4	19.7	Female	Yes	No	14.4	No
9	MSSA‡ pneumonia	5	18.5	Female	Yes	No	14.9	No

* BMI = Body mass index, † MRSA = methicillin resistant staphylococci aureus, ‡ MSSA = methicillin susceptible staphylococci aureus

glucose levels decreased to achieve normoglycemia. Due to this approach the tracer infusion and clamp study in these three patients was extended to four hours instead of three, to achieve steady state normoglycemia for at least one hour. Interestingly, the same situation occurred in both study days for these three patients, who appeared to be extremely insulin resistant. Two of these patients were receiving glucocorticoids.

Experimental design

The experimental design followed, involved two 24h dietary study periods in a randomized cross-over fashion, where the subjects received for 24h a specific parenteral level of amino acid intake (SAA or HAA) on study day 1, followed by the alternate parenteral level of amino acid intake in the next day (Figure 1). Each patient received two dietary study days and two tracer-clamp studies.

Parenteral dietary intake

Patients were adapted to the level of study protein intake for at least 16hs before the tracer infusion study, and continued to receive the randomized level of parenteral amino acid intake during the tracer infusion study period at baseline and during the HEC. The amino acid composition of the TPN provided to the patients is shown in table 3. Energy intake provided as parenteral glucose and lipids were prescribed by the clinical team according to standard care. The total energy intake supplied remained unchanged during both study days (Table 1).

Table 3. Amino Acid Composition of Formulas Administered to the adolescents

	Aminosyn (10%) (n=8)	Clinisol (15%) (n=1)
Essential		
Histidine	300	894
Isoleucine	660	749
Leucine	1000	1040
Lysine	1050	1180
Methionine	172	749
Phenylalanine	298	1040
Threonine	400	749
Tryptophan	200	250
Valine	500	960
Non-essential		
Arginine	1018	1470
Alanine	993	2170
Asparagine	0	0
Aspartate	700	434
Cysteine	0	0
Glutamine	0	0
Glutamate	738	749
Glycine	500	1040
Proline	722	894
Serine	530	592
Tyrosine	270	39
Taurine	0	0

All values are mg/100mL. Composition provided by Aminosyn (Hospira, Lake Forest, IL), and Clinisol (Baxter, Deerfield, IL).

Tracer infusion studies

On each study day the patients received a 7 hour, primed, continuous, stable isotope tracer infusion study (Figure 2). The first 4 hours were the basal period and during the last 3 hours of the tracer study the patients received the HEC. Isotope tracers were purchased from Cambridge Isotope Laboratories (Andover, MA) and were tested for sterility and pyrogenicity. On each tracer study the patients received an intravenous, primed, continuous, 7 hour tracer infusion of L-[1-^{13}C]leucine (6 μmol.kg^{-1}; 6 μmol.kg^{-1}.h^{-1}), D-[6,6-$^{2}H_2$]glucose (25 μmol.kg^{-1}; 30 μmol.kg^{-1}.h^{-1}), and [1,1,2,3,3-$^{2}H_5$]glycerol (30 μmol.kg^{-1}; 3 μmol.kg^{-1}.h^{-1}) for prime and constant infusion rates, respectively. The bicarbonate pool was primed with 2.1 μmol.kg^{-1} of ^{13}C sodium bicarbonate as previously described [29]. Four hours after initiation of the tracer infusions, a HEC was conducted as described below (Figure 2). At the end of the first study day, the parenteral protein intake was changed to the alternate level of protein intake and again, the tracer infusion protocol was repeated (Figure 1).

Hyperinsulinemic Euglycemic Clamp (HEC)

Four hours after the initiation of the tracer infusions a HEC was conducted as previously described[28, 30]. In brief, a 3h infusion of insulin (Actrapid, Novo Nordisk Inc., Princeton, NJ), dissolved in sterile isotonic NaCl was started at 80 $mU.m^{-2}.min^{-1}$ in order to achieve both normoglycemia between 90-110 $mg.dL^{-1}$ (5.0 – 6.1 mM) and a plasma insulin concentration greater than 100 $\mu U.mL^{-1}$. During the insulin infusion, whole blood glucose concentration was monitored at the bedside every 5-10 min. In order to maintain the plasma glucose concentration between 90-110 $mg.dL^{-1}$ and the enrichment of D-[6,6-$^2H_2$]glucose at steady state for the duration of the study, a 30% glucose solution (Baxter, Deerfield, IL) enriched at 3.5% with D-[6,6-$^2H_2$]glucose was infused as previously described ("hot Ginf")[30].

Measurements and sample analysis

Arterial blood samples were obtained at frequent intervals (Figure 2), centrifuged (12 min 3000 rpg at 4°C) and frozen at -80°C until analysis. We used plasma alpha-ketoisocaproate (α-KIC) enrichment, which is intracellularly produced from the infused leucine tracer. Plasma isotope enrichment of [1-^{13}C]α-KIC, D-[6,6-$^2H_2$]glucose and [1,1,2,3,3-$^2H_5$]glycerol were determined as previously described[31-34]. Carbon dioxide production (VCO_2) was obtained with the respiratory profile monitor (CO_2SMO Plus, Novametrix Medical System, Wallingford, CT) during the last 40 minutes of the baseline period and during the last 30 minutes of the HEC period. Enrichment of $^{13}CO_2$ in whole blood was obtained as previously described[29, 35]. Plasma samples for insulin were analyzed with standard human insulin specific RIA techniques. Amino acids in plasma were determined as previously described[36] by anion exchange chromatography with ninhydrin detection on a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, England).

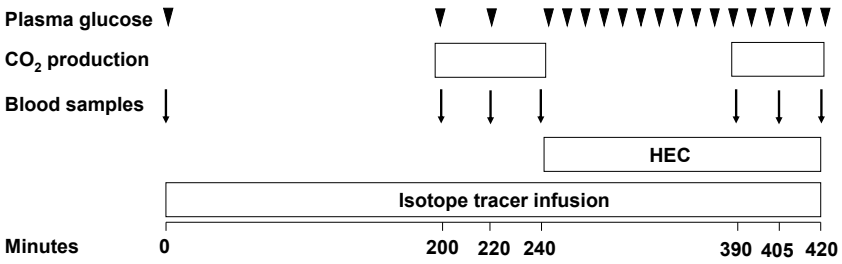


Figure 2. Schematic presentation of the tracer infusion studies during both study days in nine critically ill septic adolescents receiving either standard or high parenteral amino acid intake. Black triangles indicate time points of plasma glucose measurements, arrows indicate time points for plasma collection for isotopic enrichment measurements and square boxes represent the time period in which carbon dioxide production measurements take place.
HEC = Hyperinsulinemic Euglycemic Clamp.

Calculations

Whole body kinetics of protein and lipids were calculated by conventional isotope dilution equations using a stochastic model during steady state enrichment[37] and glucose kinetics were estimated using the Steele equation[38]. The rate of appearance (Ra) of unlabeled substrate can be derived from the plasma isotope enrichment calculated by:

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

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 (Mole fraction per cent excess), respectively.

$^{13}CO_2$ recovery during 30% Dextrose infusion

To determine the contribution of ^{13}C originating from the 30% dextrose infused during the HEC to $^{13}CO_2$ produced during leucine oxidation, we conducted an identical clamp study without the tracer infusion[28], in four patients with similar characteristics. Using linear regression we established the $^{13}CO_2$ background enrichment in relation to the elevation of glucose infusion rate during HEC. For every additional $mg.kg^{-1}.min^{-1}$ of glucose infused during HEC in our pilot studies, $^{13}CO_2$ enrichment increased by 0.31 APE*10^3. Thus for every additional $mg.kg^{-1}.min^{-1}$ of glucose infused during HEC in the patients enrolled in the actual study, we subtracted 0.31 APE*10^3 enrichment of the $^{13}CO_2$ blood enrichment (APE*10^3), because this amount was contributed by the 30% dextrose infused, and the remaining enrichment originated from L-[1-^{13}C]leucine oxidation.

Glucose metabolism

Estimates of whole-body glucose kinetics were made at isotopic steady-state, effectively attained during the last 40 min of the basal period and the last 30 min of the HEC. Mean values of plasma D-[6,6-$^2H_2$]glucose enrichment (Mole fraction percent excess) and of exogenous glucose infusion rate were used for data calculation. Under steady state conditions total glucose rate of appearance (Ra) is equal to the rate of disappearance (Rd)[38]. The rates of glucose disappearance reflect glucose utilization.
Total endogenous glucose production (EGP) rate was calculated during the last 40 min of the basal period and the last 30 min of the HEC by subtracting the exogenous glucose infusion rate from the glucose rates of appearance (Ra)[39].

$$EGP\ (mg.kg^{-1}.min^{-1}) = Glucose\ Ra - Glucose\ infusion\ rate \quad (2)$$

Insulin sensitivity ($mg.kg^{-1}.min^{-1}/\mu U.mL^{-1}$) as measured with the HEC and pointed out as M-value, is the rate of glucose disappearance (Rd) divided by plasma insulin concentration during steady state[40].

$$\text{Insulin sensitivity } (mg.kg^{-1}.min^{-1}/\mu U.mL^{-1}) = Rd/\text{Plasma insulin} \quad (3)$$

Insulin stimulated glucose disposal ($mg.kg^{-1}.min^{-1}$) was considered equal to the sum of EGP and GIR during steady state glucose rate of disappearance[41].

$$\text{Insulin stimulated glucose disposal} = EGP + GIR \quad (4)$$

Protein Metabolism

Whole body plasma leucine flux, an index of protein metabolism, was calculated using plasma α-ketoisocaproate (α-KIC) enrichment expressed as mole fraction per cent excess during the last 40 min of the baseline period and the last 30 min of the HEC period, as described previously[34]. Leucine Oxidation was obtained as follows:

$$VCO_2 \times (E^{13}CO_2/69.18)/^{13}C\ \alpha\text{-KIC} \quad (5)$$

where VCO_2 is the rate of carbon dioxide elimination measured in milliliters per minute and converted to millimoles per hour by multiplying by 60 min and dividing by 22.4, which is the number of 1 in 1 mole of an ideal gas at standard temperature and pressure. We have previously shown that 69.18 is the correction factor for $^{13}CO_2$ under recovery obtained from parenterally fed critically ill children[35].

Under the assumption that 1 gram of mixed muscle protein contains approximately 621 μmol of leucine[42], whole body protein turnover was calculated from the model described by Golden and Waterlow[43]. Briefly, the model is described in the following equation;

$$Ra = S + O = B + I. \quad (6)$$

Where Ra is the rate of appearance into the plasma pool, S corresponds to protein synthesis and O to oxidation, and therefore losses from the plasma amino acid pool, while B represents protein breakdown and I dietary intake, and therefore entry of amino acids into the plasma pool[29].

Protein synthesis (S) was calculated by subtracting the rates of leucine oxidation (O) from the plasma leucine flux (Ra).

$$\text{Prot Synthesis} = \text{Leucine Ra} - \text{Leu ox} \quad (7)$$

Protein breakdown (B) was calculated by subtracting the leucine intake (I; tracer infusion + leucine content of parenteral nutrition) from the leucine flux (Ra).

$$\text{Prot Breakdown} = \text{Leu flux} - \text{Leu intake} \quad (8)$$

Protein balance was then calculated subtracting whole body protein breakdown from whole body protein synthesis.

$$\text{Prot bal} = \text{Prot Synthesis} - \text{Prot Breakdown} \quad (9)$$

Lipid Metabolism

Glycerol flux was calculated during the last 40 min of the baseline period, and during the last 30 min of the hyperinsulinemic euglycemic clamp according to the steady state tracer dilution equations reported previously[32]. As part of their parenteral nutrition the patients were provided with lipids intravenously which contained 2.25 $mg.mL^{-1}$ glycerol. Lipolysis was calculated by subtracting the glycerol intake (tracer infusion + glycerol intake through parenteral nutrition) from the glycerol flux (Ra).

$$\text{Lipolysis} = \text{Glycerol Ra} - \text{Total glycerol intake} \quad (10)$$

Statistical analysis

From our previous data on protein turnover at random different levels of protein intake in critically ill children[42], we estimated that 8 patients with complete data, would detect a difference of 20% (80% power, type I error of 5%) on protein balance. The Shapiro-Wilk normality test was used to determine data normality. Comparison between the two different amino acid intakes at baseline and during the hyperinsulinemic euglycemic clamp were made using the repeated measurements ANOVA, after which a student's paired *t test* was used for normally distributed data. For non-parametric data the Wilcoxon matched pairs test was used. Data are presented as the mean ± standard deviation. Statistical significance was considered at $p < .05$. Repeated measures ANOVA were used to analyze the effect of insulin on parameters of glucose, lipid and protein metabolism over time and between normal and high amino acid intake. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, La Jolla, CA., USA).

Results

Patients

Between the two study days, there were no differences in PRISM, PELOD scores, CRP, PICU length of stay at start of study (PICU LOS) or highest glucose values prior to the onset of the tracer and clamp studies. There were no differences in the total energy intake provided in both study days (Table 1).

Insulin sensitivity and glucose kinetics

The maximum plasma glucose concentrations did not differ between both study days prior to the onset of the tracer studies (Table 1). As expected, plasma glucose concentration differed between the baseline and HEC periods, respectively for SAA and HAA intakes ($p < .001$; Table 1). Plasma insulin concentrations for the baseline period did not differ between the dietary groups (Table 1). However, there was considerable variability in the plasma insulin concentrations during the HEC period in both, the SAA and HAA groups because of three highly insulin resistant patients.

Endogenous glucose production (EGP) decreased significantly with insulin administration during the HEC, both at the SAA and the HAA intakes ($p < .05$) but it was not fully suppressed during the HAA intake (Figure 3; Panel A).

Insulin stimulated glucose disposal, an index of peripheral insulin sensitivity[40] was not different between the dietary groups ($p > .05$). However, these values were about 30 - 35% of reported values of 14.2 $mg.kg^{-1}.min^{-1}$ in healthy children[44], confirming that our patients presented significant peripheral insulin resistance (Figure 3; Panel B).

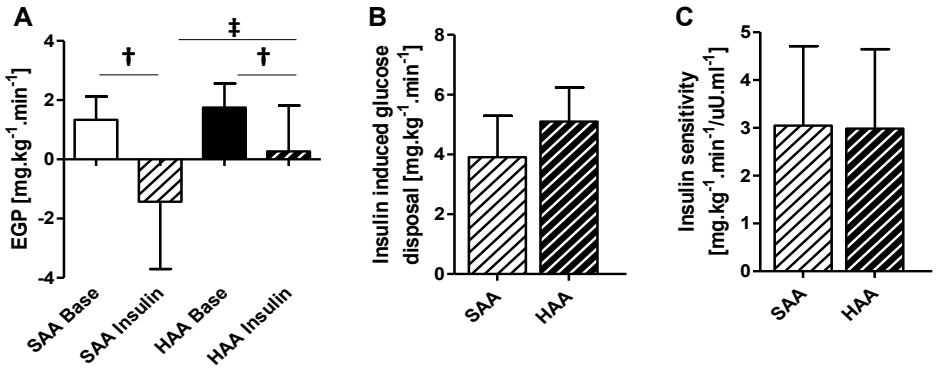


Figure 3. Glucose kinetics in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during HEC. Panel A; EGP ($mg.kg^{-1}.min^{-1}$), Panel B; Insulin induced glucose disposal ($mg.kg^{-1}.min^{-1}$), Panel C; Insulin sensitivity depicted as M-value ($mg.kg^{-1}.min^{-1}/\mu U.mL^{-1}$). SAA = Standard amino acid intake, HAA = High amino acid intake, HEC = hyperinsulinemic euglycemic clamp, EGP = Endogenous glucose production. Values are mean ± SD, † $p < .05$; baseline vs. HEC, ‡ $p < .05$; SAA vs. HAA.

There was no difference in the glucose infusion rate required during the HEC to maintain normoglycemia at steady state (6.2 ± 1.9 mg.kg^{-1}.min^{-1} vs. 5.6 ± 2.2 mg.kg^{-1}.min^{-1}, respectively for SAA and HAA; $p > .05$). Insulin sensitivity did not differ between study days (M-value 3.1 ± 1.7 vs. 3.0 ± 1.7 mg.kg^{-1}.min^{-1}/μU.mL^{-1}, $p > .05$, respectively for SAA and HAA) (Figure 3; Panel C).

Protein turnover

Plasma amino acid concentrations

As expected, the HAA intake resulted in higher ($p < .05$) plasma amino acid concentrations for many but not all amino acids. Insulin administration only decreased tyrosine levels at SAA ($p < .05$), while none of the measured plasma amino acids concentrations decreased under hyperinsulinemia at HAA intake (Table 4).

Table 4. Plasma amino acid concentrations*

	SAA[a]		HAA[b]	
	Baseline	HEC	Baseline	HEC
Leucine	134 ± 50	122 ± 42	197 ± 82 ‡	185 ± 91 ‡
Isoleucine	77 ± 28	73 ± 26	120 ± 48 ‡	112 ± 51 ‡
Valine	193 ± 74	174 ± 64	268 ± 102 ‡	256 ± 114 ‡
Alanine	296 ± 115	295 ± 116	363 ± 142 ‡	364 ± 180 ‡
Arginine	110 ± 57	133 ± 85	149 ± 61 ‡	172 ± 87
Ornithine	91 ± 38	87 ± 33	127 ± 53	120 ± 47
Citrulline	13 ± 10	12 ± 10	16 ± 17	16 ± 18
ASPX [c]	48 ± 14	45 ± 13	54 ± 19	52 ± 16
GLUX [d]	456 ± 131	455 ± 148	517 ± 103	504 ± 146
Glycine	203 ± 65	208 ± 70	236 ± 72	243 ± 96
Methionine	25 ± 28	22 ± 28	28 ± 26	24 ± 19
Cystine	6 ± 5	8 ± 6	8 ± 6	9 ± 8
Phenylalanine	104 ± 44	95 ± 38	109 ± 37	111 ± 41
Tyrosine	64 ± 33	55 ± 32 †	57 ± 27 ‡	59 ± 36
Lysine	222 ± 98	231 ± 97	293 ± 82 ‡	305 ± 116 ‡
Histidine	63 ± 21	66 ± 20	78 ± 19	76 ± 23
Threonine	154 ± 80	155 ± 76	162 ± 74	172 ± 71
Serine	99 ± 31	102 ± 29	128 ± 36 ‡	131 ± 49 ‡
Proline	236 ± 85	239 ± 62	376 ± 124 ‡	361 ± 131 ‡
Taurine	39 ± 36	30 ± 28	27 ± 20	30 ± 20

*Plasma amino acids depicted as μmol.L^{-1}; values are mean ± SD, † $p < .05$; Baseline vs HEC, ‡ $p < .05$; SAA vs HAA
[a]SAA = Standard amino acid intake,
[b]HAA = High amino acid intake,
[c]ASPX = Asparagine and Aspartate,
[d]GLUX = Glutamine and Glutamate

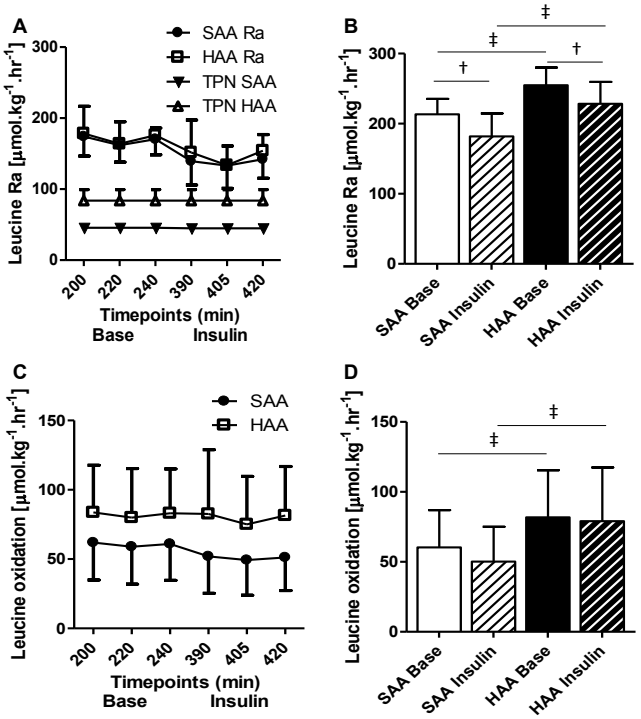


Figure 4. Leucine kinetics in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during HEC. Panel A; Time curve of leucine rate of appearance through TPN and endogenous leucine appearance on both study days, Panel B; Bars of total leucine rate of appearance during the study periods, Panel C; Time curve of Leucine oxidation on both study days, Panel D; Total leucine rate of oxidation during the study periods, TPN = total parenteral nutrition, SAA = Standard amino acid intake, HAA = High amino acid intake, HEC = hyperinsulinemic euglycemic clamp. Values are µmol.kg^{-1}.h^{-1}; mean ± SD. † p < .05; baseline vs. HEC, ‡ p < .05; SAA vs. HAA.

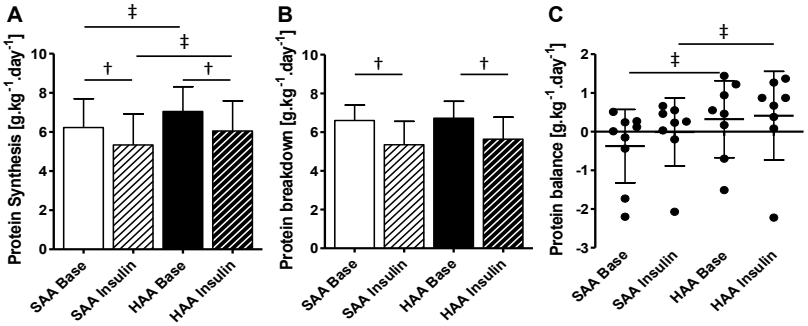


Figure 5. Protein metabolism in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during HEC. Panel A; Protein synthesis, Panel B; Protein breakdown, Panel C; Protein balance, SAA = Standard amino acid intake, HAA = High amino acid intake, HEC = hyperinsulinemic euglycemic clamp. Values are g.kg^{-1}.d^{-1}, mean ± SD. † p < .05; baseline vs. HEC, ‡ p < .05; SAA vs. HAA.

Leucine Rate of appearance and oxidation

As shown in Figure 4 Panel A, plasma leucine Ra increased when amino acid intake was higher, as it would be expected, both at baseline and during HEC ($p < .01$ SAA vs. HAA), and during HEC the rate of appearance of leucine decreased both at SAA intake and HAA intake ($p < .05$) (Figure 4; Panel A and B).

The oxidation rates of leucine were not affected by insulin infusion, but did increase with the higher amino acid intake at baseline ($p < .001$) and during HEC ($p < .001$) (Figure 4; Panel C and D).

Protein metabolism

As shown in Figure 5 Panel A, whole body protein synthesis rates significantly decreased when insulin was administered at SAA intake ($p < .01$), and this effect persisted even in the presence of HAA intake ($p < .01$). The protein synthesis rates were improved by the HAA intake during the baseline and the HEC period ($p < .05$). In contrast, as shown in Figure 5 Panel B, whole body protein breakdown was not affected by the amino acid intake, but significantly decreased ($p < .05$) with insulin administration during the SAA intake and the HAA intake. Protein balance improved ($p < .05$) when the HAA intake was given, but insulin administered during the HEC at the HAA intake did not further improve protein balance. Likewise, insulin administration during SAA intake failed to significantly improve protein balance (Figure 5; Panel C).

Lipolysis

The rates of lipolysis were not affected by insulin during SAA or HAA amino acid intake. Interestingly, the high amino acid intake increased lipolysis ($p < .05$) (Figure 6).

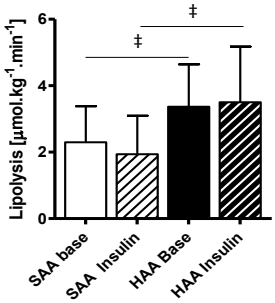


Figure 6. Rate of lipolysis in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during HEC. SAA = Standard amino acid intake, HAA = High amino acid intake, HEC = hyperinsulinemic euglycemic clamp. Values are µmol. $kg^{-1}.min^{-1}$; mean ± SD. ‡ $p < .05$; SAA vs. HAA.

Discussion

Protein turnover

The negative whole body protein balance observed at standard amino acid intakes in the present study is consistent with previous data by us and others[45, 46]. Our data showed that parenteral amino acids intakes of 1.5 gr.kg^{-1}.day^{-1} as per recommended guidelines[21] are insufficient to support protein turnover and balance in septic, insulin resistant adolescents at baseline conditions and while receiving insulin.

In critically ill adults, increasing protein intake from about 1 to 1.5 gr.kg^{-1}.day^{-1} improved but did not normalize whole body protein balance, while a further increase to 2 gr.kg^{-1}.day^{-1} did not lead to a further improvement[47]. In contrast, our study demonstrated that in these septic adolescents, increasing parenteral amino acid intakes from 1.5 to 3.0 gr.kg^{-1}.day^{-1} in the presence of adequate caloric intake, showed a strong trend towards stimulation of protein synthesis and significantly improved whole body protein balance, even in the absence of insulin administration.

It is not surprising that whole body protein synthesis was increased with higher amino acid intakes, since amino acids, especially branched-chain amino acids such as L-leucine, and cationic amino acids such as L-arginine act as nutrient signals themselves, modulating cellular processes that lead to protein synthesis via augmented mRNA translation initiation[20]. Amino acids are known to alter the phosphorylation status of mammalian target of rapamycin (mTOR) associated signaling proteins in muscle, and inhibiting AMP-activated protein kinase (AMPK) signaling, resulting in increased protein synthesis via enhanced translation initiation and translation elongation[20]. The mTOR pathway is a key regulator of cell growth and proliferation[48].

Under physiological conditions, the major regulator of muscle protein synthesis is increased amino acid availability (particularly leucine), rather than insulin[49]. Hence, dietary amino acid intake and intracellular transport play a key role in this process. However, under conditions of sepsis and cytokine release, muscle protein synthesis is inhibited by decreased mTOR kinase activity in muscle, as evidenced by reduced phosphorylation of both eukaryotic initiation factor (eIF)4E-binding protein (BP)-1 and ribosomal S6 kinase (S6K)1, which are mTOR downstream signaling proteins[6]. The ability of leucine to increase 4E-BP1 and S6K1 phosphorylation is greatly attenuated by TNF and glucocorticoids, which results in “leucine resistance”[6]. This effect, however, may be overcome at some extent by administration of amino acids, as demonstrated in an endotoxemic piglet model whereby supplying amino acids at concentrations comparable to those found in the fed state, rescued the inhibitory response of sepsis on muscle protein synthesis[50]. The same effect has been observed in an adult rat model of chronic sepsis by feeding oral leucine[51], and is observed in our current data where the septic adolescents receiving a high amino acid intake, showed a strong trend towards increased protein synthesis.

In regard to the effects of insulin on protein synthesis during sepsis, our data showed that during sepsis and perhaps exacerbated by the use of glucocorticoids, insulin decreased whole body protein synthesis, even when a high amino acid intake was provided. These data are in agreement with that of septic animal models[6], insulin dependent diabetics[52] and critically ill newborns[53]. Lang and coworkers[6] reported that insulin failed to stimulate protein synthesis in an animal model of sepsis via a defect in insulin signaling to a step in translation initiation mediating the assembly of the active eukaryotic initiation factor 4 F complex (eIF4F), which is a key protein complex in translation initiation. Therefore, sepsis induces insulin resistance to protein synthesis.

We observed that insulin administration decreased protein breakdown, which is consistent with previous reports in critically ill newborns[53, 54] and in adults with diabetes mellitus[52]. Using leucine kinetics in healthy humans, it has been shown that hyperinsulinemia decreased proteolysis but did not stimulate protein synthesis. By contrast, elevation of plasma levels of amino acids, by infusion of an amino acid mixture, stimulated the protein synthesis, but did not suppress endogenous proteolysis[55]. Thus, amino acids and insulin appear to exert different and complementary effects in stimulating protein anabolism. In healthy humans insulin is “permissive” for protein synthesis and suppressive for protein breakdown[56].

However, during sepsis and insulin resistance, the high amino acid intake did not overcome insulin resistance. It appears then that in septic adolescents insulin suppresses protein synthesis and breakdown, while high amino acid intake enhances protein synthesis but diminishes insulin effect on protein breakdown.

Furthermore, there is evidence that insulin resistance is associated with muscle breakdown. During inflammation, insulin resistance decreases Phosphoinositide 3-kinase (PI3K) activity and this subsequently reduces the level of phosphorylated protein kinase B (Akt)[13]. A low Akt relieves the inhibition of the expression of specific E3 ubiquitin-conjugating enzymes atrogin-1/MAFbx and MuRF1 in muscle. Expression of these E3 enzymes is found in conditions causing loss of lean body mass[13]. The Ubiquitin Proteasome Pathway together with autophagy, are the main routes that cells use for degrading intracellular proteins.

Endogenous glucocorticoids and impaired insulin signaling are required for stimulation of muscle breakdown in inflammation[57]. These conditions were found in our patients, and currently, a large proportion of critically ill patients are managed with glucocorticoids which exacerbates insulin resistance and protein breakdown.

Insulin did not have an additive effect to the HAA intake, in terms of net protein balance in the septic adolescents. This was mainly due to a less pronounced suppression of protein breakdown with insulin at HAA intake, while protein synthesis was simultaneously decreased at HAA intake. High protein intakes are known to induce insulin resistance[58].

Glucose metabolism and insulin sensitivity

All septic patients showed considerable insulin resistance with about 65% decrease in insulin sensitivity when compared to values of ~11 mg.kg^{-1}.min^{-1}/μU.mL^{-1} reported in fasted healthy adolescents[59, 60], and the degree of insulin resistance is comparable to that observed in critically ill adults (~ 4.6 mg.kg^{-1}.min^{-1}/μU.mL^{-1})[61]. Despite significant insulin resistance, when the standard amino acid intake was supplied in the presence of insulin, endogenous glucose production was suppressed, which is in agreement with previous studies in critically ill adults[62-65].

Interestingly, when a high amino acid intake was provided to our patients, insulin failed to suppress the rates of endogenous glucose production and lipolysis rates were increased. This observation, in addition to the lack of insulin suppression of protein breakdown at high amino acid intakes raised concern that a high amino acid intake may exacerbate insulin resistance, and that a greater supply of gluconeogenic amino acids could preferentially trigger their use over glycogen for glucose production[66]. Amino acid signaling is integrated by mTOR which operates a negative feedback loop toward insulin receptor substrate 1 (IRS-1) signaling, promoting insulin resistance for glucose metabolism[67]. Amino acid infusion in healthy subjects to achieve hyperaminoacidemia decreases insulin sensitivity[68]. In contrast, we observed no effect of increased amino acid intake on insulin plasma levels or insulin sensitivity, but our sample size was limited. Hence, although a high amino acid intake increased protein synthesis, at the amounts supplied may have negatively impacted glucose homeostasis.

Lipolysis

Insulin did not decrease lipolysis and this is consistent with data in critically ill adults[64, 69]. Furthermore, lipolysis increased at HAA intakes, again raising concern for increased insulin resistance when the HAA intake was provided.

Conclusion

We confirmed that critically ill septic adolescents are markedly insulin resistant and conclude that recommended parenteral amino acid intakes in these patients are insufficient to maintain protein balance. Increasing amino acid intakes to 3 g.kg^{-1}.d^{-1} improved protein synthesis and balance, but it may have favored gluconeogenesis and stimulated lipolysis, raising concern that this level of amino acid intake may have enhanced insulin resistance. Insulin infusion and tight glucose control at standard amino acid intakes decreased both protein synthesis and breakdown, and did not affect protein balance. Dose-response studies on protein administration and their effects on energy metabolism and insulin resistance are needed.

Acknowledgements

We are indebted to the patients and their families at Texas Children's Hospital for their selfless contribution to this study. We thank the nursing staff, Debra Griffin, David Zurakowski, Wim Hop, Chaji Chacko, Gardi Minderman-Voortman and Kristien Dorst for their contribution to these studies and Dr. Morey Haymond for his advice.

References

1. Matsuda N, Yamamoto S, Yokoo H, Tobe K, Hattori Y. Nuclear factor-kappaB decoy oligodeoxynucleotides ameliorate impaired glucose tolerance and insulin resistance in mice with cecal ligation and puncture-induced sepsis. Crit Care Med 2009;37(10):2791-9.
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. Hasselgren PO, Pedersen P, Sax HC, Warner BW, Fischer JE. Current concepts of protein turnover and amino acid transport in liver and skeletal muscle during sepsis. Arch Surg 1988;123(8):992-9.
4. Vary TC. Regulation of skeletal muscle protein turnover during sepsis. Curr Opin Clin Nutr Metab Care 1998;1(2):217-24.
5. Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol 1992; 262(2 Pt 1):C445-52.
6. Lang CH, Frost RA, Vary TC. Regulation of muscle protein synthesis during sepsis and inflammation. Am J Physiol Endocrinol Metab 2007;293(2):E453-9.
7. Cooney RN, Kimball SR, Vary TC. Regulation of skeletal muscle protein turnover during sepsis: mechanisms and mediators. Shock 1997;7(1):1-16.
8. Vary TC, Kimball SR. Sepsis-induced changes in protein synthesis: differential effects on fast- and slow-twitch muscles. Am J Physiol 1992;262(6 Pt 1):C1513-9.
9. Kimball SR, Orellana RA, O'Connor PM, et al. Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs. Am J Physiol Endocrinol Metab 2003;285(3):E637-44.
10. Orellana RA, Kimball SR, Nguyen HV, et al. Regulation of muscle protein synthesis in neonatal pigs during prolonged endotoxemia. Pediatr Res 2004;55(3): 442-9.
11. 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-37S.
12. Voisin L, Breuille D, Combaret L, 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.
13. Wang X, Hu Z, Hu J, Du J, Mitch WE. Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling. Endocrinology 2006;147(9):4160-8.
14. Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, Greenhaff PL. A potential role for Akt/FOXO signalling in both protein loss and the impair-

ment of muscle carbohydrate oxidation during sepsis in rodent skeletal muscle. J Physiol 2008;586(Pt 22):5589-600.
15. 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.
16. Wilson M, Weinreb J, Hoo GW. Intensive insulin therapy in critical care: a review of 12 protocols. Diabetes Care 2007;30(4): 1005-11.
17. Verbruggen S, Sy J, Arrivillaga A, Joosten K, van Goudoever J, Castillo L. Parenteral Amino Acid Intakes in Critically Ill Children: A Matter of Convenience. JPEN J Parenter Enteral Nutr 2010;34(3): 329-40.
18. Verbruggen S, Sy J, Gordon WE, et al. Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am J Physiol Endocrinol Metab 2009(297):E1046-E55.
19. Davis TA, Fiorotto ML, Burrin DG, et al. Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs. Am J Physiol Endocrinol Metab 2002;282(4): E880-90.
20. Fujita S, Dreyer HC, Drummond MJ, et al. Nutrient signalling in the regulation of human muscle protein synthesis. J Physiol 2007;582(Pt 2):813-23.
21. 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.
22. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001; 345(19):1359-67.
23. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005;6(1):2-8.
24. Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003;362(9379):192-7.
25. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
26. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44(235):291-303.
27. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970;45(239):13-23.
28. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237(3): E214-23.
29. 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.
30. Finegood DT, Bergman RN, Vranic M. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. Diabetes 1987;36(8): 914-24.
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. Beylot M, Martin C, Beaufrere B, Riou JP, Mornex R. Determination of steady state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer. J Lipid Res 1987;28(4):414-22.
33. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, Haymond MW. Measurement

of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide. J Appl Physiol 2008;104(4):944-51.
34. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. 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, et al. Bicarbonate kinetics and predicted energy expenditure in critically ill children. Am J Clin Nutr 2008;88(2):340-7.
36. Moore S, Spackman DH, Stein WH. Automatic recording apparatus for use in the chromatography of amino acids. Fed Proc 1958;17(4):1107-15.
37. 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.
38. 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.
39. Bier DM, Leake RD, Haymond MW, et al. Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. Diabetes 1977; 26(11):1016-23.
40. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22(9):1462-70.
41. Jani R, Molina M, Matsuda M, et al. Decreased non-insulin-dependent glucose clearance contributes to the rise in fasting plasma glucose in the nondiabetic range. Diabetes Care 2008;31(2):311-5.
42. Argaman Z, Young VR, Noviski N, et al. Arginine and nitric oxide metabolism in critically ill septic pediatric patients. Crit Care Med 2003;31(2):591-7.
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. Danadian K, Balasekaran G, Lewy V, Meza MP, Robertson R, Arslanian SA. Insulin sensitivity in African-American children with and without family history of type 2 diabetes. Diabetes Care 1999; 22(8):1325-9.
45. Castillo L, Yu YM, Marchini JS, et al. Phenylalanine and tyrosine kinetics in critically ill children with sepsis. Pediatr Res 1994;35(5):580-8.
46. van Waardenburg DA, Deutz NE, Hoos MB, et al. Assessment of whole body protein metabolism in critically ill children: can we use the [15N]glycine single oral dose method? Clin Nutr 2004;23(2): 153-60.
47. 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.
48. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006;124(3):471-84.
49. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab 2006;291(4):E745-54.
50. Orellana RA, Kimball SR, Suryawan A, et al. Insulin stimulates muscle protein synthesis in neonates during endotoxemia despite repression of translation initiation. Am J Physiol Endocrinol Metab 2007;292(2):E629-36.
51. Vary TC. Acute oral leucine administration stimulates protein synthesis during chronic sepsis through enhanced asso-

ciation of eukaryotic initiation factor 4G with eukaryotic initiation factor 4E in rats. J Nutr 2007;137(9):2074-9.
52. Charlton M, Nair KS. Protein metabolism in insulin-dependent diabetes mellitus. J Nutr 1998;128(2 Suppl):323S-7S.
53. Agus MS, Javid PJ, Piper HG, et al. The effect of insulin infusion upon protein metabolism in neonates on extracorporeal life support. Ann Surg 2006;244(4): 536-44.
54. Agus MS, Javid PJ, Ryan DP, Jaksic T. Intravenous insulin decreases protein breakdown in infants on extracorporeal membrane oxygenation. J Pediatr Surg 2004;39(6):839-44; discussion -44.
55. Tessari P, Inchiostro S, Biolo G, et al. Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. Evidence for distinct mechanisms in regulation of net amino acid deposition. J Clin Invest 1987;79(4): 1062-9.
56. Greenhaff PL, Karagounis LG, Peirce N, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol Endocrinol Metab 2008;295(3):E595-604.
57. Hu Z, Wang H, Lee IH, Du J, Mitch WE. Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. J Clin Invest 2009;119(10):3059-69.
58. Krebs M, Krssak M, Bernroider E, et al. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes 2002;51(3):599-605.
59. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in african-american children: decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes 2002;51(10):3014-9.
60. Moran A, Jacobs DR, Jr., Steinberger J, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999;48(10):2039-44.
61. Zauner A, Nimmerrichter P, Anderwald C, et al. Severity of insulin resistance in critically ill medical patients. Metabolism 2007;56(1):1-5.
62. Shangraw RE, Jahoor F, Miyoshi H, et al. Differentiation between septic and postburn insulin resistance. Metabolism 1989;38(10):983-9.
63. Chambrier C, Laville M, Rhzioual Berrada K, Odeon M, Bouletreau P, Beylot M. Insulin sensitivity of glucose and fat metabolism in severe sepsis. Clin Sci (Lond) 2000;99(4):321-8.
64. Whyte MB, Jackson NC, Shojaee-Moradie F, et al. Metabolic effects of intensive insulin therapy in critically ill patients. Am J Physiol Endocrinol Metab 2009;298(3):E697-705.
65. Thorell A, Rooyackers O, Myrenfors P, Soop M, Nygren J, Ljungqvist OH. Intensive insulin treatment in critically ill trauma patients normalizes glucose by reducing endogenous glucose production. J Clin Endocrinol Metab 2004; 89(11):5382-6.
66. Chevalier S, Burgess SC, Malloy CR, Gougeon R, Marliss EB, Morais JA. The greater contribution of gluconeogenesis to glucose production in obesity is related to increased whole-body protein catabolism. Diabetes 2006;55(3):675-81.
67. Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 2001;276(41):38052-60.
68. Tessari P, Inchiostro S, Biolo G, et al. Hyperaminoacidaemia reduces insulin-mediated glucose disposal in healthy man. Diabetologia 1985;28(11):870-2.

69. Schricker T, Carli F, Lattermann R, Wachter U, Georgieff M. Glucose infusion does not suppress increased lipolysis after abdominal surgery. Nutrition 2001; 17(2):85-90.

Chapter 8

Albumin synthesis rates in post-surgical infants and septic adolescents; influence of amino acids, energy, and insulin

Sascha CAT Verbruggen
Henk Schierbeek
Jorge Coss-Bu
Koen FM Joosten
Leticia Castillo
Johannes B van Goudoever

Submitted

Abstract

Background and aims

To investigate the effects of glucose, parenteral amino acids, and intravenous insulin on albumin synthesis rates in critically ill children.

Methods

Two studies were performed in 8 post-surgical infants (age 9.8 ± 1.9 months; weight 9.5 ± 1.1 kg) and 9 septic adolescents (age 15 ± 1 yr.; BMI 20 ± 4 $kg.m^{-2}$), respectively. All received a primed, constant, tracer infusion with [1-^{13}C]Leucine. The infants in study 1 were randomized to receive low (2.5 $mg.kg^{-1}.min^{-1}$) and standard (5.0 $mg.kg^{-1}.min^{-1}$) glucose intake in a crossover setting of two periods of 4 hours each. The adolescents in study 2 were randomized to receive total parenteral nutrition with standard (1.5 $g.kg^{-1}.day^{-1}$) and high (3.0 $g.kg^{-1}.day^{-1}$) amino acid intake in a two day crossover setting. On both study days, during the last 3 hours of the tracer study, they received insulin infused at 80 $mU{\cdot}m^{-2}{\cdot}min^{-1}$.

Results

The post-surgical infants and the septic adolescents were mildly hypoalbuminemic (~2.5 $g.dL^{-1}$) with high synthesis rates, which were not affected by different intakes of glucose, amino acids, or insulin infusion.

Conclusions

Albumin synthesis rates in hypoalbuminemic critically ill children appear to be upregulated through pathways other than nutrient signaling. In septic adolescents, albumin synthesis rates appear insulin resistant.

Introduction

Albumin is the most abundant protein in human plasma with a normal plasma concentration of around 4.0 g.dL^{-1}, while about 60% of the total albumin pool is located in the interstitial space[1]. Albumin holds several important functions, both in health as well as in critically ill patients. It is the main preserver of colloid oncotic pressure (~75%), it functions as an anticoagulant and anti-oxidant and it is an important binding transporter of metabolites and drugs[1, 2].

Critically ill patients are often hypoalbuminemic, primarily due to dilution and redistribution secondary to an altered vascular permeability[1]. In critically ill patients hypoalbuminemia has been documented as a marker for disease severity, nutritional status, prolonged ventilator support and prolonged length of stay[3, 4]. In critically ill adults and children, low albumin plasma levels (< 3.3 g.dL^{-1}) are inversely related to morbidity and mortality, where in adults each 1.0 g.dL^{-1} drop in serum albumin raised the odds of morbidity by 87% and mortality by 137%[3-5]. However, plasma concentrations are static measurements. Dynamic measurements by means of albumin synthesis rates actually show a consistent increase in critically ill adults[6, 7].

Despite the clear association between hypoalbuminemia and poor outcome, there is still a debate on the benefits and safety of intravenous albumin administration, partially due to the increased risk of escape in the extravascular space and inflammation[2, 5]. Therefore, stimulation of endogenous albumin synthesis seems an appealing alternative.

Albumin synthesis can be stimulated by an increase in energy (glucose and fat) but is particularly responsive to amino acid intake[8-10]. Hyperinsulinemia has shown to increase albumin synthesis as well, with an additive effect of increased amino acids in healthy adults[10]. This latter intervention is of particular interest as insulin is more frequently used to treat hyperglycemia in critically ill adults and children[11]. Moreover, the increase in synthesis rates in response to these interventions is immediate and fast. Albumin synthesis rates increased by 40% within 2 h following intravenous endotoxin in healthy volunteers[12]. Furthermore, nutritional supplementation[13, 14] and hyperinsulinemia[15] increased albumin synthesis rates, within 4, 6, and 3 h respectively.

The effect of various nutritional interventions on albumin synthesis rates have not been investigated in critically ill children, other than in premature infants[16]. Increasing amino acid availability resulted in higher albumin synthesis rates, although not as high as reached in utero[16]. Given the limited knowledge of albumin synthesis rates and the impact of nutrition in the critically ill pediatric population, we set out to ascertain these. We hypothesized that in critically ill children albumin synthesis rates are increased and responsive to nutrients and hyperinsulinemia. Therefore, our first objective was to quantify albumin synthesis rates in critically ill infants and adolescents. Our second

objective was to determine the impact of nutrients on albumin synthesis rates in these children, with special emphasis on parenteral glucose and amino acids. Our third objective was to determine whether additional hyperinsulinemia in combination with parenteral glucose and amino acid intake would increase albumin synthesis rates through a synergistic fashion. The here described studies were part of two larger studies aiming to investigate the effect of reduced glucose intake on glucose homeostasis and protein catabolism (Study 1) and the effect and interactions of increased amino acid intake and hyperinsulinemia on substrate metabolism and insulin resistance (Study 2).

Methods

Patients

Study 1 was a one day study with infants (0.5 – 1.0 y) admitted after surgical repair of non-syndromal craniosynostosis to the pediatric intensive care unit (PICU) at Erasmus MC – Sophia Children's Hospital in Rotterdam, The Netherlands. Study 2 was a two day study in adolescents (13 – 18 y) who were admitted with a diagnosis of severe sepsis or Systemic Inflammatory Response Syndrome (SIRS), as defined by the criteria of the First International Pediatric Sepsis Forum[17], to the PICU at Texas Children's Hospital, Houston, Texas. All patients had drawing and infusing catheters in place for clinical purposes. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded. The study protocols were approved, respectively by the Institutional Review Board of Erasmus Medical Center, Rotterdam, The Netherlands and by the Institutional Review Board of Baylor College of Medicine, Houston, Texas. Studies were carried out after written informed consent from the parents.

Study design and data collection

The experimental design of study 1 is shown in figure 1 and consisted of an 8 hr glucose infusion in a randomized, cross-over design, four hours with low (LG; 2.5 $mg.kg^{-1}.min^{-1}$) and four hours with standard (SG; 5.0 $mg.kg^{-1}.min^{-1}$) glucose intake rates. Patients were randomized for the order of glucose intake through a computer generated envelop. Laboratory personnel, the clinical team and investigators were blinded for glucose intake until after analyses were finished. Prior to the study, infants did not receive (par) enteral nutrition other than intravenous glucose infusion in the range of 4-6 $mg.kg^{-1}.min^{-1}$ as per standard care before start of the study. Eight hours after admission to the PICU and after obtaining baseline blood samples, the intravenous glucose intake as per standard care (4.0 – 6.0 $mg.kg^{-1}.min^{-1}$) was stopped and the study glucose intake started (t=0), after which the patients received a primed, continuous, 8 h intravenous tracer infusion with L-[1-^{13}C]leucine at 8 $\mu mol.kg^{-1}$ and 8 $\mu mol.kg^{-1}.h^{-1}$ respectively. Four

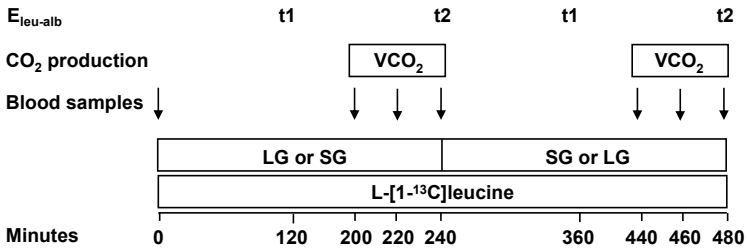


Figure 1; Experimental design of study 1 in 8 post-surgical infants admitted to the pediatric intensive care unit receiving only parenteral glucose as nutrition, LG = low glucose intake (2.5 $mg.kg^{-1}.min^{-1}$), SG = standard glucose (5.0 $mg.kg^{-1}.min^{-1}$), $E_{leuc-alb}$ = Enrichment of [1-13C]Leucine incorporated into albumin, VCO_2 = Carbon dioxide production

hours after start of the study (t=240) the study glucose was switched as per cross-over design (Figure 1).

Study 2 consisted of 2 study days in a randomized cross-over fashion, i.e. one with standard (SAA; 1.5 $g.kg^{-1}.day^{-1}$) and one with high (HAA; 3.0 $g.kg^{-1}.day^{-1}$) amino acid intake via total parenteral nutrition (TPN) (Figure 2a). Patients received full parenteral nutrition (Aminosyn (Hospira Inc. Lake Forest, IL.) or Clinisol (Baxter, Deerfield, IL)) for at least 24 hours before the start of the study. Both days consisted of two experimental periods, a basal and a period with insulin infusion (see subsequent description at the end of the paragraph) (Figure 2b). Amino acid intake was randomized by pharmacy through a computer generated envelop. Insulin infusion always followed a baseline period because of the expected wash-out effect of insulin on protein metabolism. Laboratory personnel were blinded for both the amino acid intake and the insulin allocation, while investigators were blinded for amino acid intake, but could not be blinded for insulin allocation due to the risk of hypoglycemia. Energy intake provided as parenteral glucose and lipids were prescribed by the clinical team according to standard care. The total energy intake supplied remained unchanged during both study days. After an adaptation period of at least twelve hours of the randomized TPN, the patients received a primed, continuous, 7 h infusion with L-[1-13C]leucine at 6 $\mu mol.kg^{-1}$ and 6 $\mu mol.kg^{-1}.h^{-1}$ respectively, of which the last three hours in combination with a hyperinsulinemic euglycemic clamp as previously described[18] (Figure 2b). Briefly, a 3 h infusion of insulin (Actrapid, Novo Nordisk Inc., Princeton, NJ), dissolved in sterile isotonic NaCl was started at 80 $mU.m^{-2}.min^{-1}$ in order to achieve both normoglycemia between 90-110 $mg.dL^{-1}$ and a plasma insulin concentration greater than 100 $\mu U.mL^{-1}$. During insulin infusion, small blood samples were obtained from the indwelling arterial catheter every 5 - 10 minutes to monitor whole blood glucose concentration, at the bedside with the aid of a Y.S.I. 2300 STAT Plus analyzer (YSI Life Sciences, Yellow Springs, OH). To maintain the plasma glucose between 90-110 $mg.dL^{-1}$ (5.0 – 6.1 mM) for the duration of the study, a 30% glucose solution was infused with a Harvard syringe pump (PHD 22/2000, Harvard apparatus, Holliston, MA)[18].

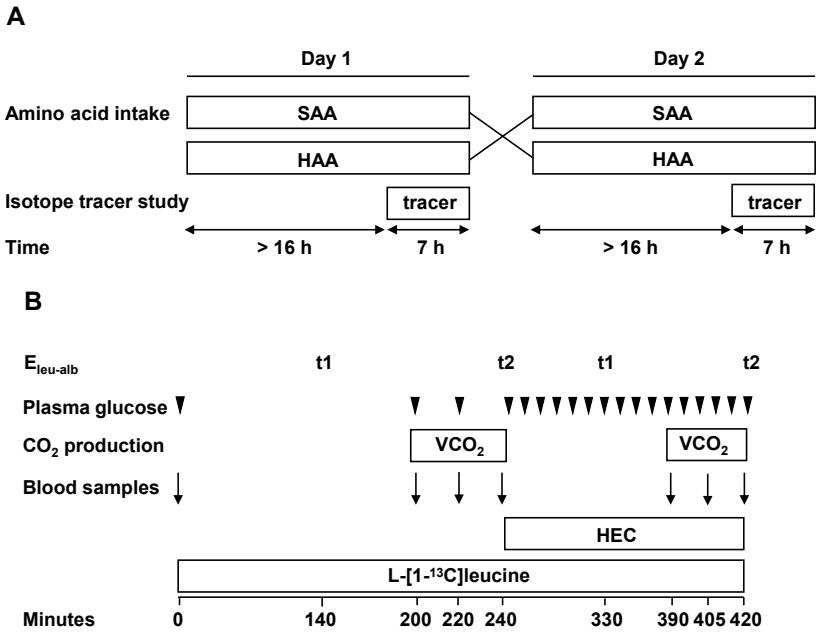


Figure 2; Experimental design of study 2 in 9 adolescents admitted to the pediatric intensive care unit receiving full parenteral nutrition with two different amino acid intakes at baseline and during hyperinsulinemia, Panel A Two day study protocol with two different amino acid intakes in a randomized cross-over fashion, Panel B Isotope tracer infusion on both days during baseline and during hyperinsulinemia, SAA = Standard amino acid intake (1.5 g.kg^{-1}.day^{-1}), HAA = High amino acid intake (3.0 g.kg^{-1}.day^{-1}), $E_{leuc-alb}$ = Enrichment of [1-^{13}C]Leucine incorporated into albumin, VCO_2 = Carbon dioxide production

Materials

L-[1-^{13}C]leucine (99 atom%) was purchased from Cambridge Isotope Laboratories (Andover, MA) and tested for sterility and pyrogenicity after they were compounded at the investigational pharmacy at Texas Children's Hospital or Erasmus MC – Sophia Children's Hospital.

Measurements and sample analysis

Basal metabolic rate was predicted using the Schofield equation[19]. All were assessed for severity of disease by the Pediatric Logistic Organ Dysfunction (PELOD) score[20] and the Pediatric Risk of Mortality III (PRISM III) score[21].

Blood samples were obtained from the arterial line at standard frequent intervals (Figure 1) and immediately centrifuged and frozen at -80°C until samples were analyzed. To isolate albumin from plasma, we used anti-human serum albumin affinity resin kits (Vivascience; Sartorius Group, Hannover, Germany). Enclosed spin columns were filled with 400 µL affinity resin and 25 µL of thawed plasma. The column was washed 3 times with a tris-buffer, and albumin was thereafter eluted from the affinity resin with 0.1 mol

glycine.L^{-1} (acidified to pH 2.5 with HCl). Eluted albumin was precipitated with 750 µL of 2 mol $HClO_4$.L^{-1}. A washing step was performed with 0.2 mol $HClO_4$.L^{-1} by resuspending and precipitating the pellet again. The protein pellet was then hydrolyzed in 140 µL of 6 mol HCl.L^{-1} for 22 h at 110°C. After hydrolyzation, the acid was evaporated by using a speedvac, after which the dried amino acids were dissolved in H_2O. Samples were derivatized using propylchloroformate (commercial kits: Phenomenex for hydrolysates, EZ:Faast, Bester BV, Amstelveen, the Netherlands) and measured in triplicate on a gas chromatograph–combustion–isotope ratio mass spectrometer (GC-C-IRMS; Delta XP, Thermo Electron, Bremen, Germany)[22]. As albumin precursor, we used plasma [1-^{13}C] α-ketoisocaproate (α-KIC; the keto-acid of leucine, a measure of intracellular leucine enrichment) enrichment at a plateau. Liver amino acyl-tRNA enrichment forms the true precursor, but its use requires tissue biopsies and technically demanding assays. Nevertheless, α-KIC enrichment adequately represents leucyl-tRNA enrichment and is valuable in this type of research[23, 24]. Plasma isotope enrichment of [1-^{13}C]α-KIC were, after derivatization to butyldimethyl-silylquinoxalinol derivatives, determined by gas chromatography mass spectrometry (GC-MS) as previously described[25]. Plasma albumin concentrations were routinely measured on a Hitachi 912 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Carbon dioxide production (VCO_2) was obtained with a respiratory profile monitor (Deltatrac™ I MBM-200, Datex Division Instrumentarium Corp. Finland / CO_2SMO Plus, Novametrix Medical System, Wallingford, CT, USA) during the last 30 minutes of each study period. To determine the enrichment of $^{13}CO_2$ in whole blood, 1 mL of perchloric acid 10% was added to 1.0 mL of whole blood in a vacutainer to release the CO_2. The $^{13}CO_2$ in gas was subsequently determined on gas combustion isotope ratio mass spectrometry (GC-IRMS)[26, 27].

Plasma glucose level > 110 mg.dL^{-1} (~ > 6.1 mmol.L^{-1}) were considered hyperglycemic and plasma albumin levels < 3.5 g.dL^{-1} were considered hypoalbuminemic.

Calculations

Whole body kinetics of leucine were calculated by conventional isotope dilution technique using a stochastic model during steady state plateau[28]. The rate of appearance (Ra) of unlabeled leucine can be derived from the plasma isotope enrichment calculated by:

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

where *i* is the infusion rate of [1-^{13}C]Leucine, E_{inf} is the tracer enrichment of the infusate and E_{pl} the tracer enrichment in plasma, respectively. At steady state plateau rate of appearance (Ra) is equal to rate of disappearance (Rd).

The fractional albumin synthesis rate (FSR) reflects the fraction of the intravascular albumin pool that is renewed per unit of time (%.d^{-1}) and can be calculated by using the following equation[16]:

$$FSR = (E_{leu\text{-}alb,t2} - E_{leu\text{-}alb,t1})/E_{\alpha\text{-}kic} \times (24 \times 60)/(t2 - t1) \times 100\% \quad (2)$$

where $E_{leu\text{-}alb}$ is the enrichment in mole percent excess (MPE.) of incorporated leucine in albumin at t1 and t2 (Figure 1 and 2), and $E_{\alpha\text{-}kic}$ is the mean enrichment in MPE of the precursor, ie, plasma α-KIC, at these time points in minutes.
The absolute albumin synthesis rate (ASR) represents the absolute amount of albumin that is produced per day (mg.kg^{-1}.d^{-1}), and it can be calculated by using the following equation[16]:

$$ASR = FSR \times C_{alb} \times vol_{bl} \times (1 - Ht) \times weight^{-1} \quad (3)$$

where C_{alb} is the plasma albumin concentration in gL^{-1}, vol_{bl} is the child's total blood volume in mL (assumed to be 75 and 70 mL/kg body wt for the infants and adolescents respectively), Ht stands for hematocrit and (1 – Ht) is the fraction of blood that is plasma.
We also calculated the contribution (%) of albumin ASR in relation to whole-body protein synthesis in percentage on the basis of measured leucine turnover data. To do so we needed to determine leucine oxidation and the non-oxidative leucine disposal (NOLD) representing leucine utilized into whole-body protein synthesis (in μmol.kg^{-1}.h^{-1}).
Leucine oxidation rates were calculated as follows;

$$\text{Leucine Oxidation} = VCO_2 \times (E^{13}CO_2/69.18)/[^{13}C]\alpha\text{-KIC} \quad (4)$$

NOLD is the leucine oxidation subtracted from the leucine rate of disappearance;

$$NOLD = Ra_{leu} - \text{Leucine Oxidation} \quad (5)$$

where 69.18 is the $^{13}CO_2$ refraction correction factor for critically ill children[29]. VCO_2 is measured in milliliters per minute and converted to millimoles per hour by multiplying by 60 min and dividing by 22.4, which is the number of 1 in 1 mole of an ideal gas at standard temperature and pressure to convert to milliliters per minute.
This allows us to calculate the contribution of albumin synthesis in ratio to the whole body protein synthesis according to the following equation[16]:

$$\text{Contribution} = [(ASR \times 0.104)/(NOLD \times 131.2 \times 24 \times 0.001)] \times 100\% \quad (6)$$

where 0.104 represents the fraction of leucine residues in albumin on a weight basis, 131.2 is the mole mass of leucine, and 24 and 0.001 convert to day and milligram, respectively.

Statistical analysis

A prospective power analysis on our previous data on albumin synthesis rates[16], revealed that 8 patients with complete data, would detect a difference of 20% (80% power, type I error of 5%) in synthesis rates. Data are presented as the mean ± standard deviation unless non-parametric, in which case they are presented as median and 95% CI. Due to the variation between the two studies, not only in nutritional and metabolic support, but moreover in age and diagnosis, statistical comparison would not be justified. Differences within study groups were tested with the repeated measurements ANOVA, after which a paired student's *t test* was used. For non-parametric data the Wilcoxon matched pairs or Mann Whitney was used. Statistical significance was considered at $p < 0.05$. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, La Jolla, CA., USA).

Results

Patients

The demographic characteristics of all patients are described in table 1.

Study 1. Study 1 included 8 post-surgical infants (age 0.8 ± 0.2 months; 9.5 ± 1.1 kg). All patients were hemodynamically stable without vasoactive drugs and all patients were breathing spontaneous with an inspiratory fraction of oxygen less than 0.6.

Table 1. Patient demographics of 8 infants and 9 adolescents admitted to the PICU*

	Study 1	Study 2	
Age (years)	0.8 ± 0.2	15.0 ± 1.2	
Gender (male:female)	6 : 2	3 : 6	
Tanner score		4.0 ± 0.9	
Body Mass Index ($kg.m^{-2}$)		20 ± 4	
Weight (kg.)	9.5 ± 1.1	49.1 ± 13.1	
Length (cm)	74.3 ± 3.0	154.3 ± 11.6	
		SAA [a]	HAA [b]
PICU LOS [c] (days)	1	5.9 ± 3.3	6.3 ± 3.6
C-Reactive Protein ($mg.dL^{-1}$)	2.4 ± 1.3	16.5 ± 9.4	15.1 ± 12.0
PELOD [d]	10 ± 9	9 ± 11	6 ± 7
PRISM III [e]	7 ± 4	10 ± 4	8 ± 4
Catecholamines (n=)	0	1	0
Glucocorticoids (n=)	0	4	3

* All values are mean ± SD, [a] SAA = standard amino acid intake ($1.5\ g.kg^{-1}.d^{-1}$), [b] HAA = high amino acid intake ($3.0\ g.kg^{-1}.d^{-1}$), [c] PICU LOS = Length of stay in the PICU at start of the study, [d] PELOD = Pediatric Logistic Organ Dysfunction [20], [e] PRISM III = Pediatric Risk of Mortality III [21]

Study 2. Study 2 included nine septic adolescents (age 15 ± 1 yr.; BMI 20 ± 4 kg.m^{-2}, Tanner-score 4.0 ± 0.9). All patients were mechanically ventilated and hemodynamically stable. One patient received epinephrine as vasopressor therapy and 4 patients received glucocorticoids as additional therapy (Table 1). One patient randomized to start first with the standard amino acid intake group (SAA) died the next day after the first study (SAA) was completed, therefore the high amino acid intake (HAA) part of the study could not be conducted. In a second patient a technical error occurred during the insulin infusion protocol on the study day with SAA intake. Therefore, complete two-day study data were available on 7 patients. Data available from the two incomplete studies were included according to intention-to-treat principle.

Nutritional interventions

The nutritional characteristics of all patients are described in table 2.

Study 1. The infants received glucose intake in two different amounts and did not receive any amino acids. As anticipated, the caloric intake was below the caloric requirements according to the Schofield equations[19], although significantly higher

Table 2. Nutritional parameters of 8 infants and 9 adolescents admitted to PICU*

	Study 1		Study 2			
	LG[a]	SG[b]	SAA[c]		HAA[d]	
			Base	Insulin	Base	Insulin
Schofield[e] (kcal.kg^{-1}.day^{-1})	53.7 ± 5.3		29.3 ± 6.0		29.3 ± 6.0	
Caloric intake (kcal.kg^{-1}.day^{-1})	12.7 ± 0.2	25.3 ± 0.5 ^	32.7 ± 10.0	41.8 ± 8.4	37.8 ± 9.9	43.0 ± 14.3
Caloric intake (% of Schofield)	24 ± 1	47 ± 2 ^	115 ± 39	147 ± 44	136 ± 31	156 ± 55
Glucose intake (mg.kg^{-1}.min^{-1})	2.6 ± 0.1	5.2 ± 0.1 ^	4.5 ± 1.7	6.2 ± 1.9	4.6 ± 1.5	5.6 ± 2.2
Protein intake (gr.kg^{-1}.day^{-1})	0	0	1.5 ± 0.2	1.5 ± 0.2	2.8 ± 0.4‡	2.8 ± 0.4‡
Glucose calories (kcal.kg^{-1}.day^{-1})	12.7 ± 0.2	25.3 ± 0.5 ^	21.8± 8.1	30.9 ± 8.9	22.4 ± 7.6	27.6 ± 10.8
Amino acid calories (kcal.kg^{-1}.day^{-1})	0	0	5.8 ± 0.8	5.8 ± 0.8	11.2 ± 1.4 ‡	11.2 ± 1.4 ‡
Lipid calories (kcal.kg^{-1}.day^{-1})	0	0	5.7 ± 3.9	5.7 ± 3.9	4.7 ± 3.5	4.7 ± 3.5
Glucose plasma level (mg.dL^{-1})	105 ± 10	133 ± 30 ^	168 ± 58	98 ± 6 †	172 ± 62	101 ± 15†
Insulin plasma levels (µU.mL^{-1})	54 (19 -159)	73 (24 – 186)	32 (17 – 122)	144 (94 – 2385)†	51 (29 – 153)	168 (93 – 2239)†

* All values are mean ± SD, [a] LG = low glucose (2.5 mg.kg^{-1}.min^{-1}), [b] SG = Standard glucose (5.0 mg.kg^{-1}.min^{-1}), [c] SAA = standard amino acid intake (1.5 g.kg^{-1}.d^{-1}), [d] HAA = high amino acid intake (3.0 g.kg^{-1}.d^{-1}), [e] Resting Energy Expenditure according to Schofield equations [19], ^ LG vs. SG, $p < .05$, † Base vs. Insulin $p < .05$, ‡ SAA vs. HAA, $p < .05$

during SG. During LG the infants were normoglycemic, and during SG glucose levels were higher and hyperglycemic, while the insulin plasma levels did not significantly differ.

Study 2. The adolescents received full parenteral nutrition in two different amounts of amino acids (Table 2). The caloric intake was adequate or above requirements according to the Schofield equations[19] (Table 2), but did not significantly differ between protocols. During baseline the adolescents were hyperglycemic, while during insulin infusion they were normoglycemic and had significantly higher insulin concentrations (Table 2).

Albumin synthesis rates

All patients were hypoalbuminemic (Table 3).

Study 1. Albumin synthesis rates, non-oxidative leucine disposal (NOLD), and the contribution of albumin synthesized in relation to the whole body protein synthesis were not affected with different glucose intakes (Figure 3, Table 3).

Study 2. Neither an increased amino acid intake nor the supraphysiological insulin concentrations affected albumin synthesis rates. NOLD was not significantly affected by the different amino acid intakes, but was lower ($p < .05$) during insulin infusion in the adolescents (Table 3). The contribution of albumin synthesized in relation to the whole body protein synthesis was comparable between protocols and not affected by either intervention (Figure 3, Table 3).

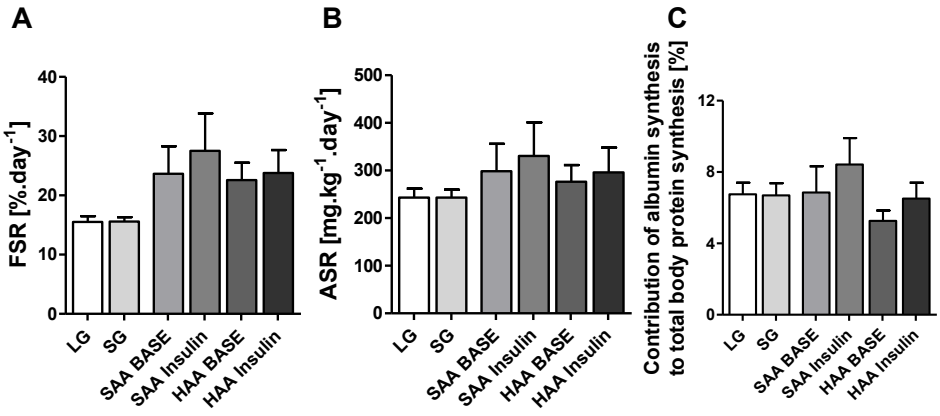


Figure 3; Albumin synthesis in 8 infants and 9 adolescents receiving various nutritional support and insulin, Panel A Albumin fractional synthesis rate (%.day^{-1}), Panel B Albumin absolute synthesis rate (mg.kg^{-1}.day^{-1}), Panel C Contribution of albumin synthesis as ratio to total whole body protein synthesis per day LG = low glucose intake (2.5 mg.kg^{-1}.min^{-1}), SG = standard glucose (5.0 mg.kg^{-1}.min^{-1}), SAA = Standard amino acid intake (1.5 g.kg^{-1}.day^{-1}), HAA = High amino acid intake (3.0 g.kg^{-1}.day^{-1}), Base = during baseline, Insulin = during insulin infusion (80 mU.m^{-2}.min^{-1}).

Table 3. Albumin synthesis of 8 infants and 9 adolescents admitted to the PICU*

	Study 1		Study 2			
	LG [a]	SG [b]	SAA [c]		HAA [d]	
			Base	Insulin	Base	Insulin
Albumin concentration ($g.dL^{-1}$)	2.6 ± 0.4		2.6 ± 0.6		2.5 ± 0.3	
FSR [e] ($\%.d^{-1}$)	16.3 ± 3.2	16.0 ± 2.2	23.7 ± 13.8	27.5 ± 19.0	22.6 ± 8.3	23.8 ± 11.0
ASR [f] ($mg.kg^{-1}.day^{-1}$)	243 ± 45	243 ± 47	299 ± 172	330 ± 211	276 ± 99	296 ± 148
Non-oxidative leucine disposal ($\mu mol.kg^{-1}.h^{-1}$)	122 ± 14	124 ± 20	153 ± 36	128 ± 38 †	173 ± 31 #	149 ± 38 †‡
Contribution to total protein synthesis (%)	6.7 ± 1.9	6.7 ± 1.9	6.9 ± 4.4	8.4 ± 4.4	5.3 ± 1.7	6.5 ± 2.5

* All values are mean ± SD, , [a] LG = low glucose (2.5 $mg.kg^{-1}.min^{-1}$), [b] SG = Standard glucose (5.0 $mg.kg^{-1}.min^{-1}$), [c] SAA = standard amino acid intake (1.5 $g.kg^{-1}.d^{-1}$), [d] HAA = high amino acid intake (3.0 $g.kg^{-1}.d^{-1}$), [e] FSR = fractional synthesis rate, [f] ASR = absolute synthesis rate; † Base vs. Insulin; $p < .05$, ‡ SAA vs. HAA; $p < .05$, # SAA vs. HAA, $p = .05$

Discussion

Our study provides insight in albumin synthesis rates in critically ill children of two different age groups. The data obtained in our study are consistent with previous observations in critically ill adults that, although in a hypoalbuminemic condition, albumin synthesis rates were high during inflammation and metabolic stress. Nutritional interventions with various levels of glucose and amino acid intake, and insulin infusion did not further stimulate albumin synthesis rates, nor did they increase the contribution of albumin to whole body protein synthesized.

It has been shown previously that fractional synthesis rates are higher in neonates (12.5-23%)[16, 22] and infants (15-20%)[30, 31] than healthy adults (6-15%)[7-10, 14, 32, 33]. This is however not reflected in our study, where increased synthesis rates were more obvious in the septic adolescents. The synthesis rates measured in our infants were consistent with those measured in age-related peers[30, 31]. The synthesis rates in the adolescents were even slightly higher than those in (critically) ill adults (11-18%)[7, 34-37]. These relative high synthesis rates might be explained by their condition, as an inflammatory and catabolic insult, which usually follows after trauma, surgery or infection, is a strong stimulant for albumin synthesis in patients[12]. These findings suggest that the adolescents were in a higher inflammatory and catabolic state, compared to the infants, explaining their relative high synthesis rates for their age. The latter is supported with a higher leucine flux in the adolescents (*data not shown*), an indicator for amino acid turnover as a derivative measure of catabolism. Furthermore, the C-reactive protein, a hepatic acute phase protein, was substantially higher in the septic adolescents.

In contrast to our hypothesis and data in healthy adults and neonates[9, 14, 16], increasing both parenteral energy (glucose) as well as amino acid intake did not affect synthesis rates. Although an unexpected finding at first, it is nevertheless consistent with pub-

lished data in healthy[38] as well as post-surgical[37] adults receiving intravenous nutrients. Albumin synthesis rates are less responsive to parenteral than enteral nutrients[39, 40]. Parenteral nutrition bypasses the splanchnic uptake and amino acids are presented to the liver through the portal venous circulation in lower concentrations[41]. Studies in piglets suggest that after enteral feeding, portal rather than arterial amino acids are preferentially used for hepatic protein synthesis[41]. Therefore, the parenteral route of nutrient supply in our children might be responsible for the lack of effect of our nutritional interventions, stressing the need for early enteral nutrition in critically ill children even more.

The lack of nutritional effect could also have been caused by the medical or surgical condition our patients were in. In health, the major regulator of (muscle and hepatic) protein synthesis is increased amino acid availability, since amino acids themselves modulate cellular processes leading to protein synthesis via enhanced translation initiation as well as through promoting translation elongation[42, 43]. Under conditions of inflammation and cytokine release, muscle protein synthesis is inhibited and less responsive to amino acid supply, while hepatic synthesis of immune/inflammatory proteins is greatly increased[44-46]. An increased supply of amino acids moderately attenuates the inhibitory response of inflammation on muscle protein synthesis[47]. In contrast, a pathway other than nutrient signaling is responsible for the increased hepatic protein synthesis during an inflammatory insult[48]. Our study now shows that albumin synthesis, already amplified in our patients, was also not further affected with changes in nutrient supply.

Of further interest, administration of insulin, a strong anabolic hormone, previously reported to stimulate albumin synthesis[15, 49], did not have any effect. Insulin decreased whole body protein synthesis, indicated by the NOLD, even when a high amino acid intake was provided. Lang and coworkers[50] reported that insulin failed to stimulate protein synthesis in an animal model of sepsis via a defect in insulin signaling to a step in translation initiation. Inflammation induces insulin resistance to protein synthesis, which might explain why hyperinsulinemia in our septic adolescents did not increase albumin synthesis rates.

There are some limitations to our study which need to be taken into account, some of them inherent to studying critically ill children. As mentioned in the statistical description, statistical comparison between the two protocols was not justified due to the wide variations in between the two groups. Furthermore, absolute albumin synthesis rates are calculated with albumin plasma levels. However, as we mentioned in the introduction, hypoalbuminemia is primarily caused by dilution and redistribution secondary to an altered vascular permeability. Therefore, we potentially underestimated the absolute synthesis rates as our study was not able to correct for dilution and redistribution. However, the data within the groups are consistent and justify our conclusions that

synthesis rates were high, and unresponsive to change in parenteral nutrients as well as hyperinsulinemia. We further acknowledge that we only performed short term nutritional changes in our study. However, as has been shown in previous studies, albumin synthesis rates can react acute and fast to these interventions[12-15]. Finally, we acknowledge that 4 septic adolescents received glucocorticoids as adjuvant therapy. Glucocorticoids impair protein anabolism, also in the pediatric population[51], and are capable of obstructing the anabolic effects of insulin[52]. However, the catabolic properties are exerted primarily through increased proteolysis, more than suppression of protein synthesis[51]. We did not find a difference in synthesis rates or effect of insulin therapy in the 4 adolescents who received glucocorticoids, although our study was not powered to discover such an effect.

Conclusion

Although mildly hypoalbuminemic, albumin synthesis rates are increased in post-surgical infants and even more in septic adolescents. Synthesis rates did not respond to short term changes in parenteral intakes of glucose, amino acids, and insulin. The data from our study, although the first in the critically ill pediatric population, confirm previous observations that the low plasma albumin levels are not due to decreased synthesis rates. Furthermore, critical illness appears to regulate albumin synthesis rates through other pathways than nutritional signaling, and induces insulin resistance to protein synthesis.

Acknowledgements

Our gratitude goes out to the patients and their families at Texas children's hospital and Erasmus MC Sophia Children's hospital for their selfless contribution to this study. We thank the nursing staff, Gardi Minderman-Voortman and Kristien Dorst for their contribution.

References

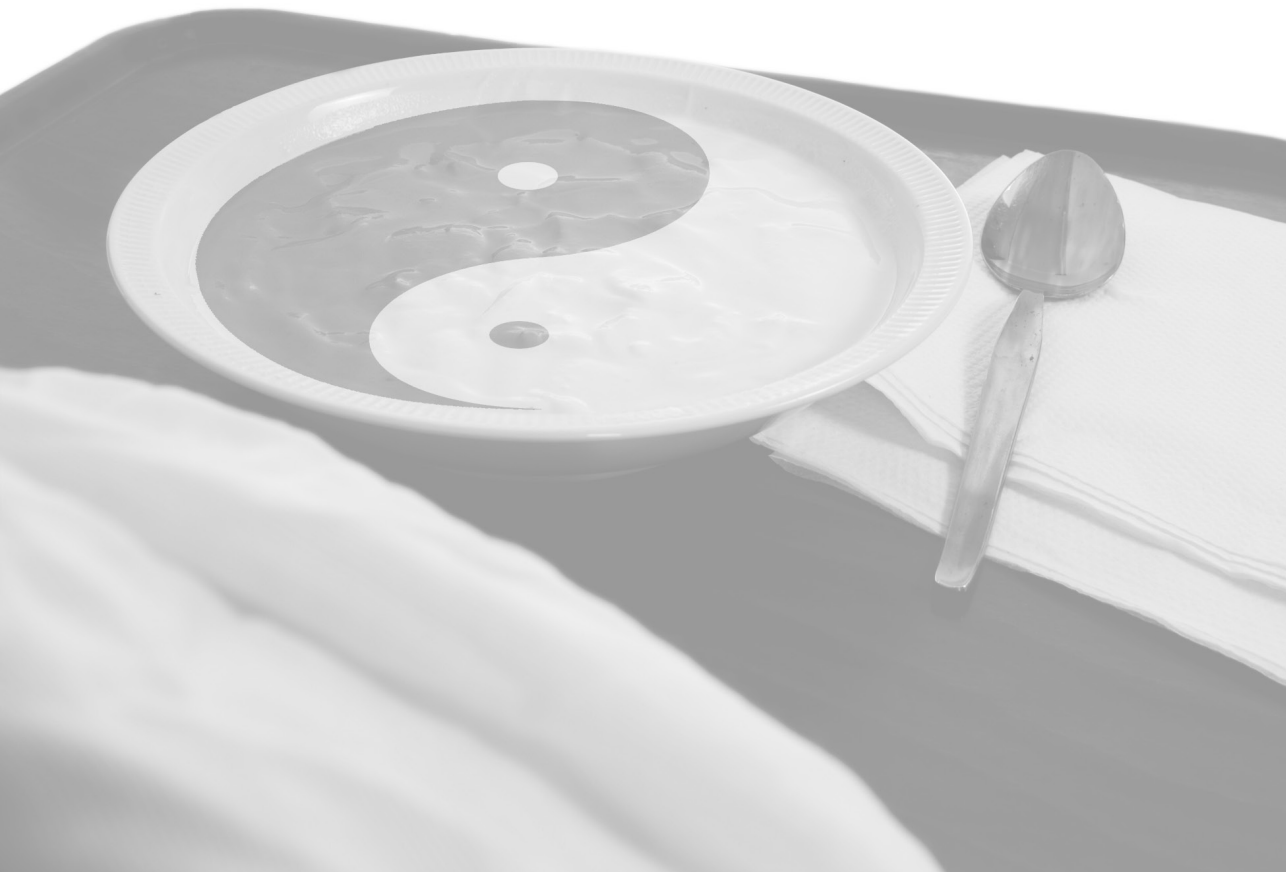
1. Margarson MP, Soni N. Serum albumin: touchstone or totem? Anaesthesia 1998; 53(8):789-803.
2. Quinlan GJ, Martin GS, Evans TW. Albumin: biochemical properties and therapeutic potential. Hepatology 2005; 41(6):1211-9.
3. Horowitz IN, Tai K. Hypoalbuminemia in critically ill children. Arch Pediatr Adolesc Med 2007;161(11):1048-52.
4. Sung J, Bochicchio GV, Joshi M, et al. Admission serum albumin is predicitve of outcome in critically ill trauma patients. Am Surg 2004;70(12):1099-102.
5. Vincent JL, Dubois MJ, Navickis RJ, Wilkes MM. Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. Ann Surg 2003; 237(3):319-34.
6. Ruot B, Breuille D, Rambourdin F, Bayle G, Capitan P, Obled C. Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. Am J Physiol Endocrinol Metab 2000;279(2):E244-51.
7. Mansoor O, Cayol M, Gachon P, et al. Albumin and fibrinogen syntheses increase while muscle protein synthesis decreases in head-injured patients. Am J Physiol 1997;273(5 Pt 1):E898-902.
8. Caso G, Feiner J, Mileva I, et al. Response of albumin synthesis to oral nutrients in young and elderly subjects. Am J Clin Nutr 2007;85(2):446-51.
9. Thalacker-Mercer AE, Johnson CA, Yarasheski KE, Carnell NS, Campbell WW. Nutrient ingestion, protein intake, and sex, but not age, affect the albumin synthesis rate in humans. J Nutr 2007; 137(7):1734-40.
10. Volpi E, Lucidi P, Cruciani G, et al. Contribution of amino acids and insulin to protein anabolism during meal absorption. Diabetes 1996;45(9):1245-52.
11. Verbruggen SC, Joosten KF, Castillo L, van Goudoever JB. Insulin therapy in the pediatric intensive care unit. Clin Nutr 2007;26(6):677-90.
12. Barle H, Januszkiewicz A, Hallstrom L, et al. Albumin synthesis in humans increases immediately following the administration of endotoxin. Clin Sci (Lond) 2002;103(5):525-31.
13. Pupim LB, Flakoll PJ, Ikizler TA. Nutritional supplementation acutely increases albumin fractional synthetic rate in chronic hemodialysis patients. J Am Soc Nephrol 2004;15(7):1920-6.
14. De Feo P, Horber FF, Haymond MW. Meal stimulation of albumin synthesis: a significant contributor to whole body protein synthesis in humans. Am J Physiol 1992; 263(4 Pt 1):E794-9.
15. Tessari P, Kiwanuka E, Millioni R, et al. Albumin and fibrinogen synthesis and insulin effect in type 2 diabetic patients with normoalbuminuria. Diabetes Care 2006;29(2):323-8.
16. van den Akker CH, te Braake FW, Schierbeek H, 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.
17. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005;6(1):2-8.
18. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237(3):E214-23.
19. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39 Suppl 1:5-41.

20. Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003;362(9379):192-7.
21. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24(5):743-52.
22. Bunt JE, Rietveld T, Schierbeek H, Wattimena JL, Zimmermann LJ, van Goudoever JB. Albumin synthesis in preterm infants on the first day of life studied with [1-13C]leucine. Am J Physiol Gastrointest Liver Physiol 2007;292(4):G1157-61.
23. Ahlman B, Charlton M, Fu A, Berg C, O'Brien P, Nair KS. Insulin's effect on synthesis rates of liver proteins. A swine model comparing various precursors of protein synthesis. Diabetes 2001;50(5): 947-54.
24. Barazzoni R, Meek SE, Ekberg K, Wahren J, Nair KS. Arterial KIC as marker of liver and muscle intracellular leucine pools in healthy and type 1 diabetic humans. Am J Physiol 1999;277(2 Pt 1):E238-44.
25. Matthews DE, Schwarz HP, Yang RD, Motil KJ, Young VR, Bier DM. 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.
26. 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.
27. Spear ML, Darmaun D, Sager BK, Parsons WR, Haymond MW. Use of [13C] bicarbonate infusion for measurement of CO2 production. Am J Physiol 1995; 268(6 Pt 1):E1123-7.
28. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C] leucine. Am J Physiol 1980;238(5):E473-9.
29. Sy J, Gourishankar A, Gordon WE, et al. Bicarbonate kinetics and predicted energy expenditure in critically ill children. Am J Clin Nutr 2008;88(2):340-7.
30. Jahoor F, Abramson S, Heird WC. The protein metabolic response to HIV infection in young children. Am J Clin Nutr 2003;78(1):182-9.
31. Morlese JF, Forrester T, Badaloo A, Del Rosario M, Frazer M, Jahoor F. Albumin kinetics in edematous and nonedematous protein-energy malnourished children. Am J Clin Nutr 1996;64(6):952-9.
32. Barle H, Nyberg B, Essen P, et al. The synthesis rates of total liver protein and plasma albumin determined simultaneously in vivo in humans. Hepatology 1997;25(1):154-8.
33. Ballmer PE, McNurlan MA, Milne E, et al. Measurement of albumin synthesis in humans: a new approach employing stable isotopes. Am J Physiol 1990;259(6 Pt 1):E797-803.
34. Dahn MS, Mitchell RA, Lange MP, Smith S, Jacobs LA. Hepatic metabolic response to injury and sepsis. Surgery 1995;117(5):520-30.
35. Essen P, McNurlan MA, Gamrin L, et al. Tissue protein synthesis rates in critically ill patients. Crit Care Med 1998;26(1): 92-100.
36. Barle H, Gamrin L, Essen P, McNurlan MA, Garlick PJ, Wernerman J. Growth hormone does not affect albumin synthesis in the critically ill. Intensive Care Med 2001;27(5):836-43.
37. Barle H, Nyberg B, Andersson K, et al. The effects of short-term parenteral nutrition on human liver protein and amino acid metabolism during laparoscopic surgery. JPEN J Parenter Enteral Nutr 1997;21(6):330-5.
38. Ballmer PE, McNurlan MA, Essen P, Anderson SE, Garlick PJ. Albumin synthesis

rates measured with [2H5ring]phenylalanine are not responsive to short-term intravenous nutrients in healthy humans. J Nutr 1995;125(3):512-9.
39. Bower RH, Talamini MA, Sax HC, Hamilton F, Fischer JE. Postoperative enteral vs parenteral nutrition. A randomized controlled trial. Arch Surg 1986;121(9): 1040-5.
40. Tsujinaka T, Morimoto T, Ogawa A, et al. Effect of parenteral and enteral nutrition on hepatic albumin synthesis in rats. Nutrition 1999;15(1):18-22.
41. Stoll B, Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 2006; 84 Suppl:E60-72.
42. Wilson FA, Suryawan A, Orellana RA, Gazzaneo MC, Nguyen HV, Davis TA. Differential effects of long-term leucine infusion on tissue protein synthesis in neonatal pigs. Amino Acids 2010.
43. Fujita S, Dreyer HC, Drummond MJ, et al. Nutrient signalling in the regulation of human muscle protein synthesis. J Physiol 2007;582(Pt 2):813-23.
44. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340(6): 448-54.
45. Lang CH, Frost RA. Sepsis-induced suppression of skeletal muscle translation initiation mediated by tumor necrosis factor alpha. Metabolism 2007;56(1):49-57.
46. 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.
47. Orellana RA, Kimball SR, Suryawan A, et al. Insulin stimulates muscle protein synthesis in neonates during endotoxemia despite repression of translation initiation. Am J Physiol Endocrinol Metab 2007;292(2):E629-36.
48. Morlese JF, Forrester T, Jahoor F. Acute-phase protein response to infection in severe malnutrition. Am J Physiol 1998; 275(1 Pt 1):E112-7.
49. De Feo P, Volpi E, Lucidi P, et al. Physiological increments in plasma insulin concentrations have selective and different effects on synthesis of hepatic proteins in normal humans. Diabetes 1993;42(7):995-1002.
50. Lang CH, Frost RA, Vary TC. Regulation of muscle protein synthesis during sepsis and inflammation. Am J Physiol Endocrinol Metab 2007;293(2):E453-9.
51. Van Goudoever JB, Wattimena JD, Carnielli VP, Sulkers EJ, Degenhart HJ, Sauer PJ. Effect of dexamethasone on protein metabolism in infants with bronchopulmonary dysplasia. J Pediatr 1994;124(1): 112-8.
52. van Beek RH, Zimmermann LJ, Vergunst van Keulen JG, et al. Leucine kinetics during simultaneously administered insulin and dexamethasone in preterm infants with severe lung disease. Pediatr Res 2001;49(3):373-8.

Part IV

General discussion & summary



Chapter 9

General Discussion

As summarized in chapter 1, critically ill children require specific nutritional and metabolic support to counteract ongoing catabolism, regain control of glucose homeostasis, and supply sufficient substrates to help recover from their disease process, while maintaining growth and development[1-5]. Nonetheless, it remains a poorly investigated area with little evidence, and nutritional goals[6] as well as glycemic control[7] are still not met in the Pediatric Intensive Care Unit (PICU). This leads to increased morbidity and mortality in critically ill children[5, 8-13].

This thesis aimed to contribute to the development of future guidelines for the nutritional and metabolic support in critically ill children of all age groups.

It was clear from the start that we needed to focus on the deficiencies of the support currently provided and the impact this has on interactions between energy (glucose and insulin metabolism) and protein (i.e. amino acids) in critically ill children. The main hypotheses we set out to investigate were;

- Hyperglycemia in critically ill children is caused by insulin resistance and can be treated according to age-related glucose control protocols.
- Glucose and protein/amino acids are currently provided in inadequate amounts to critically ill children of different age groups.
- Insulin exerts its anabolic properties also in critically ill children and can be used as an additive tool to deflect catabolism.

Role of insulin therapy on the PICU

Sir Frederick G. Banting received the Nobel prize for the discovery of insulin and during his acceptance speech in 1925 he stated; *"Insulin is not a cure for diabetes, it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life"*.

The story on adult ICU's so far...

In 2006, we developed the hypotheses on which the present thesis is based. By then it had become clear that critical illness hyperglycemia, or "diabetes of the ill", was not merely an adaptive stress response, as was thought before. In 2001, a landmark paper by van den Berghe and colleagues showed that a tight glucose regimen with insulin to maintain blood glucose concentrations at 4.5 – 6.1 mmol.L^{-1} reduced the morbidity and mortality in a surgical intensive care unit (ICU)[14]. Since a subgroup analysis showed the greatest gain was achieved in patients with sepsis and multisystem organ failure, medical ICU patients were expected to benefit as well[15]. In 2004, the Surviving Sepsis Campaign recommended glucose control for all patients with sepsis and explicitly

stated; “There is no reason to think that these data are not generalizable to all severely septic patients”[16]. Because few interventions in critically ill patients improved outcome to this extent, the results were enthusiastically embraced, and many ICU’s, both surgical and medical, sought to institute intensive glucose control measures[17-19].
Also on our pediatric ICU (PICU), a step-wise nurse driven tight glucose control protocol to treat hyperglycemia was initiated in 2006. At that time, a subsequent large trial by van den Berghe and colleagues was presented. It was a similar study on a medical ICU and showed comparable beneficial organ protective results, although less striking as in the surgical setting, revealing no mortality benefit from intensive glucose control, except in a subgroup requiring critical care for 3 or more days[20]. This was an indication that tight glucose control, which was thought to become one of the most successful intensive care treatments, was more complex than anticipated. Since then, a number of subsequent studies reported various results, some were terminated prematurely due to the unacceptable high incidence of hypoglycemia[21], others reported disparate results[22, 23]. A large meta-analysis which includes a recent international multi-center trial (NICE-SUGAR), the largest to date, showed that intensive insulin therapy had no effect on overall risk of death, although it suggested that surgical patients do benefit from tight glucose control (Figure 1)[45].
Various arguments can be given for these differences in outcome; variation in population, blood glucose target ranges, accuracy of insulin infusion and glucose monitoring protocols, the form of (parenteral) nutrition, and methods used to measure blood glucose levels[46]. Notwithstanding the ongoing debate, it has become undeniable that both low as well as high blood glucose levels worsen outcome[47], and several medical advisory boards recommend routine glycemic control as standard care[48, 49]. The debate regarding the tight glucose control in adults is targeted on two major issues; 1) uncertainty about the optimal glucose target ranges, and 2) the unacceptable high risk of developing hypoglycemia.

What to expect from “tight glucose control” on the PICU?

These two issues are of even greater concern in the pediatric population. So where do we stand from a pediatric point of view?
In **chapter 2** we have given a comprehensive overview of the causes and consequences of hyperglycemia and the potential benefits of insulin therapy in critically ill children. Similar to the pathofysiology in adults, hyperglycemia in critically ill children is primarily caused by insulin resistance, as we have demonstrated in **chapter 7** in septic adolescents, by means of a Hyperinsulinemic Euglycemic Clamp (HEC), which is considered the “gold standard” to determine insulin resistance. Even the post-surgical infants described in **chapter 4** appeared in an insulin resistant condition, although their hyperglycemic state was shown to be aggravated predominantly

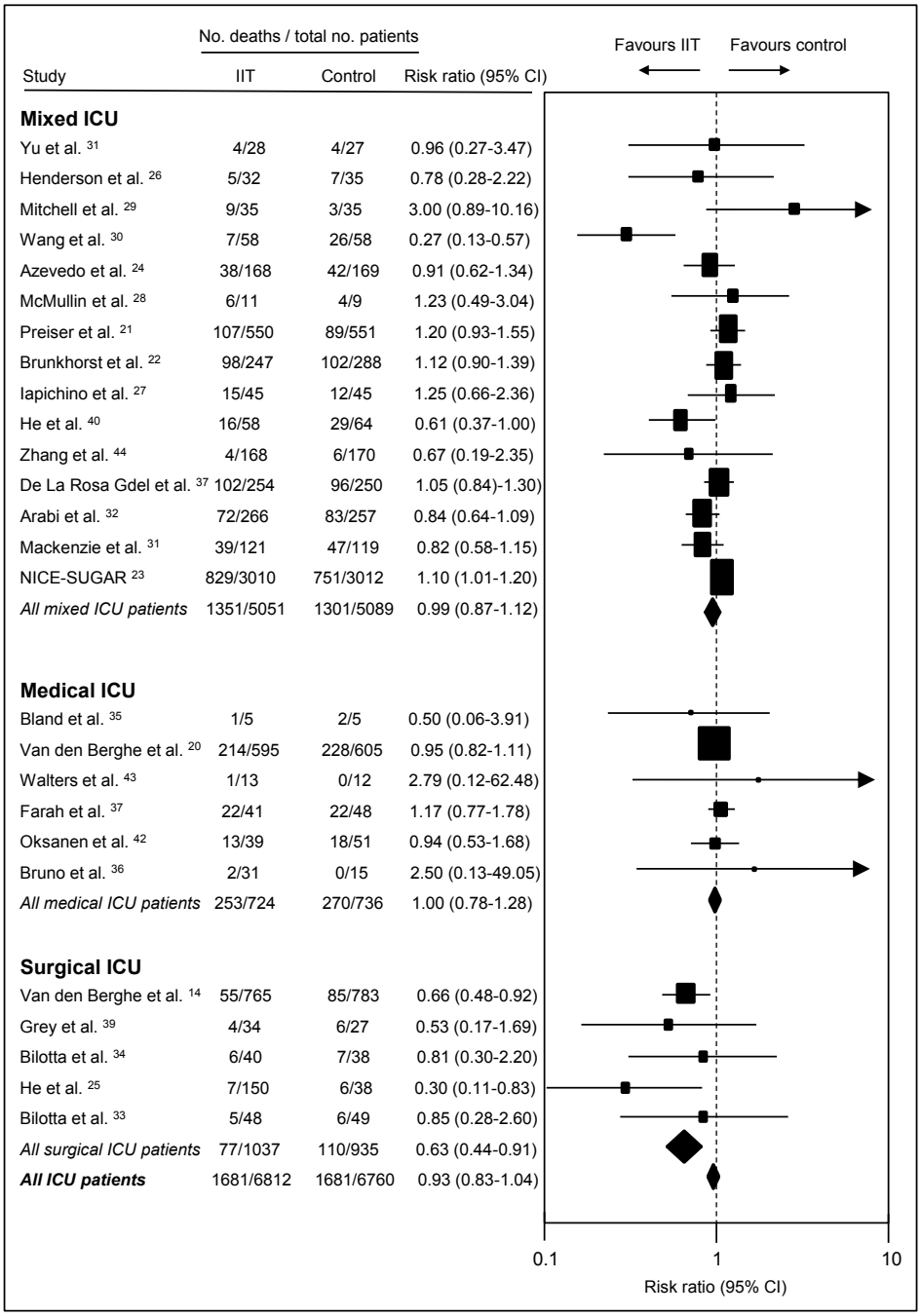


Figure 1. Meta-analysis from adult studies showing the risk-ratios of mortality in clinical trials comparing intensive insulin therapy (IIT) to conventional glycemic control stratified by type of ICU.
CI; confidence interval. Adapted from reference *45*.

through their exogenous glucose intake, provided according to age-specific recommendations[50, 51].

In **chapter 2** we review several studies that associated hyperglycemia in the pediatric intensive care population with increased morbidity; increased rate of infections, prolonged mechanical ventilation and stay on the PICU and in the hospital, and worsened neurological outcome[9, 12, 52-57].

Hyperglycemia, through intracellular glucose overload, causes more excessive glycolysis and oxidative phosphorylation resulting in an increased generation of oxygen radicals such as peroxynitrite and superoxide in these cells. These reactive oxygen species cause mitochondrial dysfunction and disturbed energy metabolism, which leads to increased apoptosis and might explain cellular and organ system failure in critically ill patients[58-60]. To date, no studies have explored the (intra)cellular mechanisms of hyperglycemia in children. However, it is not likely that there will be substantial differences from adults, and recently two small mechanistic studies of insulin therapy in the pediatric population have been reported. Intensive insulin therapy in burned children has shown to improve insulin sensitivity, possibly through improved mitochondrial function and oxidative capacity of fatty acids[61]. Additionally, it has been shown that in infants undergoing congenital heart surgery prevention of hyperglycemia, independent of insulin signaling pathways, protects myocardial cells and function, and reduces the inflammatory response[62]. Yet, these mechanistic benefits in critically ill children do not guarantee beneficial clinical implications.

A large proportion of **chapter 2** consists of the review we published in 2007, in which we could only report on smaller trials with insulin therapy, showing an improved glycemic control, protein and lipid metabolism, caloric intake, and inflammatory response[63-68]. Since then, two large outcome studies of tight glucose protocols specifically designed for the pediatric population have been published. A study in hyperglycemic very low birth weight (VLBW) neonates was discontinued prematurely because of an increased incidence of hypoglycemia (29% vs. 17%) and parenchymal abnormalities detected with cranial ultrasound in those treated with insulin[69]. A large trial to study the outcome of tight glucose control in critically ill children (< 16 years old) started recently, and the results are eagerly anticipated for[70]. In the mean time, available data are limited to one large prospective randomized trial, which enrolled 700 critically ill children, and showed that tight glucose control with intensive insulin therapy shortened duration of PICU stay (5.5 vs. 6.2 days) and decreased mortality (3% vs. 6%) as compared with conventional treatment[71]. However, hypoglycemia (≤ 2.2 mmol.L^{-1}) occurred in 25% of the children and severe hypoglycemia (≤ 1.7 mmol.L^{-1}) occurred in 5% of the children. Although no severe clinical symptoms were reported, long-term follow up and neurocognitive development of these children are eagerly awaited for.

Is insulin therapy in critically ill children dangerous?

There are several explanations for the high incidence of hypoglycemia as we summarize in **chapter 2**. First, target ranges in the study by Vlasselaers and colleagues[71] were low (2.8–4.4 mmol.L^{-1} for infants and 3.9–5.6 mmol.L^{-1} for children), and as the ideal target range of tight glucose regimens still needs to be established less strict protocols might be sensible. We currently treat hyperglycemia with help of a glucose target range of 4.0-8.0 mmol.L^{-1}. Second, the starting dose of insulin was high 0.1-0.2 IU.kg^{-1}.h^{-1}, whereas a lower starting dose ranging from 0.02-0.05 IU.kg^{-1}.h^{-1} appears to be effective and safe[72]. Third, the insulin protocol was adjusted by nurses based on their experience. Simple model-based, computer-assisted or nurse driven protocols have been reported in critically ill adults to increase safety[73-78]. There have been a few studies which report tight glucose control protocols with a very low incidence of hypoglycemia (0-4%), one of which was the step-wise nurse driven protocol that started on our PICU in 2006[72, 79, 80]. These studies report effectivity and safety in older children, while it drew our attention that the majority (> 80%) of the (severe) hypoglycemic incidents in the study by Vlasselaers and colleagues[71] occurred in infants. In **chapter 3** we evaluated our glucose protocol specifically in this young population. Although our protocol was equally effective in newborn infants as in older children[72], hypoglycemia occurred more frequently in the infants, potentially caused by less mature regulatory capacities in glucose and/or insulin metabolism[81-83]. This makes us aware that infants, and possibly also young children, are a more vulnerable population where additional safety approaches are necessary. For instance, tools that help identify individuals with increased risk of developing hypoglycemia are warranted. In addition, we await the development and implementation of (near-) continuous glucose monitoring devices and "closed-loop" glycemic control systems for critically ill children. These would provide additional safety in preventing hypoglycemic incidents. Moreover, combined with validated protocols, these devices could help prevent glucose variability, which would also be useful in older children. Fluctuations in blood glucose levels are highly associated with increased mortality and may actually be worse than constant moderate hyperglycemia[84-87]. This novel aspect of tight glucose control therefore needs to be taken into account in future tight glucose control studies. Furthermore, we observed that overall insulin treatment in the infants in **chapter 3** was short (median duration of 27h, range 10 – 57h). Obviously it remains uncertain whether insulin therapy for such a short period of time can be expected to exert any beneficial effect on outcome. An alternative to insulin therapy in the initial phase of hyperglycemia might be to reduce the glucose intake. We described this approach in **chapter 4** and it concerns the principal question what the specific requirements are for energy (glucose) in the initial phase of injury (both trauma, surgery as well as infection). This subject is discussed below in the paragraph "*Glucose requirements; the initial phase*". Finally, it is important

to understand that the optimal glucose target ranges are still uncertain and that 'hypoglycemia' in children and infants is still a controversial issue in regard to its definition[88]. The complexity of the optimal target ranges becomes evident in patients with traumatic brain injury, which is one of few specific diagnoses that have been investigated in this setting. In this population, a reduction of systemic glucose below 6 mmol.L^{-1} with exogenous insulin has been found to exacerbate brain metabolic distress and increase mortality, and a less restrictive target for systemic glucose control (6–10 mmol.L^{-1}) seems more appropriate[89, 90]. Whether other diagnoses should be treated according to specific target ranges is uncertain and also deserves further attention.

Is insulin an anabolic tool during critical illness?

One of the main hypotheses of this thesis was that insulin exerts an anabolic function in critically ill children, which at least partially could explain the beneficial effects of insulin therapy. In healthy humans insulin is "permissive" for protein synthesis and suppressive for protein breakdown[91]. During critical illness, insulin has multiple metabolic effects, and insulin resistance affects both protein and energy metabolism, in addition to several other processes as we summarized in **chapter 2**. Some of the earlier studies on the effects of insulin therapy in children receiving intensive care actually used this pleiotropic property of insulin as outcome variable. These studies showed that besides glycemic control insulin therapy improved inflammatory response, caloric intake, dyslipidemia and growth, although no data on protein anabolism were available[63, 64, 66, 68, 92, 93].

In contrast to our hypothesis, however, we found that whole body protein balance was not affected during hyperinsulinemia, as described in **chapter 7**. Interestingly, although hyperinsulinemia did reduce whole body proteolysis, it simultaneously blunted whole body protein synthesis. Whole body protein synthesis can roughly be divided into muscle and splanchnic protein synthesis, which largely consists of hepatic protein synthesis; acute phase proteins and constitutive proteins. Although insulin has a differential effect on muscle and splanchnic protein synthesis[94], our study was not designed to make a differentiation for the effect of insulin on protein synthesis in these specific organs. However, we did focus on one of the major hepatic constitutive proteins. In **chapter 8** we described fractional (FSR) and absolute (ASR) albumin synthesis rates in post-surgical infants and septic adolescents. Both the FSR and the ASR were not affected with plasma insulin changes. Interestingly, the contribution of albumin synthesis to whole body protein synthesis increased slightly during insulin infusion, although statistical significance was not achieved. Nonetheless, it appears that the decrease in whole body protein synthesis during hyperinsulinemia was based on a decrease in synthesis of hepatic acute phase proteins, a decrease in muscle protein synthesis or a combination of both.

The latter hypothesis is most likely, as both the influence on hepatic as well as muscle protein synthesis are described in previous studies. A decrease in hepatic protein synthesis would be in agreement with two clinical studies which showed that insulin therapy reduced the hepatic pro-inflammatory cascade and reduced concentrations of acute phase proteins such as c-reactive protein[66, 71]. The insulin resistance to muscle protein synthesis is supported by data from septic animal models where, via a defect in insulin signaling to mediate translation initiation, insulin failed to stimulate muscle protein synthesis[95].

Aside from the lack of protein anabolism, insulin insufficiently suppressed lipolysis in septic insulin resistant adolescents as we reported in **chapter 7**, which is in agreement with previous studies in critically ill adults[96, 97]. Therefore it appears safe to conclude that during critical illness there are no arguments to use insulin therapy as an anabolic tool to improve protein balance or the rate of lipolysis.

There are several conditions which were present in our study patients and in a large proportion of patients on our ICU's, which can be held responsible for the lack of anabolic effect of insulin during critical illness. First, it is well known that exogenous glucocorticoids induce insulin resistance both to glucose as well as protein anabolism[98, 99]. Second, immobility *per se* causes a decreased muscle protein synthesis[100-102]. Finally, nutritive blood flow, depending on the opening of a vascular network to supply nutrients and insulin to organs is essential to support protein anabolism[103], while the microcirculation is often compromised in our patients[104, 105]. These aspects should be taken into account for future studies.

Recommendations

In light of the large bulk of studies in adults, the available data in critically ill children, and from our studies presented, we propose some recommendations on glycemic control and insulin therapy on the PICU.

- In anticipation of subsequent trials identifying the optimal target range and the specific population that benefits from tight glucose control, all critically ill children requiring support for cardiac and/or respiratory failure should be treated with a moderate glucose target range of 4-8 mmol.L^{-1}. Children with traumatic brain injury should be treated with an even less strict glucose target range of 6-10 mmol.L^{-1}.
- A large proportion of children will exert hyperglycemia for a short period of time (<24 h), and insulin therapy in this population will probably not exert the same benefits, while it does hold the unnecessary risk of hypoglycemia. This population might benefit from a reduced glucose intake in the initial phase.
- Young infants and children can effectively be treated with insulin, although adjusted age-specific glucose control protocols to reduce the risk for hypoglycemia are warranted, and we recommend to lower the insulin starting dose in infants.

- Although organ specific anabolic effects cannot be ruled out, there are currently no grounds to use insulin as an anabolic tool in insulin resistant critically ill children.

Macronutrient requirements

Despite the clear association with in-hospital protein-energy malnutrition and increased morbidity, the knowledge on specific requirements is poor and routine nutritional assessment on the PICU is scarce[5].

Energy requirements and the importance of its assessment.

The energy requirement of a healthy child is the sum of basal metabolic rate, diet induced thermogenesis, the energy needed for physical activity and the energy needed for growth. Although the metabolic response is enhanced in critical illness, studies in critically ill children have reported increased[2, 106], but also equal[107] or even decreased energy expenditures[108]. These variations can be explained by the heterogeneous nature of critical illness. The energy expenditure is impacted by diagnosis[109, 110], therapy (sedatives, mechanical ventilation[111, 112]) and age[113]. Different methods for the assessment of energy requirements in critically ill children have been developed. To estimate energy requirements, two mathematical methods have been developed for children older than 2 months of age. The predictive equation according to Schofield is based upon a large number of healthy subjects[114]. An alternative equation based upon 100 mechanically ventilated children was developed by White[115]. However, the predicted energy expenditure remains inaccurate in critically ill children due to the abovementioned wide variation in metabolic response. It is therefore preferred to measure energy requirements. For instance with the indirect calorimetry, which is a non-invasive validated method that can be used at the PICU bedside. This method has an additional advantage as it also provides the respiratory quotient (RQ), which is the calculated quotient between volume produced carbon dioxide (VCO_2) and volume consumed oxygen (VO_2). A RQ < 0.8 indicates underfeeding with gluconeogenesis and protein catabolism and a RQ > 1.0 suggests carbohydrate overfeeding with lipogenesis[116]. For most children, a single measurement of the total daily energy expenditure is sufficient to provide a good insight into energy requirements, although serial measurements are preferred due to the effects of treatment, physical activity and type of nutrition[117]. The major limitation of the indirect calorimetry is that it cannot be performed when patients are mechanically ventilated with an endotracheal tube leak (>10%) and/or a FiO_2 > 60%[118]. Also, time limitations and financial restraints prevent this method to be used on a wide scale in every day practice. However, considering the valuable information on both the energy requirements as well as the evaluation of the adequacy of the nutrients provided, it is

a highly recommended tool to establish appropriate nutritional and metabolic support on an individual basis.

Glucose requirements; the initial phase.

Directly after children are admitted to a PICU with acute injury, regardless of the etiology (e.g. trauma, surgery or infection), they are given intravenous glucose to provide their fluid and glucose intake. Due to obvious reasons, such as insufficient intravenous access, low priority in an acute situation, and lack of awareness, additional amino acids and/or lipids are usually not provided in the initial phase. Hyperglycemia is a frequent complication of excessive glucose intake in this initial phase. This led us to reassess the glucose requirements in critically ill children. Due to the paucity of data in children it is difficult to define lower limits of glucose intake. The current recommendations are based largely upon "circumstantial evidence" such as the relation between glucose intake and rate of glucose production, induced catabolism, and cerebral glucose availability and utilization[50]. The latter is in essence the most crucial as hypoglycemia primarily leads to neurological damage in children[119-121]. In **chapter 4** we showed that hyperglycemia could safely be prevented by a reduced glucose intake, half of what is considered standard practice for age[50], without occurrence of hypoglycemia. The children were well capable of sustaining normoglycemic blood glucose levels due to an increased glucose production, primarily almost entirely through gluconeogenesis. We acknowledge that our study only provides data for post-surgical infants, and that it is premature to state that current glucose guidelines for children in the initial phase after injury are too high. However, present recommendations on glucose requirements in various age groups and diagnoses need to be reassessed, also in light of prevention of hyperglycemia. We are currently performing confirmatory studies in children undergoing congenital heart surgery and have planned additional studies in septic children.
Aside from the necessity to confirm the safety of reduced glucose in different age groups and diagnoses, it is important to emphasize that the results from chapter 4 cannot be used for recommendations of glucose or energy requirements in critically infants. Beyond the initial phase on injury it is still highly recommended to use the abovementioned assessments to measure energy/glucose requirements on a regular base. With use of the RQ, excessive glucose intake can be prevented and might help avert complications such as hyperglycemia, fatty liver and increased oxygen consumption or carbon dioxide production.

Amino acid requirements; providing the right quantity.

It is well recognized that malnutrition and protein catabolism in critically ill patients lead to muscle wasting which results in physical inability[8, 10], higher risk of infections[10], prolonged mechanical ventilation[13] and hospital stay[11, 12]. The negative whole body protein

balance observed in **chapter 5 and 7** are consistent with previous data in critically ill children[122, 123]. The enteral protein provided to the infants (~ 2.3 $g.kg^{-1}.day^{-1}$), children (~ 2.0 $g.kg^{-1}.day^{-1}$) and adolescents (~ 1.4 $g.kg^{-1}.day^{-1}$) described in **chapter 5** and the standard parenteral amino acids provided to the adolescents (~ 1.5 $g.kg^{-1}.day^{-1}$) described in **chapter 7** were insufficient to prevent protein catabolism. These intakes are current standard practice as we have shown in **chapter 6** and are according to current recommended guidelines by the American Society for Parenteral and Enteral Nutrition (ASPEN), the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), and the European Society for Clinical Nutrition (ESPEN)[51, 124]. This makes us conclude that current recommendations on (par)enteral protein / amino acid requirements are inadequate to sustain an optimal protein balance.

In critically ill adults, increasing protein intake from 1.0 to about 1.5 $g.kg^{-1}.day^{-1}$ is considered optimal, but did not normalize whole body protein balance, while a further increase to about 2.0 $g.kg^{-1}.day^{-1}$ had no further benefit[125-128]. In contrast to these studies in adults, our study in **chapter 7** demonstrated that increasing parenteral amino acid intakes to 3.0 $g.kg^{-1}.day^{-1}$ in the presence of adequate caloric intake stimulated protein synthesis, and achieved a positive protein balance in septic adolescents. This result is in agreement with a previous study in critically ill children who received a higher protein intake (~ 2.8 $g.kg^{-1}.day^{-1}$) in combination with a higher ratio of energy intake to energy expenditure (~ 1.7), which led to a positive protein balance[3].

However, it is still premature to conclude that we should increase the amount of protein and amino acids to critically ill children admitted to our PICU. First of all, we have shown in **chapter 5** that amino acid metabolism greatly differs between infants, children and adolescents and confirmatory studies are therefore needed in children of all age groups. Also, optimal protein and amino acid nutrition probably will differ between different groups of diagnosis (e.g. burns, infection, trauma, post-surgery). Furthermore, although it has been a long tradition to use protein or nitrogen balance as a primary endpoint in nutritional studies evaluating protein and amino acid requirements, it needs to be acknowledged that it merely is an intermediate endpoint. Therefore, clinical outcome studies using maintenance of organ function, wound healing, length of stay on the PICU or hospital among other parameters are awaited for. Finally, excessive amounts of free amino acids may also result in toxicity, and long-term use of parenteral nutrition results on cholestasis and liver failure[129-132]. It has been demonstrated that L-methionine is hepatotoxic[133], whereas L-arginine, through L-ornithine production induces necrotizing pancreatitis in rats[134], and L-Lysine at large doses induces renal failure in dogs[135]. Moreover, we observed in **chapter 7** that the high parenteral amino acid intake provided to the adolescents may have enhanced insulin resistance. We will discuss this matter in further detail later in the paragraph "*protein and energy interactions*".

Amino acid requirements: providing the right quality.

Protein and amino acid requirements in critically ill children reach beyond the traditional areas of protein metabolism and balance. Individual amino acids exert “non-protein” functions during health and critical illness. They are precursors for the biosynthesis of substrates such as serotonin, nitric oxide and polyamines[136, 137], they can act as signaling molecules in signal transduction pathways that control the growth, proliferation, and survival of cells[138, 139], and they help protect against oxidative stress and protect endothelial health[140, 141]. This emphasizes the importance to provide each individual amino acid according to their specific requirements. Currently, however, the primary goal of protein or amino acid administration is to try and meet current recommendations of nitrogen requirements.

The amino acid composition of the (parenteral) formulas is variable and lacks scientific support as we demonstrated in **chapter 6**, and the specific amino acid requirements of critically ill children have not been defined. This latter, however, is not an easy goal, as these specific requirements of each individual amino acid differ with age, route of administration and likely also diagnosis. In **chapter 5** we demonstrated this at the hand of the essential amino acid methionine. We showed that the splanchnic uptake of enterally administered methionine decreased with age. This difference in first pass disappearance in the splanchnic area illustrates the effect of route of administration. The impact of age is clear from the differences in splanchnic uptake, but is also demonstrated by the fate of methionine. While the infants utilized a high proportion of methionine for non-oxidative disposal (synthesis of methylated compounds and protein), the adolescents utilized a high fraction for transsulfuration. This might reflect a greater need for other amino acids associated in the transsulfuration pathway and oxidative stress (e.g. cysteine, taurine, glycine and glutathione) in the adolescents. Overall, we observed that the rates of methionine transsulfuration (oxidation) in all infants, children and adolescents exceeded the dietary intake of methionine and cysteine intake. In some degree they were all in a negative balance, illustrating the age-specific inadequacies of methionine currently provided. So, we can conclude that requirement studies should focus on different age groups.

Furthermore, amino acid utilization and metabolism is directed to different pathways depending on the anatomical presentation and requirements are different when provided via the enteral vs. the parenteral route[142]. Therefore, differentiation between parenteral and enteral administration is also required. Because this is just the example for methionine, one can start to imagine the magnitude of the challenge that lies ahead of us.

The preferred technique to determine amino acid requirements is the so-called indicator amino acid oxidation (IAAO) method. This method has been used to determine almost all essential amino acid requirements in adults[143]. Regarding the pediatric

population, several essential amino acid requirements have been determined in healthy school-aged children[144-147], parenterally fed post-surgical neonates[148-150], and enterally fed premature neonates[151]. However, no data are available for critically ill children.
Aside from the lack of scientific basis on which the parenteral intakes of the essential (EAA) amino acids are given, most non-essential (NEAA) amino acids are given in limited amounts as we showed in **chapter 6**. Moreover, several NEAA, such as cysteine, taurine, glutamine, are sometimes not provided at all. It is widely assumed that the NEAA must be less important, since they are the amino acids that are simply produced by the body. However, this reasoning appears wrong from an evolutionary point of view. It appears more plausible that human ancestors would have retained the capacity to synthesize those amino acids that we now label NEAA, to maintain their supply even when nutrition is scarce. These amino acids must really be important during periods of illness, injury and catabolism. It is increasingly recognized that, both from a metabolic as well as from a functional perspective, all amino acids are essential[152-156]. Therefore, the distinction between EAA and NEAA should be reassessed, as the NEAA deserve special attention, particularly during critical illness.

Protein and energy interactions

Aside from the abovementioned arguments there is another important aspect why improving nutritional support is not simply a matter of providing more nutrients to our patients. There is an interaction between protein and energy metabolism; they depend on each other, but also influence one another.

The effect of energy intake on protein metabolism.

Under physiological conditions, an increase in energy supply will not promote nitrogen retention unless the protein supply is adequate, and conversely an increased protein supply will be useless if energy is limiting. A lack in energy supply might even induce protein catabolism to release substrates for the cost of energy. These conditions, however, might be different during conditions of critical illness. In **chapter 4** we observed that a 50% cut in energy (glucose) supply had no effect on the protein turnover in post-surgical infants. This is in agreement with a previous study in neonates, which showed that different glucose intakes did not affect protein turnover[157]. It is clear that the reduced glucose intake described in **chapter 4** was not meant for prolonged use, considering that no amino acids were provided. Yet, although one should distinguish between hypocaloric feeding (low energy, but sufficient protein delivery) and underfeeding (energy and protein restriction), it is not likely that the provision of amino acids would have affected the results. **Chapter 7** shows that the negative protein balance could not be deflected by sufficient or increased amounts of energy intake, even in combination with insulin infusion and standard provision of amino acids. So, as we have discussed

earlier, during conditions of critical illness insulin does not exert the expected anabolic properties on protein balance, nor on albumin synthesis as shown in **chapter 8**, even though the deficiencies in glucose homeostasis were largely overcome. This was in contrast with our hypothesis, also because, independent of plasma insulin levels, normoglycemia improved protein turnover after abdominal surgery in adults[158]. Our results are supported by a study in insulin resistant adult ICU patients where different amounts of glucose and insulin infusion did not affect protein metabolism[97]. Indeed, it has been recognized that a deficiency in energy supply is not solely responsible for protein catabolism[159]. Protein breakdown during critical illness is usually caused by activation of the ubiquitin-proteasome proteolytic pathway (UPP) in muscle initiated by activation of caspase 3[160, 161], and pathophysiological triggers include activation of lysosome-[162] and calpain-dependent pathways[163]. Recently, a mechanistic link has been proposed between protein catabolism and insulin resistance[164-166]. These findings suggest that in (insulin resistant) patients, regulatory pathways controlling protein turnover change and are less influenced by energy intake than in healthy individuals.

Amino acid intake affects energy metabolism

It has been a long tradition of ignoring the contribution of protein metabolism to the energy delivery of nutritional support. However a patient not only receives the energy equivalent of 100% of the amino acids given in the diet, but also energy from the net loss of nitrogen, which is even higher in a catabolic state. High amino acid feeding might therefore add to any problems associated with high glucose or lipid provision, such as increased insulin resistance. Moreover, free amino acids provided in excessive amounts or released from proteolysis, and exceeding the incorporation into proteins are oxidized and/or channeled into the gluconeogenic pathway[167]. So, increased amino acid intake potentially might be a detrimental influence on insulin resistance and glucose metabolism.

This was confirmed by the study we described in **chapter 7**. During the high amino acid intake, endogenous glucose production and protein breakdown were not fully suppressed during insulin infusion and lipolysis rates were increased. The high amino acid intake may have favored gluconeogenesis, which raises concern that it may have enhanced insulin resistance. An increased insulin resistance could also explain the increased lipolysis rate we observed. Although this was not confirmed by increased levels of plasma insulin or with our Hyperinsulinemic Euglycemic Clamp technique, there is a strong indication from other studies that links the provision of amino acids to insulin resistance. Amino acid infusion in healthy subjects to achieve hyperaminoacidemia decreases insulin sensitivity for glucose metabolism[168], possibly through a negative feedback loop toward insulin-stimulated tyrosine phosphorylation of insulin receptor substrate 1 and 2 (IRS-1, IRS-2)[169, 170].

Hence, although a high amino acid intake increased protein synthesis and improved the protein balance, there is concern that it could negatively impact glucose and lipid homeostasis. Dose-response studies on protein administration and their effects on energy metabolism and insulin resistance are needed. Furthermore, these interactions between protein and energy metabolism emphasize the importance of clinical outcome studies, as this type of study does not focus on one aspect, such as protein balance, but looks at the patient as a whole.

Recommendations

Based on the studies presented in this thesis and in reflection with the arguments put forward in the subsequent discussion we conclude and recommend the following:

- Current recommended glucose intakes can induce hyperglycemia in infants in the initial post-surgical phase and can safely be reduced, while awaiting confirmatory studies in different age groups and diagnoses.
- To evaluate glucose / energy requirements after the initial phase, routine assessments by means of techniques such as indirect calorimetry, to measure Energy Expenditure and Respiratory Quotient, are invaluable to prevent excessive glucose intake.
- Both the quantity as well as the quality of currently provided amino acid intakes are inadequate and lack scientific support. Future studies should focus on the requirements of specific amino acids based on age, diagnosis and route of administration.
- In light of protein balance as outcome variable, doubling the intake of amino acid enhances protein anabolism in septic adolescents and should be considered in future nutrition guidelines. However, before increased amino acid intakes are implemented in the clinical setting, the potential detrimental effects on insulin sensitivity and glucose and lipid metabolism demands further investigation.

Future perspectives

There are two remaining issues not discussed in this thesis, we want to bring under consideration.

First, to be worthwhile, recommendations and guidelines need to be followed by the actual practice at the bedside. This is of even greater importance when we want to demonstrate the impact on clinical outcome. However, there is an increased awareness that the implementation of nutritional guidelines on ICU's is complex and time-consuming and therefore often unsuccessful[7, 171-173]. Identifying effective methods to improve guideline implementation should go hand in hand with the development of these guidelines.

Finally, there is one other aspect which is getting increasingly more attention in nutrition science. It is the concept of using specific nutrients to help modulate key processes such as immunity, inflammation, endothelial health and oxidative stress. This concept, known as “pharmaconutrition”, is becoming more accepted to play a future role in nutritional and metabolic therapy. Several macro- as well as micronutrients have drawn attention in this matter; glutamine, cysteine, arginine, selenium, zinc, vitamin C and E, and omega-3 fatty acids. We will mention those which have been cited as most promising. First, glutamine, the most abundant amino acid in plasma, which has numerous metabolic functions[174]. It is an important fuel for rapidly dividing cells, is a precursor for glutathione, protects intestinal mucosa and augments cellular immune functions[141, 174, 175]. A meta-analysis of glutamine supplementation in critically ill patients have revealed a reduction in mortality[176]. Second, arginine, which is also an amino acid that exerts multiple functions[141]. It is a precursor for creatine, polyamines, it is an essential product in the urea cycle and it is the sole precursor for nitric oxide[141, 174]. This latter property is subject of concern, because arginine supplementation has lead to inconsistent results in clinical studies, possibly because of the potential toxic effect of arginine as a substrate for inducible nitric oxide synthase (iNOS)[175]. Increased NO production might deteriorate microcirculation and organ dysfunction. In contrast, there has been extensive evidence that certain patient groups might benefit from arginine supplementation[177]. Finally, the use of the omega-3 fatty acids (primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) is at the centre of attention. Their biological effects are related to anti-inflammatory effects. Enteral supplementation of fish oil, rich in omega-3 fatty acids, have improved outcome in patients with Acute Respiratory Distress Syndrome (ARDS)[175].
However, despite the promising results in adult patients, ‘pharmaconutrition’ studies in critically ill children are limited to three small studies in burned children[178-180]. These studies are too small and limited to draw any conclusions and larger studies are necessary.

As a rule, future perspectives and recommendations always stress the importance of forthcoming studies. Despite, and probably also *due to*, the studies and discussions presented in this thesis, this isn’t any different in case of nutritional and metabolic support in critically ill children. Therefore we want to summarize the most important issues in future studies to follow.

- In respect to the mechanisms and benefits of tight glucose control in critically ill children, a limited amount of data is available. Attention of future investigation should be focused on; identification of children at risk for prolonged periods of hyperglycemia, optimal glucose target ranges, and the efficacy and safety of protocols in specific age groups and diagnoses (e.g. post-cardiac surgery and septic shock).

- Our studies described in chapter 4, 5, 7 and 8 have focused on specific populations; varying in age, diagnosis and route of nutrition. Obviously, confirmatory studies are needed to be able to make recommendations on glucose and amino acid intakes and the impact they exert on each other for the entire pediatric population.
- Aside from well designed smaller mechanistic studies using intermediate endpoints such as protein balance or plasma concentrations, we are in need of clinical outcome studies. Since comparable pediatric ICU populations are small, multicenter studies are therefore necessary. This type of study will show us whether we can actually affect morbidity and may be even mortality, which in the end should be the ultimate goal.

These studies will provide further knowledge which is imperative to develop clear guidelines on nutritional and metabolic support on the PICU. For now, the implementation of our results, regarding the reduced glucose intake and the increased amino acid intake, would be premature for upcoming guidelines. Additionally, it is our responsibility as researchers to start providing scientific data with which the nutritional industry can improve the composition of (par)enteral formulas.
However, we did set the scene for further examination of these approaches to improve nutritional care in critically ill children. Furthermore, the recommendation to decrease the insulin starting doses in infants is a small but valuable contribution. Once we establish the effect of nutritional interventions not only on narrow endpoints but also on the interaction between the different substrates and the patient as a whole, we are confident about the future of nutritional therapy to improve the outcome of the critically ill child.

References

1. Cogo PE, Carnielli VP, Rosso F, et al. Protein turnover, lipolysis, and endogenous hormonal secretion in critically ill children. Crit Care Med 2002;30(1):65-70.
2. Coss-Bu JA, Jefferson LS, Walding D, David Y, Smith EO, Klish WJ. Resting energy expenditure and nitrogen balance in critically ill pediatric patients on mechanical ventilation. Nutrition 1998; 14(9):649-52.
3. Coss-Bu JA, Klish WJ, Walding D, Stein F, Smith EO, Jefferson LS. Energy metabolism, nitrogen balance, and substrate utilization in critically ill children. Am J Clin Nutr 2001;74(5):664-9.
4. Hulst J, Joosten K, Zimmermann L, et al. Malnutrition in critically ill children: from admission to 6 months after discharge. Clin Nutr 2004;23(2):223-32.
5. 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.
6. de Neef M, Geukers VG, Dral A, Lindeboom R, Sauerwein HP, Bos AP. Nutri-

tional goals, prescription and delivery in a pediatric intensive care unit. Clin Nutr 2008;27(1):65-71.
7. Preissig CM, Rigby MR. A disparity between physician attitudes and practice regarding hyperglycemia in pediatric intensive care units in the United States: a survey on actual practice habits. Crit Care 2010;14(1):R11.
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(3): 309-13.
9. Faustino EV, Apkon M. Persistent hyperglycemia in critically ill children. J Pediatr 2005;146(1):30-4.
10. Villet S, Chiolero RL, Bollmann MD, et al. Negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients. Clin Nutr 2005;24(4): 502-9.
11. Heyland DK, MacDonald S, Keefe L, Drover JW. Total parenteral nutrition in the critically ill patient: a meta-analysis. Jama 1998;280(23):2013-9.
12. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, Kache S, Wilson DM. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. Pediatrics 2006;118(1):173-9.
13. Pichard C, Kyle UG, Morabia A, Perrier A, Vermeulen B, Unger P. Nutritional assessment: lean body mass depletion at hospital admission is associated with an increased length of stay. Am J Clin Nutr 2004;79(4):613-8.
14. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001; 345(19):1359-67.
15. Malhotra A. Intensive insulin in intensive care. N Engl J Med 2006;354(5):516-8.
16. Dellinger RP, Carlet JM, Masur H, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med 2004;32(3): 858-73.
17. Mackenzie I, Ingle S, Zaidi S, Buczaski S. Tight glycaemic control: a survey of intensive care practice in large English hospitals. Intensive Care Med 2005; 31(8):1136.
18. McMullin J, Brozek J, Jaeschke R, et al. Glycemic control in the ICU: a multicenter survey. Intensive Care Med 2004; 30(5):798-803.
19. Schultz MJ, Spronk PE, Moeniralam HS. Tight glycaemic control: a survey of intensive care practice in the Netherlands. Intensive Care Med 2006;32(4):618-9; author reply 20-1.
20. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. N Engl J Med 2006;354(5): 449-61.
21. Preiser JC, Devos P, Ruiz-Santana S, et al. A prospective randomised multi-centre controlled trial on tight glucose control by intensive insulin therapy in adult intensive care units: the Glucontrol study. Intensive Care Med 2009;35(10):1738-48.
22. Brunkhorst FM, Engel C, Bloos F, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med 2008;358(2):125-39.
23. Finfer S, Chittock DR, Su SY, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med 2009;360(13):1283-97.
24. de Azevedo JR, de Araujo LO, da Silva WS, de Azevedo RP. A carbohydrate-restrictive strategy is safer and as efficient as intensive insulin therapy in critically ill patients. J Crit Care 2009;25(1):84-9.
25. He W, Zhang TY, Zhou H, et al. [Impact of intensive insulin therapy on surgical critically ill patients]. Zhonghua Wai Ke Za Zhi 2007;45(15):1052-4.

26. Henderson WR, Dhingra V, Chittock D, et al. The efficacy and safety of glucose control algorithms in intensive care: a pilot study of the Survival Using Glucose Algorithm Regulation (SUGAR) trial. Pol Arch Med Wewn 2009;119(7-8):439-46.
27. Iapichino G, Albicini M, Umbrello M, et al. Tight glycemic control does not affect asymmetric-dimethylarginine in septic patients. Intensive Care Med 2008; 34(10):1843-50.
28. McMullin J, Brozek J, McDonald E, et al. Lowering of glucose in critical care: a randomized pilot trial. J Crit Care 2007; 22(2):112-8; discussion 8-9.
29. Mitchell I, Knight E, Gissane J, et al. A phase II randomised controlled trial of intensive insulin therapy in general intensive care patients. Crit Care Resusc 2006;8(4):289-93.
30. Wang LC, Lei S, Wu YC, et al. Intensive insulin therapy in critically ill patients. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2006;18(12):748-50.
31. Yu WK, Li WQ, Wang XD, et al. Influence and mechanism of a tight control of blood glucose by intensive insulin therapy on human sepsis. Zhonghua Wai Ke Za Zhi 2005;43(1):29-32.
32. Arabi YM, Dabbagh OC, Tamim HM, et al. Intensive versus conventional insulin therapy: a randomized controlled trial in medical and surgical critically ill patients. Crit Care Med 2008;36(12):3190-7.
33. Bilotta F, Caramia R, Cernak I, et al. Intensive insulin therapy after severe traumatic brain injury: a randomized clinical trial. Neurocrit Care 2008;9(2):159-66.
34. Bilotta F, Spinelli A, Giovannini F, Doronzio A, Delfini R, Rosa G. The effect of intensive insulin therapy on infection rate, vasospasm, neurologic outcome, and mortality in neurointensive care unit after intracranial aneurysm clipping in patients with acute subarachnoid hemorrhage: a randomized prospective pilot trial. J Neurosurg Anesthesiol 2007;19(3): 156-60.
35. Bland DK, Fankhanel Y, Langford E, et al. Intensive versus modified conventional control of blood glucose level in medical intensive care patients: a pilot study. Am J Crit Care 2005;14(5):370-6.
36. Bruno A, Kent TA, Coull BM, et al. Treatment of hyperglycemia in ischemic stroke (THIS): a randomized pilot trial. Stroke 2008;39(2):384-9.
37. De La Rosa Gdel C, Donado JH, Restrepo AH, et al. Strict glycaemic control in patients hospitalised in a mixed medical and surgical intensive care unit: a randomised clinical trial. Crit Care 2008; 12(5):R120.
38. Farah R, Samokhvalov A, Zviebel F, Makhoul N. Insulin therapy of hyperglycemia in intensive care. Isr Med Assoc J 2007; 9(3):140-2.
39. Grey NJ, Perdrizet GA. Reduction of nosocomial infections in the surgical intensive-care unit by strict glycemic control. Endocr Pract 2004;10 Suppl 2: 46-52.
40. He W, Li N, J. X, et al. Effect of intensive insulin therapy on short-term outcome in critically ill patients. Chinese J Clin Nutr 2008;16:220-2.
41. Mackenzie I, Ercole A, Ingle S, Zaidi S, Buczaski S. Glycaemic control and outcome in general intensive care: The east anglian GLYCOGENIC study. Br J Intensive Care 2008;18:121-6.
42. Oksanen T, Skrifvars MB, Varpula T, et al. Strict versus moderate glucose control after resuscitation from ventricular fibrillation. Intensive Care Med 2007;33(12): 2093-100.
43. Walters MR, Weir CJ, Lees KR. A randomised, controlled pilot study to investigate the potential benefit of intervention with insulin in hyperglycaemic acute ischaemic stroke patients. Cerebrovasc Dis 2006;22(2-3):116-22.

44. Zhang TY, He W, Li T, et al. Evaluation of optimal goal of glucose control in critically ill patients. Chinese J Clin Nutr 2008;16:204-8.
45. Griesdale DE, de Souza RJ, van Dam RM, et al. Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data. Cmaj 2009;180(8):821-7.
46. 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.
47. Bagshaw SM, Egi M, George C, Bellomo R. Early blood glucose control and mortality in critically ill patients in Australia. Crit Care Med 2009;37(2):463-70.
48. American College of Endocrinology and American Diabetes Association consensus statement on inpatient diabetes and glycemic control. Endocr Pract 2009;15: 1-12.
49. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 2008;34(1):17-60.
50. 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.
51. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 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.
52. Alaedeen DI, Walsh MC, Chwals WJ. Total parenteral nutrition-associated hyperglycemia correlates with prolonged mechanical ventilation and hospital stay in septic infants. J Pediatr Surg 2006; 41(1):239-44; discussion -44.
53. Branco RG, Garcia PC, Piva JP, Casartelli CH, Seibel V, Tasker RC. Glucose level and risk of mortality in pediatric septic shock. Pediatr Crit Care Med 2005;6(4): 470-2.
54. Cochran A, Scaife ER, Hansen KW, Downey EC. Hyperglycemia and outcomes from pediatric traumatic brain injury. J Trauma 2003;55(6):1035-8.
55. Hall NJ, Peters M, Eaton S, Pierro A. Hyperglycemia is associated with increased morbidity and mortality rates in neonates with necrotizing enterocolitis. J Pediatr Surg 2004;39(6):898-901; discussion 898-901.
56. Srinivasan V, Spinella PC, Drott HR, Roth CL, Helfaer MA, Nadkarni V. 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.
57. Yates AR, Dyke PC, 2nd, Taeed R, et al. Hyperglycemia is a marker for poor outcome in the postoperative pediatric cardiac patient. Pediatr Crit Care Med 2006;7(4):351-5.
58. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004;114(9): 1187-95.
59. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet 2005; 365(9453):53-9.
60. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360(9328): 219-23.
61. Fram RY, Cree MG, Wolfe RR, et al. Intensive insulin therapy improves insulin

sensitivity and mitochondrial function in severely burned children. Crit Care Med 2010;38(6):1475-83.
62. Vlasselaers D, Mesotten D, Langouche L, et al. Tight glycemic control protects the myocardium and reduces inflammation in neonatal heart surgery. Ann Thorac Surg 2010;90(1):22-9.
63. Binder ND, Raschko PK, Benda GI, Reynolds JW. Insulin infusion with parenteral nutrition in extremely low birth weight infants with hyperglycemia. J Pediatr 1989;114(2):273-80.
64. Collins JW, Jr., Hoppe M, Brown K, Edidin DV, Padbury J, Ogata ES. A controlled trial of insulin infusion and parenteral nutrition in extremely low birth weight infants with glucose intolerance. J Pediatr 1991;118(6):921-7.
65. Jeschke MG, Einspanier R, Klein D, Jauch KW. Insulin attenuates the systemic inflammatory response to thermal trauma. Mol Med 2002;8(8):443-50.
66. Jeschke MG, Klein D, Herndon DN. Insulin treatment improves the systemic inflammatory reaction to severe trauma. Ann Surg 2004;239(4):553-60.
67. Pham TN, Warren AJ, Phan HH, Molitor F, Greenhalgh DG, Palmieri TL. Impact of tight glycemic control in severely burned children. J Trauma 2005;59(5):1148-54.
68. Thabet F, Bourgeois J, Guy B, Putet G. Continuous insulin infusion in hyperglycaemic very-low-birth-weight infants receiving parenteral nutrition. Clin Nutr 2003;22(6):545-7.
69. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, et al. Early insulin therapy in very-low-birth-weight infants. N Engl J Med 2008;359(18):1873-84.
70. Macrae D, Pappachan J, Grieve R, et al. Control of hyperglycaemia in paediatric intensive care (CHiP): study protocol. BMC Pediatr 2010;10:5.
71. Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. Lancet 2009;373(9663):547-56.
72. Verhoeven JJ, Brand JB, van de Polder MM, Joosten KF. Management of hyperglycemia in the pediatric intensive care unit; implementation of a glucose control protocol. Pediatr Crit Care Med 2009; 10(6):648-52.
73. Chase JG, Shaw GM, Lin J, et al. Targeted glycemic reduction in critical care using closed-loop control. Diabetes Technol Ther 2005;7(2):274-82.
74. Chase JG, Shaw GM, Lin J, et al. Adaptive bolus-based targeted glucose regulation of hyperglycaemia in critical care. Med Eng Phys 2005;27(1):1-11.
75. Chee F, Fernando T, van Heerden PV. Closed-loop control of blood glucose levels in critically ill patients. Anaesth Intensive Care 2002;30(3):295-307.
76. Lin J, Chase JG, Shaw GM, et al. Adaptive bolus-based set-point regulation of hyperglycemia in critical care. Conf Proc IEEE Eng Med Biol Soc 2004;5:3463-6.
77. Wong XW, Singh-Levett I, Hollingsworth LJ, et al. A novel, model-based insulin and nutrition delivery controller for glycemic regulation in critically ill patients. Diabetes Technol Ther 2006;8(2):174-90.
78. Vogelzang M, Loef BG, Regtien JG, et al. Computer-assisted glucose control in critically ill patients. Intensive Care Med 2008;34(8):1421-7.
79. Preissig CM, Hansen I, Roerig PL, Rigby MR. A protocolized approach to identify and manage hyperglycemia in a pediatric critical care unit. Pediatr Crit Care Med 2008;9(6):581-8.
80. Preissig CM, Rigby MR, Maher KO. Glycemic control for postoperative pediatric cardiac patients. Pediatr Cardiol 2009; 30(8):1098-104.
81. Lucas A, Bloom SR, Aynsley-Green A. Metabolic and endocrine events at the time of the first feed of human milk in

preterm and term infants. Arch Dis Child 1978;53(9):731-6.
82. 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.
83. Blanco CL, Liang H, Joya-Galeana J, DeFronzo RA, McCurnin D, Musi N. The ontogeny of insulin signaling in the preterm baboon model. Endocrinology 2010;151(5):1990-7.
84. Hermanides J, Vriesendorp TM, Bosman RJ, Zandstra DF, Hoekstra JB, Devries JH. Glucose variability is associated with intensive care unit mortality. Crit Care Med 2010;38(3):838-42.
85. Rake AJ, Srinivasan V, Nadkarni V, Kaptan R, Newth CJ. Glucose variability and survival in critically ill children: Allostasis or harm? Pediatr Crit Care Med 2010.
86. Egi M, Bellomo R, Stachowski E, French CJ, Hart G. Variability of blood glucose concentration and short-term mortality in critically ill patients. Anesthesiology 2006;105(2):244-52.
87. Hirshberg E, Larsen G, Van Duker H. Alterations in glucose homeostasis in the pediatric intensive care unit: Hyperglycemia and glucose variability are associated with increased mortality and morbidity*. Pediatr Crit Care Med 2008.
88. 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.
89. Oddo M, Schmidt JM, Carrera E, et al. Impact of tight glycemic control on cerebral glucose metabolism after severe brain injury: a microdialysis study. Crit Care Med 2008;36(12):3233-8.
90. Oddo M, Schmidt JM, Mayer SA, Chiolero RL. Glucose control after severe brain injury. Curr Opin Clin Nutr Metab Care 2008;11(2):134-9.
91. Greenhaff PL, Karagounis LG, Peirce N, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol Endocrinol Metab 2008;295(3):E595-604.
92. Meetze W, Bowsher R, Compton J, Moorehead H. Hyperglycemia in extremely- low-birth-weight infants. Biol Neonate 1998;74(3):214-21.
93. Vaucher YE, Walson PD, Morrow G, 3rd. Continuous insulin infusion in hyperglycemic, very low birth weight infants. J Pediatr Gastroenterol Nutr 1982;1(2):211-7.
94. Charlton M, Nair KS. Protein metabolism in insulin-dependent diabetes mellitus. J Nutr 1998;128(2 Suppl):323S-7S.
95. Lang CH, Frost RA. Sepsis-induced suppression of skeletal muscle translation initiation mediated by tumor necrosis factor alpha. Metabolism 2007;56(1): 49-57.
96. Chambrier C, Laville M, Rhzioual Berrada K, Odeon M, Bouletreau P, Beylot M. Insulin sensitivity of glucose and fat metabolism in severe sepsis. Clin Sci (Lond) 2000;99(4):321-8.
97. Whyte MB, Jackson NC, Shojaee-Moradie F, et al. Metabolic effects of intensive insulin therapy in critically ill patients. Am J Physiol Endocrinol Metab 2010;298(3):E697-705.
98. Short KR, Bigelow ML, Nair KS. Short-term prednisone use antagonizes insulin's anabolic effect on muscle protein and glucose metabolism in young healthy people. Am J Physiol Endocrinol Metab 2009;297(6):E1260-8.
99. Van Goudoever JB, Wattimena JD, Carnielli VP, Sulkers EJ, Degenhart HJ, Sauer PJ. Effect of dexamethasone on protein metabolism in infants with bronchopulmonary dysplasia. J Pediatr 1994;124(1): 112-8.
100. Glover EI, Phillips SM, Oates BR, et al. Immobilization induces anabolic

resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. J Physiol 2008;586(Pt 24): 6049-61.
101. Biolo G, Ciocchi B, Lebenstedt M, et al. Short-term bed rest impairs amino acid-induced protein anabolism in humans. J Physiol 2004;558(Pt 2):381-8.
102. Gibson JN, Halliday D, Morrison WL, et al. Decrease in human quadriceps muscle protein turnover consequent upon leg immobilization. Clin Sci (Lond) 1987;72(4):503-9.
103. Clark MG, Rattigan S, Barrett EJ. Nutritive blood flow as an essential element supporting muscle anabolism. Curr Opin Clin Nutr Metab Care 2006;9(3):185-9.
104. Lauten A, Ferrari M, Pfeifer R, et al. Effect of mechanical ventilation on microvascular perfusion in critical care patients. Clin Hemorheol Microcirc 2010;45(1):1-7.
105. Top AP, Ince C, van Dijk M, Tibboel D. Changes in buccal microcirculation following extracorporeal membrane oxygenation in term neonates with severe respiratory failure. Crit Care Med 2009;37(3):1121-4.
106. Tilden SJ, Watkins S, Tong TK, Jeevanandam M. Measured energy expenditure in pediatric intensive care patients. Am J Dis Child 1989;143(4):490-2.
107. Oosterveld MJ, Van Der Kuip M, De Meer K, De Greef HJ, Gemke RJ. Energy expenditure and balance following pediatric intensive care unit admission: a longitudinal study of critically ill children. Pediatr Crit Care Med 2006;7(2):147-53.
108. Briassoulis G, Venkataraman S, Thompson AE. Energy expenditure in critically ill children. Crit Care Med 2000;28(4): 1166-72.
109. Mlcak RP, Jeschke MG, Barrow RE, Herndon DN. The influence of age and gender on resting energy expenditure in severely burned children. Ann Surg 2006; 244(1):121-30.
110. Phillips R, Ott L, Young B, Walsh J. Nutritional support and measured energy expenditure of the child and adolescent with head injury. J Neurosurg 1987;67(6): 846-51.
111. Vernon DD, Witte MK. Effect of neuromuscular blockade on oxygen consumption and energy expenditure in sedated, mechanically ventilated children. Crit Care Med 2000;28(5):1569-71.
112. 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.
113. Jaksic T, Shew SB, Keshen TH, Dzakovic A, Jahoor F. Do critically ill surgical neonates have increased energy expenditure? J Pediatr Surg 2001;36(1):63-7.
114. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39 Suppl 1:5-41.
115. White MS, Shepherd RW, McEniery JA. Energy expenditure in 100 ventilated, critically ill children: improving the accuracy of predictive equations. Crit Care Med 2000;28(7):2307-12.
116. Curley MA, Castillo L. Nutrition and shock in pediatric patients. New Horiz 1998;6(2):212-25.
117. 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.
118. Joosten KF, Verhoeven JJ, Hazelzet JA. Energy expenditure and substrate utilization in mechanically ventilated children. Nutrition 1999;15(6):444-8.
119. 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.

120. Vannucci RC, Vannucci SJ. Glucose metabolism in the developing brain. Semin Perinatol 2000;24(2):107-15.
121. Flykanaka-Gantenbein C. Hypoglycemia in childhood: long-term effects. Pediatr Endocrinol Rev 2004;1 Suppl 3:530-6.
122. Castillo L, Yu YM, Marchini JS, et al. Phenylalanine and tyrosine kinetics in critically ill children with sepsis. Pediatr Res 1994;35(5):580-8.
123. van Waardenburg DA, Deutz NE, Hoos MB, et al. Assessment of whole body protein metabolism in critically ill children: can we use the [15N]glycine single oral dose method? Clin Nutr 2004;23(2):153-60.
124. 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.
125. 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.
126. Shaw JH, Wildbore M, Wolfe RR. Whole body protein kinetics in severely septic patients. The response to glucose infusion and total parenteral nutrition. Ann Surg 1987;205(3):288-94.
127. Wolfe RR, Goodenough RD, Burke JF, Wolfe MH. Response of protein and urea kinetics in burn patients to different levels of protein intake. Ann Surg 1983; 197(2):163-71.
128. Larsson J, Lennmarken C, Martensson J, Sandstedt S, Vinnars E. Nitrogen requirements in severely injured patients. Br J Surg 1990;77(4):413-6.
129. Kelly DA. Intestinal failure-associated liver disease: what do we know today? Gastroenterology 2006;130(2 Suppl 1): S70-7.
130. Baker DH. Comparative species utilization and toxicity of sulfur amino acids. J Nutr 2006;136(6 Suppl):1670S-5S.
131. Garlick PJ. Assessment of the safety of glutamine and other amino acids. J Nutr 2001;131(9 Suppl):2556S-61S.
132. Munro IC, Renwick AG. The 5th workshop on the assessment of adequate intake of dietary amino acids: general discussion 2. J Nutr 2006;136(6 Suppl):1755S-7S.
133. Moss RL, Haynes AL, Pastuszyn A, Glew RH. Methionine infusion reproduces liver injury of parenteral nutrition cholestasis. Pediatr Res 1999;45(5 Pt 1):664-8.
134. Rakonczay Z, Jr., Hegyi P, Dosa S, et al. A new severe acute necrotizing pancreatitis model induced by L-ornithine in rats. Crit Care Med 2008;36(7):2117-27.
135. Asanuma K, Adachi K, Sugimoto T, Chiba S. Effects of lysine-induced acute renal failure in dogs. J Toxicol Sci 2006;31(2): 87-98.
136. Morris SM, Jr. Arginine: beyond protein. Am J Clin Nutr 2006;83(2):508S-12S.
137. Savelieva KV, Zhao S, Pogorelov VM, et al. Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. PLoS One 2008;3(10):e3301.
138. Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr 2007; 27:293-310.
139. Tokunaga C, Yoshino K, Yonezawa K. mTOR integrates amino acid- and energy-sensing pathways. Biochem Biophys Res Commun 2004;313(2):443-6.
140. Biolo G, Antonione R, De Cicco M. Glutathione metabolism in sepsis. Crit Care Med 2007;35(9 Suppl):S591-5.
141. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009; 37(1):1-17.
142. Stoll B, Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 2006; 84 Suppl:E60-72.

143. Elango R, Ball RO, Pencharz PB. Individual amino acid requirements in humans: an update. Curr Opin Clin Nutr Metab Care 2008;11(1):34-9.
144. Elango R, Humayun MA, Ball RO, Pencharz PB. Lysine requirement of healthy school-age children determined by the indicator amino acid oxidation method. Am J Clin Nutr 2007;86(2): 360-5.
145. Humayun MA, Turner JM, Elango R, et al. Minimum methionine requirement and cysteine sparing of methionine in healthy school-age children. Am J Clin Nutr 2006;84(5):1080-5.
146. Pillai RR, Elango R, Muthayya S, Ball RO, Kurpad AV, Pencharz PB. Lysine requirement of healthy, school-aged Indian children determined by the indicator amino acid oxidation technique. J Nutr 2010; 140(1):54-9.
147. Turner JM, Humayun MA, Elango R, et al. Total sulfur amino acid requirement of healthy school-age children as determined by indicator amino acid oxidation technique. Am J Clin Nutr 2006;83(3): 619-23.
148. Chapman KP, Courtney-Martin G, Moore AM, Ball RO, Pencharz PB. Threonine requirement of parenterally fed postsurgical human neonates. Am J Clin Nutr 2009;89(1):134-41.
149. Chapman KP, Courtney-Martin G, Moore AM, et al. Lysine requirement in parenterally fed postsurgical human neonates. Am J Clin Nutr 2010;91(4):958-65.
150. Courtney-Martin G, Chapman KP, Moore AM, Kim JH, Ball RO, Pencharz PB. Total sulfur amino acid requirement and metabolism in parenterally fed postsurgical human neonates. Am J Clin Nutr 2008; 88(1):115-24.
151. Riedijk MA, Voortman G, van Beek RH, Baartmans MG, Wafelman LS, van Goudoever JB. Cyst(e)ine requirements in enterally fed very low birth weight preterm infants. Pediatrics 2008;121(3):e561-7.
152. Riedijk MA, van Beek RH, Voortman G, de Bie HM, Dassel AC, van Goudoever JB. Cysteine: a conditionally essential amino acid in low-birth-weight preterm infants? Am J Clin Nutr 2007;86(4):1120-5.
153. Weitzel LR, Wischmeyer PE. Glutamine in critical illness: the time has come, the time is now. Crit Care Clin 2010;26(3): 515-25, ix-x.
154. Luiking YC, Poeze M, Dejong CH, Ramsay G, Deutz NE. Sepsis: an arginine deficiency state? Crit Care Med 2004; 32(10):2135-45.
155. Furst P, Stehle P. What are the essential elements needed for the determination of amino acid requirements in humans? J Nutr 2004;134(6 Suppl):1558S-65S.
156. Reeds PJ. Dispensable and indispensable amino acids for humans. J Nutr 2000;130(7):1835S-40S.
157. Hertz DE, Karn CA, Liu YM, Liechty EA, Denne SC. Intravenous glucose suppresses glucose production but not proteolysis in extremely premature newborns. J Clin Invest 1993;92(4):1752-8.
158. Biolo G, De Cicco M, Lorenzon S, et al. Treating hyperglycemia improves skeletal muscle protein metabolism in cancer patients after major surgery. Crit Care Med 2008;36(6):1768-75.
159. Jahoor F, Shangraw RE, Miyoshi H, Wallfish H, Herndon DN, Wolfe RR. 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.
160. 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-37S.
161. Voisin L, Breuille D, Combaret L, 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.
162. Deval C, Mordier S, Obled C, et al. Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting. Biochem J 2001;360(Pt 1):143-50.
163. Smith IJ, Lecker SH, Hasselgren PO. Calpain activity and muscle wasting in sepsis. Am J Physiol Endocrinol Metab 2008;295(4):E762-71.
164. Wang X, Hu Z, Hu J, Du J, Mitch WE. Insulin resistance accelerates muscle protein degradation: Activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling. Endocrinology 2006;147(9):4160-8.
165. Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, Greenhaff PL. 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.
166. 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.
167. Chevalier S, Burgess SC, Malloy CR, Gougeon R, Marliss EB, Morais JA. The greater contribution of gluconeogenesis to glucose production in obesity is related to increased whole-body protein catabolism. Diabetes 2006;55(3): 675-81.
168. Tessari P, Inchiostro S, Biolo G, et al. Hyperaminoacidaemia reduces insulin-mediated glucose disposal in healthy man. Diabetologia 1985;28(11):870-2.
169. Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 2001;276(41):38052-60.
170. Patti ME, Brambilla E, Luzi L, Landaker EJ, Kahn CR. Bidirectional modulation of insulin action by amino acids. J Clin Invest 1998;101(7):1519-29.
171. Doig GS, Simpson F, Finfer S, et al. Effect of evidence-based feeding guidelines on mortality of critically ill adults: a cluster randomized controlled trial. Jama 2008; 300(23):2731-41.
172. Jain MK, Heyland D, Dhaliwal R, et al. Dissemination of the Canadian clinical practice guidelines for nutrition support: results of a cluster randomized controlled trial. Crit Care Med 2006;34(9):2362-9.
173. Jones NE, Suurdt J, Ouelette-Kuntz H, Heyland DK. Implementation of the Canadian Clinical Practice Guidelines for Nutrition Support: a multiple case study of barriers and enablers. Nutr Clin Pract 2007;22(4):449-57.
174. Obled C, Papet I, Breuille D. Metabolic bases of amino acid requirements in acute diseases. Curr Opin Clin Nutr Metab Care 2002;5(2):189-97.
175. Jones NE, Heyland DK. Pharmaconutrition: a new emerging paradigm. Curr Opin Gastroenterol 2008;24(2):215-22.
176. Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. Crit Care Med 2002;30(9): 2022-9.
177. Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. Jama 2001;286(8):944-53.
178. Marin VB, Rodriguez-Osiac L, Schlessinger L, Villegas J, Lopez M, Castillo-Duran C. Controlled study of enteral arginine supplementation in burned children:

impact on immunologic and metabolic status. Nutrition 2006;22(7-8):705-12.
179. Sheridan RL, Prelack K, Yu YM, et al. Short-term enteral glutamine does not enhance protein accretion in burned children: a stable isotope study. Surgery 2004;135(6):671-8.
180. Mayes T, Gottschlich MM, Kagan RJ. An evaluation of the safety and efficacy of an anti-inflammatory, pulmonary enteral formula in the treatment of pediatric burn patients with respiratory failure. J Burn Care Res 2008;29(1):82-8.

Chapter 10

Summary of the thesis

Chapter 1

Optimal nutritional and metabolic support to counteract ongoing catabolism and regain control of glucose homeostasis is essential in critically ill children. Nevertheless, nutritional goals as well as glycemic control are still not met in the Pediatric Intensive Care Unit (PICU), resulting in increased morbidity and mortality.

In the introduction of this thesis an overview is given on the major metabolic changes occurring during critical illness. Subsequently, the challenges and pitfalls in the development of recommendations and guidelines for nutritional and metabolic support on the PICU are described. This is followed by a summary on glucose metabolism and the potential benefits and risks of tight glucose control regimens. Finally, an overview is provided on the function of protein and amino acids, and the diverse variables that should be taken into account when investigating their specific requirements.

The first chapter ends with the aims and outline of the thesis.

Chapter 2

As is often the case in research as well as in clinical practice, the foundations are laid in adult medicine. This also applies to tight glucose control by means of insulin therapy. Based on the promising results of glycemic control on adult ICU's, we provided an extensive review that describes the causes and consequences of hyperglycemia in critically ill children. Additionally we provided an overview of the diverse (non)metabolic properties of insulin and speculated of their potential benefits to critically ill children. From a mechanistic point of view, several benefits are to be expected from insulin therapy and tight glucose control on the PICU. Finally, we provided a summary of the studies that have investigated insulin therapy in the pediatric population, with special emphasis on the single large outcome trial performed in critically ill children. This trial showed that although tight glucose control with insulin improves outcome in a similar fashion as reported in adult ICU's, hypoglycemia was a serious and frequent complication.

Chapter 3

Despite the early striking results of the tight glucose regimens in adult ICU's, there is still an ongoing debate focusing on the unacceptable high risk of hypoglycemia. The same reaction followed when the results of the large trial on the PICU reported an incidence of hypoglycemia of 25%, which occurred predominantly (>80%) in infants. We evaluated a step-wise nurse driven glucose protocol on our PICU and focused specifically on infants less than one month old. We concluded that the young infants could be treated according to the protocol equally effective as older children. However, in contrast to studies evaluating glucose control protocols in older children we found a higher incidence of hypoglycemia (6.8%). Based on previous studies in infants as well

as animal models we proposed that infants are more vulnerable to develop hypoglycemia following insulin administration, due to a wide variation in the capacity to regulate both glucose and insulin metabolism. Therefore, infants and potentially also young children require a more cautious approach when treated for hyperglycemia with insulin. We adjusted the insulin starting dose for infants in our protocol.

Chapter 4

One of the observations in chapter 3 was that a large proportion of the infants treated according to the tight glucose protocol required insulin therapy for a short period of time. It is less likely that this subset of infants and children can benefit from this short treatment period, while they still are put at increased risk to develop hypoglycemia. In chapter 4 we described a study that showed that a reduced glucose intake, half of the current recommendations, could prevent hyperglycemia effectively and safely. By means of isotope tracer techniques we showed that the post-surgical infants (n=8) were well capable to sustain normoglycemia, and increased their endogenous glucose production. We also showed, with help of the relatively novel average deuterium enrichment method, that these infants predominantly used gluconeogenesis to produce glucose. Furthermore, we showed that reducing the glucose intake did not exacerbate the mild catabolic state these infants were in. This approach could therefore be useful to prevent or treat hyperglycemia in the initial phase of treatment when children are admitted to the PICU.

Chapter 5

This chapter showed the ontogeny of the splanchnic uptake and utilization of the essential amino acid methionine in critically ill children (n=24). We used stable isotope techniques in enterally fed infants, children and adolescents, and applied two kinetic models: the plasma methionine enrichment and the “intracellular” homocysteine enrichment. The plasma model underestimated methionine kinetics in children and adolescents but not in the infants.

The rates of methionine splanchnic uptake were higher in infants than adolescents. We also showed that the fate of methionine utilization in critically ill children differed with age. In the infants methionine was used primarily for synthesis of proteins and methionine-derived compounds, while the adolescents had high transsulfuration (oxidation) rates. All patients were in a negative methionine balance, a clear indication that the enteral supply of this specific amino acid is currently insufficient.

Chapter 6

In this chapter, we provided an exploratory overview of the amino acid intake of children of all age groups receiving full parenteral nutrition who were admitted to the

PICU during a 12-month period. Using the recommended intakes from the Institute of Medicine (IOM) as reference we compared the intakes of the essential amino acids (EAA) to those actually provided to critically ill children. The non-essential amino acids (NEAA) were compared with the content of breast milk (infants) or mixed muscle protein (older children).. As the recommended intakes from the IOM are not developed for critically ill children we further contrasted EAA and NEAA intakes with the composition of representative inflammatory/immune proteins, because these proteins, not muscle proteins, are the major source of amino acid utilization for protein synthesis during critical illness. Our data showed that currently, EAA as well as NEAA are not provided through evidence based guidelines but based on convenience. This results in both insufficient, but also excessive amounts of intakes. The latter is also of importance as amino acids potentially can be toxic.

Chapter 7

In the study described in this chapter, the effect of an increased parenteral amino acid intake, with and without insulin infusion, on glucose metabolism, protein kinetics and lipolysis was investigated. On two subsequent days a stable isotope tracer infusion in combination with a hyperinsulinemic euglycemic clamp (HEC) was performed in nine septic adolescents. This two day study allowed us to show that providing parenteral amino acids, twice the amount currently recommended, improved whole body protein balance independent of hyperinsulinemia.

By means of the HEC technique we showed that hyperglycemic septic adolescents were insulin resistant. Moreover, insulin infusion in these adolescents did not lead to whole body protein anabolism and did not suppress lipolysis.

Despite the positive effect on whole body protein balance, the increased parenteral amino acid intake also led to increased lipolysis and potentially enhanced gluconeogenesis. These aspects make us aware that increasing the amino acid intake can potentially lead to increased insulin resistance.

Chapter 8

The same eight post-surgical infants and nine septic adolescents described in chapter 4 and 7 were used for this study. In these two separate studies the stable isotope tracer of the amino acid leucine was infused to measure leucine and protein kinetics. In the study described in chapter 8 albumin synthesis rates were calculated by measuring the increase of incorporated tracer leucine into albumin over time. This allowed us to describe the albumin synthesis rates in post-surgical infants as well as septic adolescents. We described that both the infants as well as the adolescents were slightly hypoalbuminemic, but exerted high synthesis rates. This latter was more obvious in the septic adolescents, which is in agreement with their higher catabolic state. In the

infants, the intake of glucose changed over time. In the adolescents, the amount of parenteral amino acids as well as the plasma insulin levels changed over time. We observed no differences in fractional as well as absolute albumin synthesis rates with any of these interventions. We concluded that the synthesis rates are regulated through other pathways than nutrient signaling.

Chapter 9

This chapter provides a general discussion in which the major findings of the studies presented in this thesis are critically analyzed and put into a wider perspective with help of the current literature. With help of these considerations some recommendations and future perspectives for the clinicians as well as researchers are presented.

Chapter 11

Samenvatting voor niet-medici

Hoofdstuk 1

Kritisch zieke kinderen op de intensive care (IC) worden behandeld voor hun ziekte, terwijl ze vaak met beademing en medicijnen ondersteund worden voor hun ernstig zieke organen zoals longen, hart, nieren en lever. Kinderen die zo ziek zijn kunnen niet op een normale manier eten en drinken, terwijl een goede voeding wel belangrijk is voor hun herstel. Het voeden van kritisch zieke kinderen wordt nog verder gecompliceerd omdat ook hun stofwisseling van suikers, eiwitten en vetten niet meer normaal verloopt. In hoofdstuk 1 wordt kort een overzicht gegeven van de belangrijkste stofwisselingsproblemen bij kritisch zieke kinderen op de IC. Dit zijn een te hoge bloedsuiker, en afbraak van vetten en lichaamseiwitten, voornamelijk de eiwitten in spieren. Deze problemen kunnen worden veroorzaakt door medicijnen, door onvoldoende (goede) voeding, maar ook door veranderingen van het lichaam als reactie op het kritisch ziek zijn. Eén van deze veranderingen is een verminderde gevoeligheid voor het hormoon insuline, wat belangrijk is voor een normale stofwisseling. Door deze veranderingen in de stofwisseling worden de patiënten nog zieker, waardoor ze vaak langer op de intensive care of in het ziekenhuis behandeld moeten worden, of waardoor ze soms zelfs overlijden. Optimale voeding en ondersteuning van de stofwisseling zijn daardoor van het grootste belang. Echter, de kennis van voeding voor kinderen op de IC is nog onvoldoende, en veel voedingsproducten worden waarschijnlijk niet in de goede hoeveelheid gegeven of zijn van onvoldoende kwaliteit. De samenstelling en de hoeveelheid van veel van de huidige voedingen voor kritisch zieke kinderen zijn gebaseerd op het beleid van volwassenen of op kleine en soms zelfs verouderde studies. De belangrijkste problemen en vraagtekens die er momenteel nog zijn rondom de voeding en ondersteuning van de stofwisseling bij kritisch zieke kinderen worden samengevat in hoofdstuk 1. Allereerst in een korte uitleg van de verstoorde suiker stofwisseling en de mogelijke voordelen maar ook risico's van de behandeling met het hormoon insuline, zoals dat bij suikerpatiënten wordt gedaan. Van insuline is bekend dat het bloedsuikerwaarden kan normaliseren, maar ook dat het de afbraak van eiwitten en vetten kan tegen gaan. Er zijn ook risico's verbonden aan een dergelijke behandeling, zoals te lage bloedsuiker waarden. Een te lage bloedsuiker waarde kan gevaarlijk zijn, terwijl het niet eens zeker is of insuline hetzelfde gunstige effect heeft bij kritisch zieke kinderen als dat het heeft bij kritisch zieke volwassenen. Daarna wordt in hoofdstuk 1 kort ingegaan op de functie van eiwitten en aminozuren (dit zijn de bouwstenen van eiwitten), welke belangrijk zijn voor kritisch zieke kinderen. Het bestuderen van de voedingsbehoeften van eiwitten en aminozuren is lastig en er wordt kort uitgelegd waar men bij dit soort onderzoeken rekening mee moet houden. Het eerste hoofdstuk eindigt met de doelstellingen en het overzicht van de studies verricht in dit proefschrift. Het proefschrift bestaat grofweg uit vier delen; deel 1 (hoofdstuk 2 t/m 4) gaan over de stofwisseling van suikers en de mogelijke effecten en risico's van insuline behandeling.

Deel 2 (hoofdstuk 5 en 6) gaat over de eiwitten en aminozuren in de voeding, en over de gevolgen die de leeftijd en de manier van voeden van het kind hebben op de eiwit stofwisseling en voeding. Deel 3 (hoofdstuk 7 en 8) beschrijft hoe het veranderen van de hoeveelheid energie en eiwitten in de voeding ook effect heeft op elkaar. En deel 4 (hoofdstuk 9 en 10), tot slot, geeft een uitgebreide discussie en samenvatting van de belangrijkste onderwerpen en conclusies van het gehele proefschrift.

Hoofdstuk 2

Vaak worden zowel in onderzoek, als ook in de kliniek, behandelingen en ideeën voor kritisch zieke kinderen gevolgd die succesvol zijn bij volwassen patiënten. Dit geldt ook voor de behandeling van te hoge bloedsuikers met insuline op de intensive care, ook bij patiënten die geen suikerziekte hebben. Bij kritisch zieke volwassenen is sinds een aantal jaren bekend dat het verlagen van hoge bloedsuikers met insuline zorgt voor een verbetering van het herstel en zelfs de overleving. Gebaseerd op de veelbelovende resultaten van deze behandeling bij patiënten op de volwassenen IC, geven wij in hoofdstuk 2 een uitgebreid overzicht van de oorzaken en de gevolgen van hoge bloedsuikers in kritisch zieke kinderen. Bovendien geven wij een overzicht van de diverse effecten van insuline bij kritisch zieke zuigelingen en kinderen. Op basis van deze gegevens speculeerden wij over de mogelijke voordelen van het geven van insuline aan kritisch zieke kinderen. Daarnaast vatten we de studies samen die insuline als therapie hebben onderzocht bij zuigelingen en kinderen op de IC. De nadruk van dit gedeelte van hoofdstuk 2 ligt dan op de enige grote studie die in kritisch zieke kinderen werd uitgevoerd. Deze studie toonde aan dat het behandelen van hoge bloedsuiker waarden met insuline een verbetering gaf van de overleving van kritisch zieke kinderen, zoals dat ook op de volwassen IC werd gezien. Echter, een gevolg van deze insuline behandeling was wel dat veel kinderen lage bloedsuiker waarden kregen, iets wat bij kinderen een ernstige complicatie kan zijn. De oorzaken van deze te lage bloedsuiker waarden liggen naar onze mening in de lage bloedsuiker grenzen die worden nagestreefd in deze studie, de hoge startdoseringen van insuline en het feit dat de verpleegkundigen in deze studie zelf de insuline mochten aanpassen op basis van hun ervaring.

Hoofdstuk 3

Ondanks de goede resultaten van de insuline behandelingen bij volwassen patiënten op de IC, is er nog steeds een discussie gaande over de onaanvaardbare risico's van lage bloedsuikers. Dezelfde reactie volgde toen de resultaten van de enige grote studie op de kinder IC een lage bloedsuiker bij 25% van de kinderen veroorzaakte, waarvan het merendeel (meer dan 80%) bij zuigelingen. Zoals wij al beschreven in hoofdstuk 2 zijn de oorzaken van deze lage bloedsuikers naar onze mening; de lage bloedsuiker grenzen die worden nagestreefd in deze studie, de hoge startdoseringen van insuline

en het feit dat de verpleegkundigen in deze studie zelf de insuline mochten aanpassen. Op onze kinder IC bestaat er een insuline protocol voor kritisch zieke kinderen welke effectief en veilig is gebleken bij oudere kinderen. Omdat het merendeel van de lage bloedsuikers in de beschreven studie ontstonden bij zuigelingen, evalueerden wij het protocol dat op onze eigen kinder IC wordt gebruikt, en concentreerden ons specifiek op zuigelingen jonger dan één maand oud. Wij concludeerden dat de jonge zuigelingen volgens het protocol even goed behandeld kunnen worden als de oudere kinderen. Echter, terwijl hetzelfde protocol bij de oudere kinderen geen te lage bloed suikers deed ontstaan, vonden wij bij 6,8% van de zuigelingen een te lage bloedsuiker. Door deze studie en door andere studies gedaan in zuigelingen, maar ook in dieren stelden wij dat de zuigelingen kwetsbaarder zijn voor insuline behandeling, door een onrijpe stofwisseling van suiker en het hormoon insuline. Daarom vinden wij dat de zuigelingen, en mogelijk ook jonge kinderen, voorzichtiger behandeld moeten worden met insuline. Wij verlaagden de insuline dosering in ons protocol voor zuigelingen om in de toekomst te lage bloedsuiker waarden te voorkomen.

Hoofdstuk 4

Eén van de observaties in hoofdstuk 3 was dat een groot deel van de zuigelingen die met insuline werden behandeld, maar voor een hele korte periode (minder dan een dag) te hoge bloedsuiker waarden hadden. Het is minder waarschijnlijk dat deze zuigelingen en kinderen baat hebben van deze korte behandeling, terwijl het risico op de lage bloedsuiker waarden door de insuline blijft bestaan. Veel kinderen die maar voor een korte tijd te hoge bloedsuiker waarden hebben, zijn de kinderen in de begin periode van de opname op de kinder IC. Het verlagen van de hoeveelheid suiker in het infuus is voor deze kinderen mogelijk een manier om hoge bloedsuikers te voorkomen zonder dat insuline nodig is. In hoofdstuk 4 beschrijven wij een studie waarin we minder suiker gaven via het infuus aan zuigelingen na een operatie. We gaven deze kinderen gedurende vier uur een normale hoeveelheid suiker volgens de huidige aanbevelingen en gedurende vier uur ongeveer de helft van deze hoeveelheid. Wij toonden aan dat het geven van minder suiker tot normale bloedsuiker waarden leidde, zonder dat er lage bloedsuikers ontstonden. Deze studie maakte ook gebruik van infusen van stabiele isotopen. Dit zijn natuurlijke producten die overal, ook in ieders lichaam, voorkomen en die ons helpen om te kijken wat er met eiwitten en suikers gebeuren in zieke kinderen. Door middel van deze infusen met stabiele isotopen toonden wij aan dat de zuigelingen hun eigen bloed suikers prima op peil konden houden, en zij verhoogden hun eigen suiker productie tot het niveau waarop hun bloed suiker waarden normaal waren. Wij toonden ook aan dat deze zuigelingen voornamelijk geheel nieuwe suiker aanmaakten, en niet oude opgeslagen suiker gebruikten. Als laatste toonden wij aan dat het minder geven van suiker de afbraak van eiwit (door het ziek zijn) in hun lichaam

niet verergerde. Deze manier van voorkomen van hoge bloedsuikers kan daarom nuttig zijn als alternatief voor insuline behandeling in de beginfase wanneer de kinderen op de kinder IC worden opgenomen.

Hoofdstuk 5

De studie in dit hoofdstuk gebruikt ook weer de techniek met stabiele isotopen. Ditmaal om te kijken wat de invloed van leeftijd is op het verbruik van de darm van een aminozuur (bouwsteen van eiwitten) in kritisch zieke kinderen van verschillende leeftijden. De interesse in deze studie ging vooral uit naar Methionine, een aminozuur wat behalve voor de opbouw van eiwitten in het lichaam ook wordt gebruikt voor andere functies. Zo kan Methionine ook gebruikt worden om andere bouwstoffen te maken die je lichaamscellen en bloedvaten beschermen tegen schade van buitenaf. Dit geldt voor gezonde maar ook zieke individuen. Wij hebben in onze studie onderzoek gedaan naar zieke zuigelingen, kinderen en adolescenten die gevoed werden met sondevoeding. Wij toonden aan dat het met de leeftijd verschilt hoeveel Methionine in de darm wordt opgenomen en wat de darm ermee doet. De zuigelingen namen veel meer Methionine op in hun darmen dan adolescenten. Ook zagen we dat de zuigelingen de Methionine hoofdzakelijk gebruikten voor het maken van eiwitten, terwijl de adolescenten veel methionine verbruikten om mogelijk omgezet te worden naar de bouwstoffen die beschermen tegen schade van buitenaf. Tot slot zagen wij in deze studie dat de patiënten van alle leeftijden onvoldoende Methionine kregen via de voeding. Dit is een duidelijke aanwijzing dat de samenstelling van de voeding voor dit specifieke aminozuur momenteel onvoldoende is. Met deze studie toonden we aan dat het per leeftijd verschilt hoeveel aminozuren een ziek kind nodig.

Hoofdstuk 6

In dit hoofdstuk hebben we gedurende één jaar de voedingsstoffen, en dan voornamelijk de aminozuren, van alle kinderen op de kinder IC die voeding via het infuus ontvingen, onderzocht. In totaal zijn er 20 standaard aminozuren. Deze kunnen op verschillende manieren worden ingedeeld, en één manier om dat te doen is door ze te verdelen in essentiële (EAA) en niet-essentiële aminozuren (NEAA). Essentieel zijn de 8 aminozuren die niet door het lichaam zelf gemaakt kunnen worden, en niet-essentieel zijn de overige 12 die wel door het lichaam zelf gemaakt kunnen worden. We hebben gekeken naar de hoeveelheid van elke individueel aminozuur die aan de kinderen werd toegediend via het infuus. Deze waarden hebben we vervolgens vergeleken met de voedingsadviezen van het Instituut van Geneeskunde (IOM) zoals die gelden in de Verneigde Staten. Verder hebben we gekeken naar de samenstelling van borstvoeding als vergelijkingsmateriaal voor de voeding van zuigelingen. Echter, deze voedingsadviezen zijn niet bedoeld voor kritisch zieke kinderen en de samenstelling van voeding dient

anders te zijn als je ziek bent. Daarom hebben we gekeken hoeveel aminozuren er ongeveer nodig zijn om de belangrijkste ontstekingseiwitten te maken. Hoewel dit een theoretische manier is van het vergelijken van wat de kinderen mogelijk nodig zouden hebben en wat ze daadwerkelijk binnen krijgen, is dit momenteel het best mogelijke bewijs. Onze gegevens toonden aan dat momenteel, EAA evenals NEAA, niet worden verstrekt zoals de behoefte bij de kinderen van verschillende leeftijden vermoedelijk is, maar dat de samenstelling van voedingsinfusen is gebaseerd op gemak. Gemak voor de fabrikant omdat deze rekening moet houden met hoe oplosbaar de aminozuren zijn en hoe lang houdbaar de voeding is. Hierdoor worden een aantal aminozuren te weinig of helemaal niet gegeven. Ook worden andere aminozuren in grote hoeveelheden gegeven, dit terwijl zij mogelijk giftig kunnen zijn als ze teveel gegeven worden. Hiermee onderbouwden we ons vermoeden dat de aminozuren die zieke kinderen via het infuus krijgen niet in de juiste hoeveelheid wordt gegeven en dat er voor ieder aminozuur moet worden gekeken wat een kind op een bepaalde leeftijd nodig heeft als hij of zij ziek is.

Hoofdstuk 7

In de studie van hoofdstuk 7 hebben we gekeken of het geven van meer aminozuren, via het voedingsinfuus, met of zonder het toedienen van insuline de verstoorde eiwit balans in kritisch zieke adolescenten zou verbeteren. Normaal gesproken heeft het enkel geven van meer aminozuren of eiwitten geen effect als het lichaam ook niet meer energie krijgt om wat met die extra bouwstoffen te doen. Zoals uitgelegd in hoofdstuk 2 is van insuline bekend dat het de stofwisseling van suiker maar ook van eiwitten en vetten kan verbeteren. Daarom hebben we gekeken of we insuline kunnen gebruiken om ook de stofwisseling van eiwitten weer te normaliseren. Dit hebben we wederom gedaan met gebruik van stabiele isotopen infusen. De zieke adolescenten kregen twee dagen hun voedingsinfuus, waarvan één met een normale hoeveelheid eiwit en de andere met een dubbele hoeveelheid eiwit. Op beide dagen werd een infuus van stabiele isotopen gegeven, waarvan een periode in combinatie met een insuline infuus. De resultaten van de studie toonden aan dat er momenteel nog te weinig eiwit aan zieke adolescenten wordt gegeven en dat ze een negatieve eiwit balans hadden, terwijl het voedingsinfuus met de hoge eiwit een duidelijke verbetering gaf. Er waren hoge doseringen insuline nodig voor het verbeteren van de bloedsuiker waarden, maar insuline had geen positief effect op de eiwitbalans en verminderde de vet afbraak ook niet. Dit betekent dat de adolescenten ongevoelig waren voor insuline, niet alleen voor de suiker maar nog meer voor de eiwit en vetstofwisseling. Verder zagen we nog dat het geven van meer eiwit via het voedingsinfuus de eiwit balans verbeterde maar dat het ook ervoor zorgde dat er meer suiker werd gemaakt en dat er meer vet werd afgebroken. Hierdoor kunnen we ondanks de verbetering op de eiwitbalans nog niet

zeggen dat er in de toekomst zomaar meer eiwit gegeven moet worden aan zieke kinderen, want het heeft ook een mogelijk nadelig effect op de insuline gevoeligheid en dus de suiker en vetstofwisseling.

Hoofdstuk 8

In dit hoofdstuk wordt een studie beschreven die is verricht in dezelfde postchirurgische zuigelingen en zieke adolescenten die in hoofdstuk 4 en 7 worden beschreven. In deze twee afzonderlijke studies werd gebruikt gemaakt van stabiele isotopen. Het ging onder andere om het aminozuur Leucine om naar de eiwit balans te kijken in deze patiënten. Het aminozuur Leucine is een aminozuur wat ook gebruikt wordt om eiwitten op te bouwen. Van dit stabiele isotoop van Leucine konden we in hoofdstuk 8 gebruik maken om de aanmaaksnelheid te berekenen van één van de belangrijkste eiwitten in ons lichaam; albumine. Zoals eerder uitgelegd zijn aminozuren, zoals Leucine, bouwstenen voor eiwitten, zoals albumine. Als albumine wordt gemaakt in het lichaam, dit gebeurd in de lever, dan wordt dus ook het stabiele isotoop van Leucine ingebouwd in het eiwit albumine. Door dit isotoop van het aminozuur Leucine te meten konden we de aanmaaksnelheid van albumine berekenen in de zuigelingen en de adolescenten. Wij zagen dat de zowel de zuigelingen als de adolescenten een lage bloedwaarde van albumine hadden, iets wat vaak voorkomt als je ernstig ziek bent. Echter de aanmaaksnelheid was in alle patiënten hoog, dus de lage waarden worden niet verklaard door een lage aanmaak. Omdat de studies waren gedaan in dezelfde patiënten als in hoofdstuk 4 en 7 konden we meteen zien wat het effect van het geven van suiker, eiwit en insuline was op de aanmaak snelheid van albumine. Wij zagen in de zuigelingen geen verschillen tussen de veranderde suikerinfusen, en ook bij de adolescenten veranderde de hoeveelheid eiwit in het voedingsinfuus en de insuline niets aan de albumine aanmaak snelheid. Wij denken dan ook dat de aanmaak snelheid van albumine, en mogelijk andere eiwitten, in de lever van zieke patiënten, door andere signalen in het lichaam worden aangestuurd dan door voedingsstoffen.

Hoofdstuk 9

Dit hoofdstuk bespreekt de belangrijkste resultaten van alle studies die verricht zijn in dit proefschrift. Er wordt met behulp van de studies maar ook van de huidige literatuur ingegaan op de belangrijkste conclusies. Deze zijn onderverdeeld in een gedeelte over de suiker en insuline stofwisseling en een gedeelte over de aminozuren en eiwitten. Met een slag om de arm concludeerden wij dat kinderen, ook jonge zuigelingen effectief behandeld kunnen worden met insuline om hoge bloedsuiker waarden te behandelen, maar dat er meer studies nodig zijn om het gunstige effect hiervan aan te tonen. Ook denken wij dat er extra maatregelen nodig zijn, in het bijzonder bij jonge zuigelingen, om lage bloedsuikers te voorkomen. Een voorbeeld van een dergelijke maatregel is het

verlagen van de insuline dosering zoals wij dat hebben gedaan op onze kinder IC. Een ander voorbeeld is het verminderen van het glucose infuus direct na een operatie als alternatief voor insuline. Wij toonden aan dat deze maatregel effectief en veilig was. In het gedeelte over aminozuren en eiwitten concludeerden we dat zowel de kwantiteit als ook de kwaliteit van de aminozuren in de voeding onvoldoende zijn. Daarnaast toonden wij aan dat naast de leeftijd van het zieke kind ook de manier van voeden (via infuus of sonde) vraagt om een aangepaste voeding. In de broodnodige toekomstige studies dient dan ook rekening gehouden te worden met de leeftijd van het kind en de manier van voeden. In het laatste gedeelte concludeerden wij dat het geven van insuline om de stofwisseling van vetten en eiwitten te verbeteren bij ernstig zieke kinderen geen zin heeft. Tot slot geven we aan dat we denken dat er meer eiwit gegeven kan en moet worden aan zieke kinderen om hun eiwit balans te verbeteren. Echter omdat een hogere eiwitsamenstelling in de voeding mogelijk nadelige effecten heeft op de insuline gevoeligheid en de suiker- en vetstofwisseling, zijn er meer studies nodig voordat we dit als aanbeveling kunnen opnemen in voedingsadviezen en richtlijnen. Met hulp van deze overwegingen worden enkele aanbevelingen gedaan als opzet en richting voor toekomstig onderzoek.

Words of Thanks
-
Dankwoord

"I not only use all the brains that I have, but all that I can borrow"
Woodrow Wilson, (1856-1924, 28th President of the USA)

Hoewel dit voor mij de allerlaatste woorden zijn die ik aan “mijn boekje” toevoeg, zullen velen van jullie dit als eerste lezen. Dit zegt genoeg over het belang van deze laatste woorden. Zoals zo velen al voor mij hebben geschreven; ‘een proefschrift schrijven doe je niet alleen’, en ook dat van mij heb ik te danken aan velen.

Allereerst wil ik alle kinderen en hun ouders bedanken die hebben meegewerkt aan mijn studies.

Daarnaast, al is het alleen maar omdat ik niemand wil vergeten, wil ik iedereen bedanken voor de hulp, geruststelling, felicitaties, reddingen, ideeën, bezoeken in Houston, tips, etentjes, gezelligheid of gewoon simpelweg het tonen van interesse. Niet alleen tijdens de soms frustrerende vooruitgang van de onderzoeken, maar ook tijdens de soms hectische en vermoeiende perioden van de afgelopen jaren waren deze meer dan welkom.

Een aantal mensen wil ik hierbij in het bijzonder bedanken.

Prof. Dr. van Goudoever, beste Hans, *‘die kliniek geloof ik nu wel, maar nu moet je je maar eens wetenschappelijk bewijzen’*. Zo prikkelde je mijn ambitie op een NVK-congres in 2005. Ruim een jaar later, lag er een onderzoeksplan, was ik welkom op één van ‘s werelds meest gerenommeerde voedingsinstituten (even een nachtelijke dienst email) en hadden we zelfs de financiering rond. Je ongekende optimisme, energie en drang naar nieuwe ideeën zijn ontzettend inspirerend en je vertrek uit Rotterdam zal dan ook een gemis zijn. Ik hoop dat we nog lang kunnen samenwerken en ik wil je bedanken voor de kansen en mogelijkheden die je hebt gegeven om in ieder geval deze eerste stap te maken met dit proefschrift onder jouw bezielende leiding.

Dr. Joosten, beste Koen, voeding en intensive care, ik prijs me gelukkig dat jij mijn co-promotor wilde zijn. Je rust, geduld en kritiek hebben me meermaals gedwongen om te stoppen met haasten en te kijken waar ik nu werkelijk stond met mijn studies, resultaten en nieuwe ideeën. Dat bracht dan op het goede moment de rust en het juiste overzicht, wat dit proefschrift zeker ten goede is gekomen. Ik hoop nog veel meer studies met je te kunnen doen en te zorgen dat voeding op de kinder intensive care de aandacht krijgt die het verdient.

Prof. Castillo, dear Leticia, it all started with you welcoming me to come and work with you at the CNRC and the PICU at Texas Children’s Hospital. I cannot thank you enough for that invitation and opportunity. Everyone who reads this thesis can see the influence the work performed in Houston has on my research. Thank you for your patience to make me a better researcher and for forgiving me my impatience to become one.

Dr. Coss-Bu, dear Jorge, 'amigo', thank you for all your help during the studies in Houston. I miss all our conversations during the early hours of rounds or during the clamps, on the world's economic situation, American culture, cars and of course European soccer and 'la Naranja Mechanica'. Hopefully, Patrycja and I will see you soon.

Verder wil ik de kleine commissie, Prof. Dr. van der Heijden, Prof. Dr. Tibboel en Prof. Dr. Sauer bedanken voor hun deelname in de promotiecommissie en voor hun snelle beoordeling van het manuscript.

When you spend such an important part of your life in a strange and huge city such as Houston, Texas, you get to learn the dearest, strangest and most hard-working people. First, I want to thank everyone at the CNRC and the Texas Children's Hospital who helped me survive as a 'junior' researcher. My own research group, Debra, Jama, Eddie, Danny, Manhong and Jean, thanks for all the help at work and making sure I would finish the studies in time! Doug Burrin, your calm and sharp focus on research and nutrition are a true inspiration, and I am proud that you will be part of my thesis defense committee. Barbara and mr. Bob, thanks for all the Adobe margarita nights, the cave-man experiences and getting us to eat more health food and run a bit faster. Oh, and mr. Bob, thanks for keeping my lovely wife out of prison. The 'people downstairs', Shaji, Mahmoud, Trina, Lisa, Jean, and Gert; thanks for all the answers to my questions, helping out with the samples, the Wednesday Chipotle experiences, the Friday Gingerman beers, and of course the Breckenridge snowboard event. Monique and Robert, you guys showed us that in contrast to our prejudice, Houston is actually one large culinary happening. Thanks for all the dinners, drinks, conversations and taking us to those really strange and unique spots Houston has to offer. And finally, Marta, Afonso and Gert, you have become the definition of 'love conquers all'. Thanks for all of the above and so much more! The hours at the pool, the long days at work, the Texas camping weekend, crawfish festivals, the Rodeo, the world championship BBQ, all the beers, the talks and the never-ending Portuguese Noddy-experience. What would Houston have been without you guys?

Maar ook in Rotterdam is er natuurlijk een groep mensen die ik zeer dankbaar ben voor de afgelopen jaren. Ineke, die me wegwijs heeft gemaakt in de bureaucratische machine van de METC en de CCMO. Daniëlla, die al die tijd mijn *life-line* is geweest in de communicatie en agenda van Hans. De mensen achter de schermen, of in deze dan 'op het lab', zonder wie al dit werk nooit iets tastbaars was geworden; Henk, Gardi en Kristien, hartelijk dank. En natuurlijk de onderzoekers die nu nog zitten te dromen over het schrijven van hun dankwoord; Denise, Hester, Willemijn en Carlijn, hou vol, maar geniet ook van het maken van jullie eigen boekje. Margriet, leuk om betrokken te zijn

bij jouw eigen project. En natuurlijk, de mensen die me voor gingen; 'the boys' Frans en Chris en natuurlijk Maaike. Dank voor alle borrels, humor en congresbelevenissen. En bedenk, *"We will always have Hawaii…!".*

Al mijn collega's en verpleegkundigen op de intensive care kindergeneeskunde, hartelijk dank voor alle interesse in de voortgang van mijn onderzoek en jullie medeleven als ik er weer eens iets slechter uitzag dan in mijn vorige dienst. Nu dit is afgerond kan ik me weer volledig storten op onze prachtige afdeling. Ik heb er ontzettend veel zin in.

Mijn Paranimfen, Chris en Maurits. Chris, ik ben ongelofelijk blij dat je op zo'n bijzondere dag naast me staat. Voelt meteen een stuk gemakkelijker, alsof Johan Cruijf zelf op het schoolpleintje verschijnt om te coachen. Geweldig dat je de komende jaren nog in Rotterdam bent zodat we nog maar veel kunnen samenwerken op allerlei fronten. Maurits, held, weer moet je dan voor de zoveelste keer voor me opdraven in je mooie pak. Je hebt nu vrijwel alle mijlpalen wel meegemaakt, het is dat we elkaar 'pas' 15 jaar kennen, anders zou je nog gespeecht hebben bij mijn zwemdiploma's. Ik beloof je dat dit de laatste keer is geweest. Laten we nu maar weer eens een kroeg induiken.

Pap en mam, dit boekje is niet voor niets aan jullie opgedragen. Zonder jullie steun en hulp zou ik niet zijn wat ik nu ben en niet staan waar ik nu sta. Dit geldt niet alleen voor het afgelopen hectische jaar, maar zover als ik me kan herinneren.

Er wordt vaak gezegd; "Promoveren is net als trouwen in je eentje". Nou dat lijkt me dus geen donder aan! Lieve Patriets, wat geweldig om ook dit samen gedaan te hebben. Je vroeg me om in het dankwoord niet teveel kleffe woorden voor je te schrijven…… Lief, wees niet bang, want er zijn geen woorden klef genoeg om duidelijk te maken hoe belangrijk jij voor me bent.
Allerliefste Isa, wat heerlijk dat het afgelopen half jaar lekker aan je voorbij is gegaan. Als je toch eens wist hoe ongelofelijk veel leuker jij het leven van mama en mij hebt gemaakt tussen al dat werken en schrijven door. *"Nog meer jij is fantastig toch.."*
Jet, dank dat je me zo veel achter de laptop hebt weggehaald en in beweging hebt gehouden…..*vang!!....*

Sascha

Curriculum Vitae

Sascha Verbruggen was born on the 31st of March 1976 in Nijmegen. He finished high school at the Dominicus College in Nijmegen in 1994 and obtained his medical degree at the Maastricht University in Maastricht in 2000 (clear pass). During his medical training he finished a research project at the Pediatric Intensive Care Unit of the Red Cross Children's Hospital in Cape Town, South Africa in 1998, and a clinical internship pediatrics at the Witbank Hospital in Pretoria, South Africa in 2000. He worked as a pediatric resident (ANIOS) at the department of pediatrics at the Orbis Medical Center in Sittard in 2001 and in the St. Radboud Academic Hospital in Nijmegen in 2002. In January 2003 he started as a pediatric resident (AIOS) at the Erasmus MC – Sophia Children's Hospital in Rotterdam (Prof. Dr. A.J. van der Heijden, Dr. M. de Hoog) and at the Amphia hospital in Breda (Dr. A.A.P.H. Vaessen-Verberne). After he finished his training as a pediatrician in July 2007, he started to work on his dissertation (promotor Prof. Dr. J.B. van Goudoever, co-promotor Dr. K.F.M. Joosten) on 'Nutritional and metabolic support in critically ill children' at the Children's Nutrition Research Center, Baylor College of Medicine, and the Texas Children's Hospital in Houston, Texas, USA, under supervision of Prof. Dr. L. Castillo. In September 2008 he started his clinical work as pediatrician on the Pediatric Intensive Care Unit at the Erasmusm MC – Sophia children's hospital and continued his work on his dissertation. Sascha lives with his wife Patrycja Puiman and their daughter Isa in Rotterdam.

Affiliation of co-authors

Author	*Affiliation*
Arrivillaga, Ana	Department of Critical Care Medicine, Texas Children's Hospital, Houston, TX, USA
Burrin, Douglas	Children Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
Castillo, Leticia	Division of Critical Care Medicine, Children's Medical Center Dallas, University of Texas Southwestern, Dallas, TX, USA
Chacko, Shaji	Children Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
Coss-Bu, Jorge	Department of Critical Care Medicine, Texas Children's Hospital, Houston, TX, USA
de Betue, Carlijn	Department of Pediatric Surgery, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Gordon, William E.	Department of Critical Care Medicine, Texas Children's Hospital, Houston, TX, USA
Hsu, Jean	Children Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
Joosten, Koen	Department of Pediatric Critical Care Medicine, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Landzaat, Lonneke	Department of Pediatric Critical Care Medicine, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Reiss, Irwin	Department of Pediatric Surgery, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Schierbeek, Henk	Department of Pediatrics, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Sy, Jama	Department of Critical Care Medicine, Texas Children's Hospital, Houston, TX, USA
van Adrichem, Leon	Department of Plastic surgery, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
van Goudoever, Hans	Department of Pediatrics, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands

	Department of Pediatrics, Academic Medical Center – Emma Children's Hospital, Amsterdam, The Netherlands
	Department of Pediatrics, VU University Medical Center, Amsterdam, The Netherlands
Verhoeven, Jennifer	Department of Pediatric Critical Care Medicine, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands
Wu, Manhong	Stanford University, San Francisco Bay Area, CA, USA
Zurakowski, David	Departments of Anesthesia and Surgery, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA

List of Publications

- Verbruggen SC, Wijnen RM, van den Berg P. Megacystis-microcolon-intestinal hypoperistalsis syndrome. J Matern Fetal Neonatal Med. 2004;16(2):140-1.
- Verbruggen SC, Corel LJ, Tiddens HA, Joosten KF, de Hoog M. Fataal astma op de kinderleeftijd te voorkomen door herkenning van risicofactoren en presentatiewijze. Ned Tijdschr Geneeskd. 2006;150(5):225-9
- Verbruggen SCAT, Tiddens HAWM, Hanff LM, de Hoog M. Te veel salbutamol, te weinig inhalatiesteroïden. Pharm Weekbl 2006;141(42):1416-8.
- Verbruggen SC, Catsman CE, Naghib S, Lequin MH, van der Lely N, Buysse CMP. Acute Disseminerende EncephaloMyelitis (ADEM). Ned Tijdschr Geneeskd. 2006;150(20):1134-8
- Verbruggen SC, Joosten KF, Castillo L, van Goudoever JB. Insulin therapy in the pediatric intensive care unit. Clin Nutr. 2007;26(6):677-90.
- Joosten K, Verbruggen SC, Verhoeven JJ. Glycaemic control in paediatric critical care. Lancet. 2009;373(9673):1423-4
- Verbruggen S, Sy J, Gordon WE, Hsu JW, Wu M, Chacko SK, Zurakowski D, Burrin DG, Castillo L. Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am J Physiol Endocrinol Metab. 2009;297; E1046-E1055
- Verbruggen S, Sy J, Arrivillaga A, Joosten K, van Goudoever J, Castillo L. Parenteral Amino Acid Intakes in Critically Ill Children: A Matter of Convenience? J Parenter Enteral Nutr. 2010;34(3):329-40.
- Verbruggen S, Coss-Bu J, Wu M, Schierbeek H, Joosten K, van Goudoever J, Castillo L. Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin resistant children with tight glucose control. *Provisionally accepted Crit Care Med*
- Verbruggen S, de Betue C, Schierbeek H, Chacko S, Verhoeven J, van Goudoever J, Joosten K. Reducing Glucose Intake Safely Prevents Hyperglycemia In Post-Surgical Children. *Submitted*
- Verbruggen S, Schierbeek H, Coss-Bu J, Joosten K, Castillo L, van Goudoever J. Albumin synthesis rates in post-surgical infants and septic adolescents; influence of amino acids, energy, and insulin. *Submitted*
- Verbruggen S, Landzaat L, Reiss I, van Goudoever J, Joosten K. Efficacy and safety of a tight glucose control protocol in critically ill term infants. *Submitted*

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: S.C.A.T. Verbruggen, MD.	PhD period: 2007 – 2010
Erasmus MC Department: Pediatric Intensive Care	Promotor: Prof. J.B. van Goudoever, MD. PhD.
Research School: Erasmus MC	Supervisor: K.F.M. Joosten, MD. PhD.

1. PhD training

	Year	Workload ECTS
General courses		
- Clinical Investigation Course; Baylor College of Medicine, Houston, USA	2007	2.0
- Classical Methods for Data Analysis; NIHES, Erasmus MC	2009	4.0
Specific courses		
- Isotope Tracers in Metabolic Research; University of Arkansas for Medical Sciences, Little Rock, USA	2007	2.0
Seminars		
- Research seminars, Children's Nutrition Research Center, Baylor College of Medicine, Houston, USA	2007- 2008	1.0
- Research Fellow Symposium, Baylor College of Medicine, Houston, USA	2007- 2008	0.8
- Research bespreking kindergeneeskunde, Erasmus MC	2008 - 2010	0.6
- Research bespreking 'Moeder en Kind Centrum', Erasmus MC	2008 - 2010	0.6
International conferences		
- American Society of Nephrology, San Francisco, USA	2007	1.0
- Society of Critical Care Medicine, Honolulu, USA	2008	1.0
- Federation of American Societies for Experimental Biology, San Diego, USA	2008	1.0
- Society of Pediatric Research, Honolulu, USA	2008	1.0
- European Society of Pediatric Research, Nice, France	2008	1.0
- Federation of American Societies for Experimental Biology, New Orleans, USA	2009	1.0
- European Society of Pediatric Research, Hamburg, Germany	2009	1.0
- Benelux Association of Stable Isotope Scientist, Arnhem, The Netherlands	2010	0.4
- European Society of Pediatric Research, Copenhagen, Denmark	2010	1.0

Poster Presentations		
- Methionine Cycle Kinetics and Arginine Supplementation in Endothelial Dysfunction of ESRD, American Society of Nephrology, San Francisco, USA	2007	1.0
- Effect of Arginine or Folic Acid Supplementation on Cysteine kinetics in End-Stage Renal Disease, American Society of Nephrology, San Francisco, USA	2007	1.0
- Energy Expenditure in Patients with End-Stage Renal Disease, American Society of Nephrology, San Francisco, USA	2007	1.0
- Methionine Splanchnic Uptake in Critically Ill Infants, Society of Critical Care Medicine, Honolulu, USA	2008	1.0
- Insulin Resistance and Protein Metabolism in Pediatric Critical Illness, Society of Critical Care Medicine, Honolulu, USA	2008	1.0
- Parenteral Amino Acid Intakes in Critically Ill Children, Federation of American Societies for Experimental Biology, San Diego, USA	2008	1.0
- Methionine Splanchnic Uptake is increased in Critically Ill Children, Federation of American Societies for Experimental Biology, San Diego, USA	2008	1.0
- Parenteral Amino Acid Intakes in Critically Ill Children: A Matter of Convenience?, Federation of American Societies for Experimental Biology, San Diego, USA	2008	1.0
- Arginine supplementation improves insulin resistance in Obese Adolescents, Federation of American Societies for Experimental Biology, New Orleans, USA	2009	1.0
Oral Presentations		
- Insulin Resistance and Protein Metabolism in Critically Ill Children, European Society of Pediatric Research, Nice, France	2008	1.4
- Albumin Synthesis Rates in Critically Ill Adolescents; Effect of Insulin and Protein Intake, European Society of Pediatric Research, Hamburg, Germany	2009	1.4
- Albumin Synthesis Rates in Critically Ill Adolescents; Effect of Insulin and Protein Intake, Benelux Association of Stable Isotope Scientist, Arnhem, The Netherlands	2010	1.4
- Tight glucose regimen in critically ill infants, European Society of Pediatric Research, Copenhagen, Denmark	2010	1.4

2. Teaching activities

	Year	Workload ECTS
Lecturing		
- Education for PICU nursing staff; 'Tight Glucose regimen on the PICU', Erasmus MC	2008	1.5
- Pediatric Intensive Care Fellow Course, Nijmegen; 'tight glucose control' on our PICU's, what's the evidence?'	2009	0.8
Supervising Master's theses		
- Marianne Koenraads, medical student, University of Maastricht	2009	1.0