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ON THE LONG-TERM FOOTPRINT OF PEDIATRIC CRITICAL ILLNESS AND HOW THIS IS AFFECTED BY ACUTE MACRONUTRIENT RESTRICTION

An JACOBS

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LIST OF ABBREVIATIONS

11β-HSD2 - 11β-hydroxysteroid-dehydrogenase type 2

ACTH - Adrenocorticotropic hormone

Allo-THE - 5α-tetrahydrocortisone

Allo-THF - 5α-tetrahydrocortisol

ANT - Amsterdam neuropsychological task battery

ASPEN - American Society for Parenteral and Enteral Nutrition

BMI - Body mass index

BRIEF - Behavior rating inventory of executive function

CBCL - Child behavior checklist

CBG - Cortisol-binding globulin

CI - Confidence interval

CIRCI - Critical illness related corticosteroid insufficiency

CMS - Children's memory scale

CRH - Corticotropin-releasing hormone

CS - Corticosteroids

CSPEN - Chinese society of parenteral and enteral nutrition

D1 - Type 1 deiodinase

D2 - Type 2 deiodinase

D3 - Day 3 in PICU

D3 - Type 3 deiodinase

DNA - Deoxyribonucleic acid

E - Cortisone

ECMO - Extracorporeal membrane oxygenation

EN - Enteral nutrition

EPaNIC - Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically ill Patients

ERC - European research council

ESPEN - European society for clinical nutrition and metabolism

ESPGHAN - European society for pediatric gastroenterology hepatology and nutrition

ESPNIC - European society of pediatric and neonatal intensive care

ESPR - European society of pedatric research

F - Cortisol

FWO - Fonds wetenschappelijk onderzoek

HC - hydrocortisone

HPA – Hypothalamic-pituitary-adrenal

HPT - Hypothalamic-pituitary-thyroid

HR - Hazard ratio

ICU - Intensive care unit

IFNγ - Interferon gamma

IL1α - Interleukin-1 alpha

IL1β - Interleukin-1 beta

IL6 - Interleukin-6

IQ - Intelligence quotient

IQR - Interquartile range

IU - Internation units

IV - Intravenous

IWT - Instituut voor wetenschap en technologie

LC-MS/MS - Liquid chromatography-tandem mass spectrometry

LD - Last day of PICU-stay

mRNA - Messenger ribonucleic acid

NA - Not applicable

NEJM - New England Journal of Medicine

NICU - Neonatal intensive care unit

NIS - Natrium/iodide symporter

NTI - Non-thyroidal illness syndrome

OR - Odss ratio

PCA - Principal component analysis

PeLOD score - Pediatric logistic organ dysfunction score

PEPaNIC - Early versus Late Parenteral Nutrition in the Pediatric ICU

PICU - Pediatric intensive care unit

PIM2 score - Pediatric index of mortality 2 score

PIM3 score - Pediatric index of mortality 3 score

PN - Parenteral nutrition

PRO - Patients/parents-reported outcomes

RCT - Randomized controlled trial

RIA - Radioimmunoassay

RNA - Ribonucleid acid

RT - Reaction time

rT3 - Reverse triiodothyronine

SD - Standard deviation

SEM - Standard error of the mean

STRONG - Screening tool risk on nutritional status and growth

T3 - Triiodothyronine

T4 - Thyroxine

TBG - thyroxine-binding globulin

TBI - Traumatic brain injury

TBM - Toegepast biomedisch onderzoek met een primair maatschappelijke finaliteit

TGC – Tight glycemic control

THE - Tetrahydrocortisone

THF - 5β-tetrahydrocortisol

TNFα - Tumor necrosis factor alpha

TRH - Thyrotropin-releasing hormone

TSH - Thyroid-stimulating hormone

VMI - Visuomotor integration

WAIS - Wechsler adult intelligence scale

WFPICCS - World Federation of Pediatric Intensive and Critical Care Societies

WIQS - Wechsler intelligence quotient scale

WISC - Wechsler intelligence scale for children

WPPSI - Wechsler preschool and primary scale of intelligence

CHAPTER 1 - GENERAL INTRODUCTION

Parts of this chapter have been adapted from:

Jacobs A, Vanhorebeek I, Van den Berghe G. Nonthyroidal illness in critically ill children. Curr Opin Endocrinol Diabetes Obes 2019:26;241-249.

Jacobs A, Verlinden I, Vanhorebeek I, Van den Berghe G. Early supplemental parenteral nutrition in critically ill children: an update. J Clin Med 2019;8.

1. PEDIATRIC CRITICAL ILLNESS

Critical illness is defined as any life-threatening condition that requires extensive support of vital organ functions to avoid imminent death. This therapy often consists out of combination of pharmacological and mechanical support, such as respiratory and hemodynamic support, antibiotics, and fluid and renal replacement therapy, which takes place in the pediatric intensive care unit (PICU). Reasons for admission to the PICU cover a broad range of possibilities. Elective admissions, such as after invasive congenital heart surgery, count for a relatively large part of the admissions. In addition, children admitted to the emergency department in need of intensive care and children admitted to the general pediatric ward who need escalation of care can be referred to the PICU as emergency admissions.

As compared with critically ill adults, critically ill children are characterized by both similarities and differences. In general, the idea of stabilizing the patient with supportive therapy while monitoring his or her condition closely is applicable for both adults and children. In addition, the content of this supportive therapy is often similar. Differences frequently lie in the underlying medical or surgical diagnosis. Specific pathologic entities that occur only in infants and children, such as bronchiolitis, pediatric oncological and hematological diseases, inherited metabolic problems and congenital cardiac pathologies require more specific expertise and care. Also, dissimilarities in pharmacological measures are applicable, because of differences in circulating volumes and permeability of the blood brain barrier as compared with critically ill adults. Finally, a substantial difference lies in the incorporation of the specific role of the critically ill patient as the child of his/her parents. Challenges on the psychosocial needs of parents, which mostly comprise being in the proximity to the child and getting regular access to information,[1] are an extra dimension to the care provided in the PICU.

Over the last few decades, an evolution has become apparent in the area of critical illness in children. Because of improvement in preventive measures such as increasing vaccination rates, advancements in therapies both in and outside the PICU[2] and specialist staff training and education,[3,4] mortality rates in the PICU have decreased substantially.[5] In addition, the combination of centralization of pediatric intensive care services with transport of critically ill children from other hospitals by specialist retrieval teams has contributed to improved survival.[1,6] However, among the increasing number of survivors in the PICU, the number of children who have a moderate or severe long-term disability increases.[5,7,8] Such long-term disability leads to important emotional, practical and financial implications for patients and their families. Thus, an evolution

in clinical research is warranted. The focus on preventing mortality should shift to morbidity end points and should incorporate the evaluation of the quality of life and daily functioning of PICU survivors and their families. This opened up a highly interesting field in clinical research in the PICU, in which thinking outside the box and critical re-evaluation of existing practices are warranted.

2. PARENTERAL NUTRITION IN THE PICU

Optimal nutritional support is considered of paramount importance in critically ill children admitted to the PICU, since malnutrition and inadequate nutrient delivery have been associated with worse clinical outcome.[9,10] Moreover, critically ill children have limited macronutrient stores and relatively higher energy requirements than adults admitted to the ICU, which can lead to substantial caloric and macronutrient deficits.[10-12] The feeding is thought to attenuate the metabolic stress response, prevent oxidative cellular injury and modulate immune responses, which has led to a shift from nutritional support as adjunctive care to actual therapy of the critically ill child.[13] The enteral route is preferred to provide nutrition.[13] However, critically ill children are often too ill to be fed normally by mouth and nasogastric or nasoduodenal tube feeding is often not tolerated because of gastric dysmotility or ileus. Interruption of enteral feeding also occurs frequently because of various reasons, like medical or surgical contraindications, and radiology, bedside or surgical procedures.[14] Therefore, parenteral nutrition (PN) is often initiated to supplement the insufficient enteral intake. Nonetheless, official guidelines on timing and thresholds of initiation, composition, and doses of supplemental PN are varying widely.[13,15-17] Moreover, concerns about overfeeding have led to even more uncertainty.[17] A recent survey showed significant differences in nutritional practices in PICUs worldwide, in terms of macronutrient goals, estimation of energy requirements, timing of nutrient delivery, and thresholds for starting supplemental PN.[18]

Timing of PN initiation

Several observational studies have shown that malnutrition is associated with worse clinical outcome.[9,10,19] A macronutrient deficit has been associated with infections, weakness, prolonged mechanical ventilation, and delayed recovery. Therefore, guidelines used to recommend that when provision of enteral nutrition (EN) is insufficient, impossible or contra-indicated, supplemental PN should be initiated.[15,20,21] However, observational studies cannot assign causality to an association. Hence, the association between inadequate

nutrition and worse clinical outcome might merely exist because of a non-optimal nutritional support for the sickest children, which are at the highest risk of adverse outcome. Although it seems intuitive that providing early nutrition will be beneficial, it does not necessarily mean that nutritional support in the early phase of critical illness will improve clinical outcome.[17] In critically ill adults, the large multicenter EPaNIC (Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically Ill Patients, n=4640) randomized controlled trial (RCT) showed that withholding of supplemental PN until day 8 of ICU stay (late-PN), thus accepting a substantial macronutrient deficit, was associated with fewer ICU infections, a shorter duration of mechanical ventilation and renal replacement therapy, and a shorter ICU and total hospital stay as compared with initiating supplemental PN early (within 48 hours after ICU admission).[22] Data generated by the broad international yearly survey of clinical nutrition practices "nutritionDay" revealed an important change in the pattern of PN prescription after publication of the EPaNIC results (Unpublished data in preparation for publication, kindly shared by Prof. Dr. M. Hiesmayr, nutritionDay Project Leader). As compared with adults, critically ill children have limited stores of energy, fat and protein, and they also have relatively higher energy requirements.[10,11] Since this makes them more vulnerable for a substantial caloric and macronutrient deficit, the effect of withholding supplemental PN in critically ill children could be different from adults. Therefore, the multicenter PEPaNIC RCT (Early versus Late Parenteral Nutrition in the Pediatric ICU) was conducted by our group,[23] investigating the same intervention in 1440 critically ill children aged 0-17 years in three PICUs in Belgium, The Netherlands and Canada. Withholding supplemental PN during the first week in critically ill children resulted in fewer new infections, a shorter dependency on mechanical ventilation and general intensive care, and a shorter hospital stay as compared with providing PN early, within 24 hours after PICU admission (**Figure 1**). The clinical superiority of late-PN was more pronounced in children than it was in adults, and was shown irrespective of diagnosis, severity of illness, risk of malnutrition, or age of the child.[23] This last finding was surprising since neonates are more susceptible to macronutrient deficits than older children,[15] which raised concerns by experts.[24-26] To address these concerns, a secondary analysis of the PEPaNIC RCT was performed, which investigated the effects of withholding PN for 1 week in the 209 critically ill neonates, who did not, or hardly, tolerated any EN.[27] Analyses were performed for term neonates aged up to 4 weeks, up to 1 week and younger than 1 day. Late PN resulted in fewer nosocomial infections in neonates aged up to 1 week and younger than 1 day, and in shorter dependency on intensive care and mechanical ventilation for all studied age groups of neonates. Hence, also term neonates benefited from withholding PN during the first week in PICU, in agreement with findings for older children and adults.[22,23] Moreover, there is a more pronounced benefit of late PN in the youngest children, as shown by **Figure 1**. Since a macronutrient deficit was presumed to be more detrimental during acute illness in undernourished children,[28] a second sub-analysis of the PEPaNIC RCT was performed, investigating the effects of withholding supplemental PN during

the first PICU week in the subgroup of critically ill children who were undernourished upon admission to the PICU.[29] Undernourishment was defined as a weight-for-age z score lower than -2 in children younger than 1 year, and a body mass index-for-age z score lower than -2 in children 1 year or older. This identified 289 of 1440 PEPaNIC patients (20%) with undernourishment upon PICU admission. Among the undernourished patients, late PN reduced the absolute risk of new infections and shortened the duration of PICU stay. These effect sizes of late PN were even larger than in the main trial cohort of the PEPaNIC RCT. Late PN did not affect the safety outcomes mortality, incidence of hypoglycemia and weight deterioration during PICU stay in the undernourished patients. A larger longitudinal study of all PEPaNIC patients with weight z scores available on admission and on the last day in PICU showed that weight deterioration during PICU stay was associated with worse clinical outcomes, but that withholding supplemental PN during the first week did not alter the weight z score deterioration during PICU stay.[30]

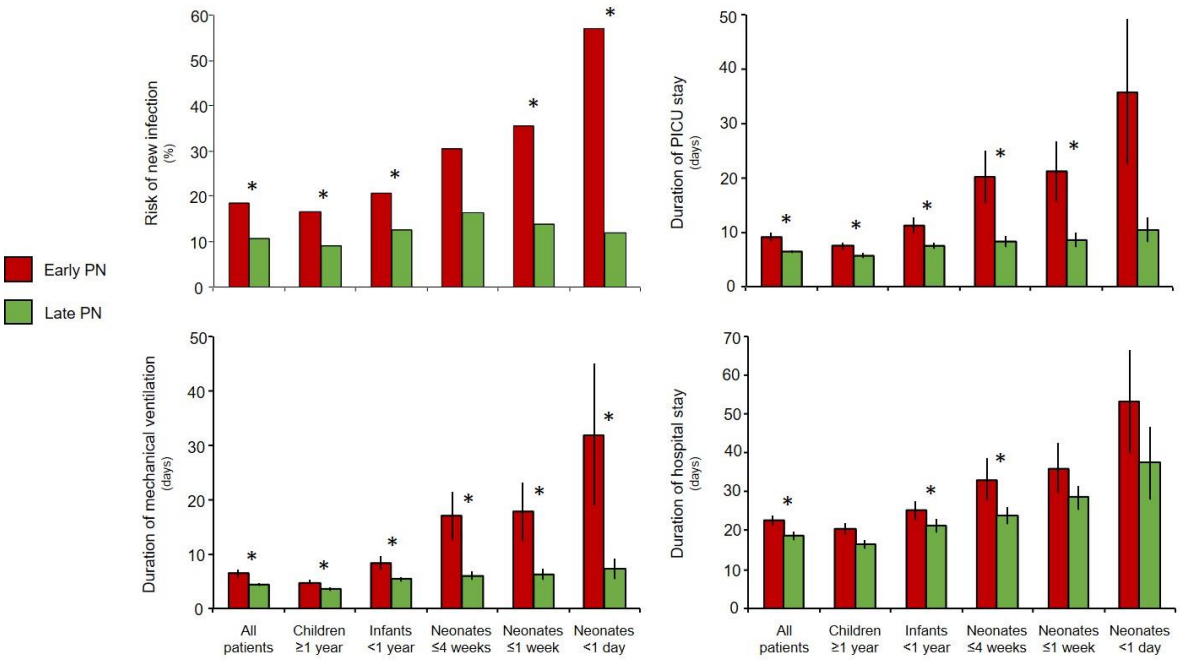

FIGURE 1 OUTCOMES OF THE PEPANIC RCT PER AGE GROUP.

Bars represent incidence of the acquisition of a new infection in PICU (percentage), and duration (days) of mechanical ventilation, PICU stay and total hospital stay. Whiskers indicate standard errors of the mean. Asterisk represent p values < 0.05, obtained with multivariable analysis adjusting for baseline risk factors [treatment center, age, risk of malnutrition (STRONGkids score), diagnosis upon admission and severity of illness (Pediatric Logistic Organ Dysfunction score PeLOD, and Pediatric Risk of Mortality 2 score, PIM2) for all patients; treatment center, risk of malnutrition, diagnosis upon admission and severity of illness for children and infants; treatment center, type of illness upon admission (medical, surgical cardiac, surgical other), severity of illness and weight for age z score for neonates).

The benefits of withholding supplemental PN during the first week in PICU appeared not only present from a clinical point of view, but also from a health-economic perspective. A cost-effectiveness study indeed

showed that the total direct medical costs were considerably lower with late PN as compared with early supplemental PN initiation.[31] This cost saving was beyond the expected lower costs for the use of PN itself, since avoidance of new infections by late PN yielded the largest cost reduction.

A possible limitation of the PEPaNIC RCT is the use of standard equations for the estimation of energy requirements, instead of indirect calorimetry.[32] However, the use of indirect calorimetry for estimating energy expenditure does not seem to be accurate,[33] or feasible[34], and is not frequently used in daily practice.[18,35]

Apart from the PEPaNIC RCT, no other RCT investigating the use or timing of supplemental PN in critically ill children has been published. A limited number of observational studies on the use of supplemental PN and over- and underfeeding in PICU showed variable results.[10,33,36] A retrospective single center study showed that late initiation of supplemental PN was associated with a higher nosocomial infection rate as compared with early initiation of supplemental PN.[36] In contrast, an observational study in 31 PICUs showed that the use of PN in general was associated with higher mortality.[10] Another retrospective study determining the incidence of over- and under-feeding in 139 children admitted to a tertiary PICU, showed that underfeeding was associated with shorter duration of PICU and hospital stay, as well as with fewer ventilation days, as compared with appropriately fed and overfed patients.[33] However, the observational design of these studies holds risk of bias by confounding variables, especially in nutritional research.[37] Therefore, comparison with the results of the PEPaNIC RCT is challenging. Further randomized controlled trials are warranted to determine the ideal time point for initiation of supplemental PN in the PICU.

Early-PN composition and the role of macronutrients

Although extensive guidelines on the composition of PN in critically ill children are available,[13] a recent survey on nutritional practices in PICUs worldwide showed a wide variation in parenterally administered doses of protein, lipids and glucose.[18] Especially the protein targets seem to be a point of discussion. Several studies assessed the association between protein delivery and clinical outcome of critically ill children.[10,38,39] In an observational international cohort study that included 500 critically ill children, mortality at 60 days was higher for patients who received PN, independently of the amount of energy or protein intake.[10] However, an important severity of illness bias has to be taken into account, since patients who are less sick are more likely to better tolerate EN. The study adjusted for severity of illness using admission scores, but data for

calculating this severity of illness score were missing in 31% of the included patients, and the choice of severity of illness score differed between the participating centers.[10] Another large multicenter observational study of the same group showed an association between a higher enteral protein intake and lower odds of mortality in more than 1200 mechanically ventilated critically ill children.[38] The effect was dose-dependent, and independent of energy intake. Again, the lack of a uniform use and lack of a complete dataset for all patients for severity of illness scores, and a substantial number of patients who received EN, could potentially create bias in these observations. The authors reasoned that an increased demand in amino acids in catabolic disease, such as critical illness, could contribute to increased higher protein degradation from muscle to ensure bodily functions,[38] which is associated with poor outcome.[40] By providing proteins, the synthesis of muscle proteins might be boosted, and thereby muscle loss could be prevented, possibly limiting the severity of intensive care unit-acquired weakness.[41] Nevertheless, a preplanned secondary analysis of the adult EPaNIC study did not support this concept, as increased macronutrient intake with early-PN, including more amino acids did not counteract muscle atrophy and actually increased the risk of developing clinically relevant muscle weakness in ICU.[42] Interestingly, in a preplanned secondary analysis of the PEPaNIC RCT, the dose of amino acids actually was associated with more infections and longer dependency on mechanical ventilation and other intensive medical care in children admitted to the PICU.[39] This risk of harm associated with early amino acid administration was elevated even at low doses of administered amino acids. A possible explanation for the difference between these results and the ones from the previously cited observational studies is the randomized design of the PEPaNIC trial, in which the doses of macronutrients differed from patient to patient and ranged widely.[39] In critically ill adults, three RCTs could not show benefit from early amino acid supplementation,[43-46] but clinical trials on the effect of amino acid administration in critically ill children in a randomized manner on clinical outcome are lacking.[47] In contrast with the harm of the amino acid administration, the secondary analysis of the PEPaNIC RCT suggested a benefit of glucose and lipid administration. Indeed, administering more glucose during the first 3 days of PICU stay was independently associated with fewer infections and administering more lipids was independently associated with earlier PICU discharge.[39] Clearly, large-scale prospective RCTs in critically ill children are needed to identify the optimal composition of supplemental PN.[17,47] Together, these findings had an important impact on recent ESPGHAN pediatric PN guidelines,[48] in which it is advised to consider withholding PN, including amino acids, for 1 week in critically ill infants, children and adolescents. Monitoring if these guidelines are actually applied in daily practice PICUs worldwide, as well as exploring the reasons for not de-implementing early-PN in the PICU, is an important step in assessing quality of care.

3. NEUROENDOCRINE ALTERATIONS DURING PEDIATRIC CRITICAL ILLNESS

Critical illness is characterized by alterations within several feedback-controlled hypothalamic-anterior pituitary axes.[49] I will focus on the changes in the hypothalamic-pituitary-thyroid (HPT) axis and in the hypothalamic-pituitary-adrenal (HPA) axis, as they both play an important role in growth and neurocognitive development and functioning of children.[50-53]

Critical illness-induced alterations in the hypothalamic-pituitary-thyroid axis

During critical illness, the acute stress generates a shift in energy expenditure and alterations in the balance between anabolism and catabolism,[54] in which the hypothalamic-pituitary-thyroid (HPT) axis plays a critical role. Pronounced HPT axis changes typically develop, in both children and adults, referred to as non-thyroidal illness syndrome (NTI).[55,56] Most characteristic of NTI are decreased T_3 levels, without a compensatory rise in TSH. The peripheral component of NTI is hallmarked by an increased conversion of circulating T_4 to the biologically inactive reverse T_3 (rT_3), whereas its conversion to biologically active T_3 is decreased, contributing to a reduced peripheral bioavailability of active thyroid hormone at the organ level.[57] In the central component of NTI, the pituitary does not respond to the decreased plasma thyroid hormone levels as it does in healthy circumstances, as TSH levels are inappropriately normal or low.

In the pediatric population, NTI mostly has been described in critically ill children after cardiac surgery,[58-62] but also in critically ill children with sepsis or septic shock,[63-66] diabetic ketoacidosis,[67] or oncological problems.[68,69] Since NTI also occurs during fasting in healthy circumstances, to reduce energy expenditure to limit catabolism,[70,71] it can also be seen in adolescents with anorexia nervosa.[72,73] In critically ill children, the severity of NTI has been associated with worse clinical outcome.[63,66,74-76] However, it remains unclear whether this association reflects an adaptive protective response or contributes to poor outcome.

The pathogenesis of NTI has only partly been unravelled but affects both central and peripheral levels. At the hypothalamic level, TRH expression is suppressed. Animal models showed a decreased TRH mRNA expression in the paraventricular nucleus after acute inflammation,[77] chronic inflammation,[78] and prolonged critical illness.[79] Local hypothalamic T_3 bioavailability rather than circulating thyroid hormones may contribute to this suppression. The expression of type 2 deiodinase (D2), which regulates local thyroid hormone availability

in the brain, is increased in rodent and rabbit NTI models.[79-81] *In vitro*, D2 activity in human glioma cells increased T_3 production, which induced T_3-responsive genes in co-cultured neurons.[82] However, in a rabbit NTI model, increased hypothalamic D2 expression did not coincide with elevated local T_3 concentrations.[79] Other factors that may contribute to the suppressed TRH expression include glucocorticoids and interleukin-1 beta (IL1β), which are both endogenously increased during critical illness.[83,84] In rats, corticosterone and dexamethasone treatment[85] and central infusion of IL1β[77] profoundly reduced TRH mRNA in the hypothalamic hypophysiotropic neurons.

TSH levels are transiently elevated but rapidly return to normal in the acute phase of illness when measured in a single daytime sample.[86] Studies in adult patients have shown that the normal nocturnal TSH surge is already absent, though, and that in the prolonged phase of critical illness pulsatile TSH secretion is virtually completely lost.[87] Low TSH mRNA expression has been observed in the pituitary of animal models.[78,80] The underlying mechanism of the inappropriately low TSH secretion by the pituitary remains unclear. Increased D2 activity in the pituitary, converting local T_4 into T_3 and thereby suppressing TSH secretion by feedback inhibition, may theoretically play a role but has not been proven.[88] In addition, the cytokine-induced increase in D1 expression in the pituitary may contribute.[89,90] Cytokines also directly suppressed TSH secretion in pituitary cell cultures.[91,92]

Also changes at the level of the thyroid gland contribute to NTI. Indeed, increased circulating levels of cytokines affect different levels of the thyroid hormone synthesis pathway, resulting in a down-regulation of T_4 and T_3 secretion. *S*tudies in cultured thyrocytes have shown suppressive effects of IL1α, IL1β, IL6, IFNγ or TNFα on thyroglobulin expression and secretion, iodide uptake by the natrium/iodide symporter (NIS), ^{125}I incorporation in thyroid hormone, and thyroid peroxidase expression.[93]

Finally, several changes in peripheral thyroid hormone availability occur. The metabolism of thyroid hormone is changed, through altered expression and activity of the deiodinating enzymes, possibly mediated by cytokines[93,94] and hypoxia.[95] D3 expression, which is virtually absent in healthy individuals, was shown to be induced in the liver and skeletal muscle of critically ill adults who died.[96] This upregulation results in more conversion of T_4 to the biologically inactive rT_3. In contrast, the hepatic expression and activity of the T_3-producing enzyme D1 was decreased.[96,97] Together, the changes in deiodinase activity diminish the T_3 availability. Altered thyroid hormone transporter and thyroid hormone receptor expression also affect thyroid hormone activity. Finally, in critically ill children with meningococcal sepsis, lower T_4 levels have been

related to lower thyroxine-binding globulin (TBG) levels, both associated with disease severity.[66] Decreased binding could therefore also contribute to the altered peripheral thyroid hormone availability.[57]

Apart from the above-described endogenously modified pathways of NTI in critical illness, iatrogenic effects on thyroid hormone secretion and metabolism should be considered. Indeed, drugs commonly used in the PICU can iatrogenically influence TSH secretion. Glucocorticoids suppress TSH release from the thyrotropes through inhibition of TRH secretion in the hypothalamus.[98-101] Also dopamine, often infused for inotropic and vasoactive support in the PICU, suppressed TSH secretion in pediatric critically ill patients.[102] Rat studies suggested that opioids affect the TRH-stimulated TSH release[103] but studies in humans revealed conflicting results.[104-106] Less commonly used drugs in PICU such as anti-epileptic medications including carbamazepine, oxcarbazepine and valproic acid, may also alter pituitary responsiveness to TRH.[101,107,108] Finally, iatrogenic iodine intoxication, via iodine containing contrast fluids or antiseptics and dressings, may also affect thyroid hormone availability and TSH secretion.[109]

Impact of metabolic interventions on non-thyroidal illness in relation to outcome

Although the association between the severity of NTI and worse clinical outcome in critically ill children is well established, an association does not clarify whether NTI is a beneficial or harmful response to critical illness. A previously published study of our group, investigating the impact of a randomized metabolic intervention in PICU on the changes in the thyroid axis in relation to acute clinical outcomes, has shed some light on this aspect. This study was a preplanned subanalysis of a large RCT performed in 700 critically ill children aged 0-16 years who had been randomized to tight glycemic control (TGC) with intensive insulin therapy or to usual care.[75] In the TGC group, insulin was infused to control blood glucose levels within age-adjusted normal fasting ranges (50-80 mg/dl for infants younger than 1 year, 70-100 for older children).[110] In the usual care group, insulin infusion was started only when the blood glucose level exceeded 215 mg/dl, and stopped when glycemia fell below 180 mg/dl. TGC decreased morbidity and mortality of critically ill children as compared with usual care, despite an increased incidence of hypoglycemia.[110] NTI was present upon PICU admission in both patients groups.[75] However, the peripheral component of NTI further aggravated over time in the TGC group as compared with the usual care group, reflected by a more pronounced further lowering of the T_3/rT_3 ratio from admission towards day 3 of PICU stay (or last day of PICU stay if patients had a shorter stay). The more pronounced peripheral inactivation of thyroid hormone may be explained by the lower blood glucose levels with TGC that are mimicking a fasting response.[75] The

further lowering of the T_3/rT_3 ratio statistically explained part of the clinical benefit of TGC.[75] This suggests that the peripheral component of NTI may be a beneficial adaptation to critical illness in children. In critically adults undergoing a similar intervention,[111] TGC did not affect the critical illness-associated changes in thyroid hormones,[112] possibly explained by selection of long-stay patients only. Also in critically ill adults, a preplanned subanalysis of the EPaNIC RCT which investigated the effect of withholding early supplemental parenteral nutrition (PN),[22] showed a similar effect on the critical illness-induced thyroid axis changes.[76] To investigate if and how the withholding of parenteral nutrition affects NTI in critically ill children, could contribute to a better understanding of the pathogenesis and possible treatment.

Critical illness-induced alterations in the hypothalamic-pituitary-adrenal axis

All causes of critical illness induce a stress response in the body, in which the HPA axis plays an important role. Theoretically, during any kind of physical or psychological stress, the hypothalamic nucleus paraventricularis releases corticotropin-releasing hormone (CRH), which activates the corticotrope cells in the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). Once ACTH reaches the zona fasciculata of the adrenal cortex via the circulation, it stimulates steroidogenesis resulting in the secretion of cortisol. In order to restore homeostasis, the increased cortisol levels exert negative feedback inhibition on the CRH and ACTH secretion by binding to the glucocorticoid receptor in the hypothalamus and pituitary gland.[113] In critical illness, the degree of increased plasma concentrations of total cortisol and free (non-protein-bound) cortisol has shown to be associated with the severity of illness, as it plays a key role in the critical illness-induced stress response.[114,115] First, in order to provide sufficient energy, cortisol suppresses anabolism and stimulates catabolism. It also has important cardiovascular effects, by increasing myocardial contractility and blood pressure, and by retaining fluids via activation of the mineralocorticoid receptor. Furthermore, cortisol plays an important role in the immune response and inflammation, as it has immune-stimulatory effects in low concentrations and immune-suppressive effects in high concentrations. Besides hypercortisolism and its effects, the stress response further consists of activation of the sympathetic nervous system and catecholamine release by the chromaffin cells in the medulla of the adrenal gland.

Until recently, it was believed that the hypercortisolism was due to a several-fold increased ACTH secretion. However, from studies in critically ill adults, it has become clear that plasma ACTH concentrations seem low rather than high during the first week in ICU, although concentrations of total and free plasma cortisol remained high.[116,117] Moreover, a previously performed tracer study of our group showed that, although total

and free plasma cortisol concentrations were several-fold increased, the cortisol production rate in critically ill adults was either not or only slightly increased as compared with the production rate of healthy adults.[118] This suggests an ACTH-cortisol dissociation, implying another mechanism for the increased free and total cortisol levels during critical illness. Both a pronounced decrease in plasma concentrations of cortisol-binding protein (CBG) and albumin, and a reduced affinity of their binding to cortisol, increase the circulating free fraction of cortisol.[119,120] Also, based on enzyme activity estimations obtained from quantification of urinary cortisol metabolites, further supported by a human biopsy study, it was shown that cortisol breakdown is decreased in the liver and kidney of critically ill adults.[118] So far, in critically ill children, the time course during the initial phase of critical illness of the systemic availability of HPA axis hormones and cortisol binding proteins and of cortisol metabolism has not yet been investigated.

Impact of metabolic interventions on the changes in the HPA-axis during critical illness

Our group previously performed a preplanned subanalysis of the large RCT investigating the effect of intensive insulin therapy to obtain TGC as compared with usual care in critically ill adults,[111] investigating the effect of intensive insulin therapy on the cortisol response to critical illness.[121] As insulin has been shown to suppress CBG levels, free cortisol levels could by higher in the TGC group.[122] In addition, insulin could alter cortisol secretion and metabolism indirectly, via its anti-inflammatory effects on cytokine levels. The results showed that intensive insulin therapy was associated with an attenuated increase in total and free cortisol in prolonged critically ill patients, statistically explaining part of the survival benefit of the therapy. It did not affect the circulating level or structure of CBG and albumin. In a subgroup analysis the effect of insulin on cortisol was present only in the surviving patients and not in the ones who died, suggesting that the effect is more likely a cause rather than a consequence of the outcome benefit.[121] In the EPaNIC RCT that showed a faster recovery and fewer complications in adult critically ill patients for whom initiation of supplemental PN was postponed until day 8 (late-PN) as compared with those for whom supplemental PN was started within 48 hours after ICU admission (early-PN),[22] the randomized intervention did not affect plasma concentrations of ACTH or total or free cortisol.[116] As in animal models and human studies outside the context of critical illness, fasting or caloric restriction seems to activate the HPA-axis, [123-126] this result was rather surprising. One possible, speculative, explanation could involve the loss of the diurnal rhythm in the HPA-axis activity in severely ill patients.[127] Also, as plasma ACTH concentrations were suppressed in these patients, a possible effect of a macronutrient restriction by late-PN on the central part of the HPA-axis may not be detectable. Finally, patients in both the late-PN and early-PN group did receive small amounts of enteral feeds

continuously, and when supplemental PN was not provided in the first week in the late-PN group, low doses of intravenous (IV) glucose were administered.[22] This could potentially have attenuated any effect of actual fasting on the HPA-axis activity. As guidelines for nutritional support in critically children are usually more aggressive as compared with critically ill adults[13], the effect of early versus late-PN on the HPA-axis activity could be different in children.

The concept of critical illness related corticosteroid insufficiency and its treatment

During critical illness in adults, both high and low cortisol levels have been associated with increased mortality.[128-130] The detrimental low cortisol levels, as well as relatively high but insufficiently elevated levels, might be a sign of HPA axis dysfunction, and are mostly present in patients with septic shock.[131,132] The concept of critical illness related corticosteroid insufficiency (CIRCI) was brought to life, which has been suggested to be present when a random total plasma cortisol concentration is lower than 10 μg/dl, or when the incremental total cortisol response to stimulation with 250 μg ACTH is lower than 9 μg/dl.[133] Also in critically ill children, one study showed that the cortisol increase after the administration of 1 μg ACTH was insufficient, which was associated with increased use of catecholamines and fluid boluses[134] In pediatric traumatic brain injury (TBI) patients, presumed adrenal insufficiency was associated with longer mechanical ventilation and higher norepinephrine use.[135] As a consequence, in both critically ill adults and children, intensivists often prescribe treatment with corticosteroids. However, this practice is not based on solid evidence and remains highly controversial, as studies have shown inconsistent and conflicting results.[136-140] Moreover, glucocorticoid administration in critically ill children could also bring about adverse effects, such as impaired insulin action and associated hyperglycemia in the short term[141] and impaired neurocognition in the longer term.[142] Therefore, more clarity on how this axis behaves during critical illness in children, and whether alterations should be treated, is essential for improving PICU care.

4. LONG-TERM SEQUELAE OF PEDIATRIC CRITICAL ILLNESS

The legacy of critical illness in children

Independent of the underlying disease, children admitted to the PICU experience a long-term legacy, years after admission. This has been most thoroughly documented for the impaired neurocognitive development,

but also includes growth retardation and may comprise poor physical functioning and reduced quality of life.[143-148] The fact that the children are treated in the PICU during crucial developmental phases likely plays a role. Interestingly, it appears that to a certain extent the neurocognitive outcome is modifiable, as shown by the attenuation of the neurocognitive impairment with the prevention of hyperglycemia during intensive care. [149] Also treatments in PICU that have been shown to cause neurodevelopmental harm, such as anesthetic and analgesic agents[150,151] and toxicants such as phthalates that leach from indwelling medical devices,[152] may be targets to find safer alternatives. Concerning nutrition in the PICU in relation to long-term outcome, experts were concerned about the safety of withholding early supplemental PN in neonates in view of the more frequent episodes of hypoglycemia observed in the late PN arm of the PEPaNIC RCT.[23,25] However, in a previous large randomized, controlled trial investigating the effect of tight glycemic control on morbidity and mortality in the PICU and on long-term neurocognitive development, a high incidence of brief hypoglycemia with tight glycemic control did not associate with harm to the neurocognitive development as documented 4 years later.[149] The proportion of neonates included in the PEPaNIC RCT was similar to that in the earlier tight glycemic control trial.[23,110] In a preplanned 2-year follow-up study, in which all patients included in the PEPaNIC RCT were approached for possible assessment of physical and neurocognitive development, the exposure to hypoglycemia also did not associate with the investigated long-term outcomes.[151] Moreover, the main results of this follow-up study showed no adverse effect of withholding supplemental PN during the first week in the PICU on survival, anthropometrics, health status and neurocognitive development. In fact, omitting early supplemental PN in the PICU improved parent-reported executive functioning (inhibition, working memory, metacognition and overall executive functioning), externalizing behavioral problems and visual-motor integration 2 years later, as compared with early supplemental PN. In particular, a better inhibitory control was observed (**Figure 2**). Since poor inhibitory control in children contributes to impulsive and destructive behaviors that upset or harm others,[153] delaying supplemental PN can have important consequences for daily life and the social environment later in life. The long-term effects of late versus early supplemental PN were more pronounced in patients who were younger than 1 year of age at the time of PICU admission as compared with older children. This age-dependent vulnerability supports the hypothesis that the harm induced by early supplemental PN might be caused by a direct metabolic insult on the developing brain, since it was not statistically explained by the acute effects of the intervention itself, such as the increased incidence of new infections or delayed recovery. However, further research is warranted to unravel underlying mechanisms that would provide support for this hypothesis. Although long-term outcome and quality-of-life years after PICU discharge gained great importance in research [154,155], investigating these outcomes is logistically challenging, expensive and time consuming. The harm induced by early-PN 2 years after admission could persist or disappear further on in

life. Moreover, impairments in other domains of development could appear which warrants a 4-year follow-up study of the PEPaNIC RCT.

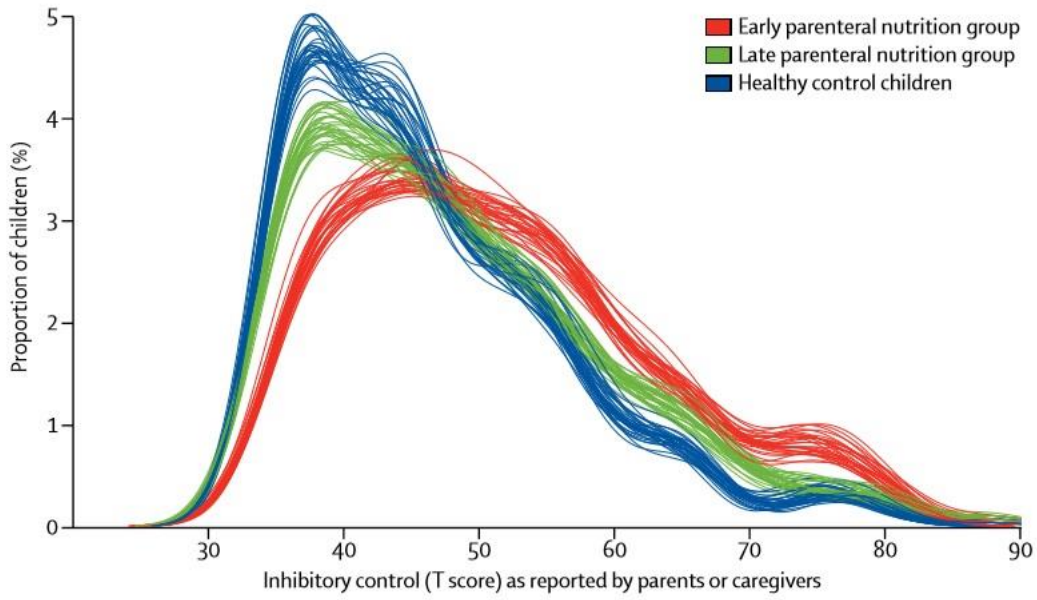

FIGURE 2 DENSITY ESTIMATES FOR INHIBITORY FUNCTION AS REPORTED BY PARENTS OR CAREGIVERS.
Densities, which correspond to the proportions of children with a certain score (equivalent to a smoothed histogram), are shown separately for healthy control children and for PEPaNIC participants who had been randomly assigned to receive late PN or early-PN. Higher scores indicate worse functioning. Each line corresponds to one of 31 imputed datasets.[151]

Underlying mechanisms of adverse long-term outcome of critically ill children

As early-PN affected both short-term and long-term outcome of the patients, carryover effects persisting in the long term must be implicated. Underlying mechanisms of the long-term harm caused by early-PN in the context of critical illness largely remain to be unraveled. The clinical benefits of late-PN observed far beyond the intervention window, suggest that early-PN induces carry-over “memory” effects with negative impact on long-term outcome. Poor long-term outcomes in other conditions have been related to accelerated telomere shortening and the induction of aberrant so-called “epigenetic” changes. Importantly, inadequate nutrition may trigger such epigenetic changes. Hence, such processes may also play a role in the developmental impairment of critically ill children and the adverse impact of early-PN on neurocognitive development.

Telomeres are nucleoprotein complexes at the end of human chromosomes that protect the DNA and that shorten with each cell cycle. Telomere shortening can be accelerated by environmental and lifestyle factors,[156,157] including excessive food consumption and/or unhealthy nutrition.[158,159] It has been demonstrated that critically ill children enter the PICU with significantly shorter leukocyte telomeres than matched healthy children.[160] More importantly, early-PN had a telomere-shortening effect as compared with late-PN in critically ill children between PICU admission and discharge, independent of baseline risk factors and post-randomization factors. Whether this accelerated telomere shortening contributes to the long-term developmental impairment, and particularly the neurocognitive impairment caused by early-PN, remains to be investigated.

Involvement of aberrant epigenetic changes in long-term consequences after acute events in life appears plausible. Epigenetics refers to the study of heritable changes in gene expression that do not involve changes in the underlying DNA sequence. Epigenetic changes play an important role in physical and neurocognitive development.[161-164] The most stable epigenetic change is the methylation or de-methylation of DNA. This is the attachment to or removal of a methyl group from a nucleotide, which occurs almost exclusively at the 5' carbon in the cytosine residue of a CpG dinucleotide.[161,162] Alterations in DNA methylation have been implicated in the adverse effects of various environmental stressors, such as inadequate nutrition (both undernutrition and overfeeding), that have shown to impact long-term health and disease.[165] Particularly during early life, DNA methylation changes may bring about long-term effects.[163,164,166] A pre-planned secondary analysis of the PEPaNIC RCT tested the hypothesis that DNA methylation changes in leukocytes occur during critical illness and that early-PN could alter these changes, explaining its negative impact on neurocognitive development 2 years later.[167] First, this study showed that DNA methylation is altered by critical illness in children, mostly towards hypomethylation. The *de novo* altered DNA methylation status of 159 identified CpG-sites statistically partially explained the impaired long-term outcome assessed 2 years after PICU admission.[167] Interestingly, a quarter of the observed changes in DNA methylation were caused by the use of early-PN. Indeed, the differential methylation of 37 of the 159 identified CpG-sites could be ascribed to the use of early-PN versus late-PN, and statistically explained the negative impact of early-PN on the neurocognitive development assessed 2 years after PICU admission (**Figure 3**). This finding was the first step in providing a molecular basis for the detrimental effect of early-PN on neurocognitive development 2 years after critical illness.

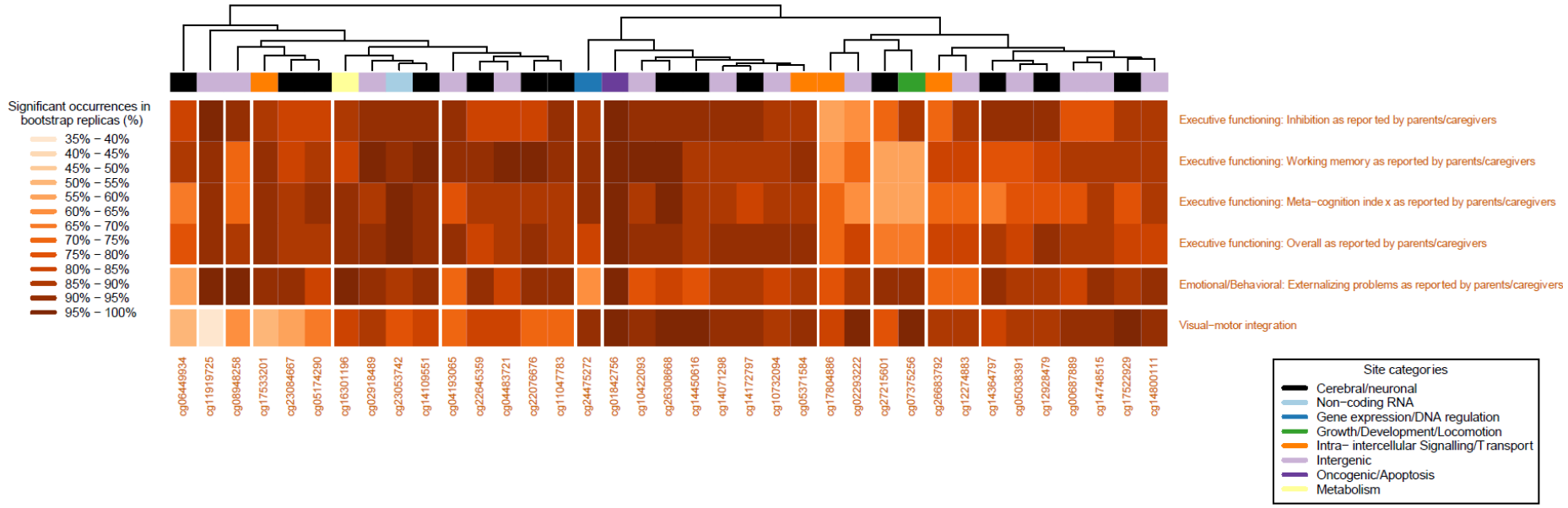

FIGURE 3 ROLE OF THE 37 DIFFERENTIALLY METHYLATED CPG-SITES EVOKED BY EARLY-PN IN EXPLAINING ITS ADVERSE IMPACT ON NEUROCOGNITIVE DEVELOPMENT IN NON-LINEAR MODELS.
Each row corresponds to 100 bootstrap replicates of multivariable non-linear models for the 6 neurocognitive outcomes negatively affected by early-PN at 2 year follow-up. Columns correspond to the 37 CpG-sites that were differentially methylated by early-PN. Color intensity of the boxes reflects the frequency with which a CpG-site was found to be independently and significantly (P<0.05) associated with the outcomes in the 100 bootstrapped replicated analyses, with darker orange colors corresponding to a higher frequency. Outcomes and CpG-sites were clustered based on these frequencies. Dendrograms show clustering hierarchy. The base of the column dendrogram is color-coded according to CpG-site functional classes "Cerebral/neuronal", 'Peripheral neuronal", "Growth/Development/Locomotion", "Metabolism", "Gene expression/DNA regulation/Epigenetic regulation", "Inflammation/immune system", "Cell structure", "Intra/intercellular Signaling/Transport", "Non-coding RNA/pseudogene", "Oncogenic/Apoptosis", or "Intergenic". The 37 CpG-sites were found to be independently and significantly associated with any of the 6 outcomes affected by early-PN in at least 50 of the 100 bootstrapped replicated analyses, confirming robustness of all associations.[167]

5. CONCLUSION

As mortality rates have dropped in the PICU and long-term developmental impairments are becoming more and more clear in children who have been critically ill, a shift towards paying more attention to morbidity endpoints should be made in evaluating new interventions or in the reassessment of current clinical practices that are not supported by solid evidence. An important part of the morbidity of critically ill children lies in the long-term footprint of impaired growth and neurocognitive development and functioning, years after PICU discharge. This legacy reveals itself independently of their pre-existing illness or condition the children suffered from. Over the last decade, progress has been made in order to identify modifiable factors that could attenuate this long-term impaired neurocognitive development, such as preventing hyperglycemia and limiting exposure to toxins such as phthalates. The previously performed large multicenter PEPaNIC RCT showed that withholding supplemental PN in the first week in PICU was clinically superior in the short-term, as compared with initiating supplemental PN within the first day after PICU admission. This important finding has been incorporated in recent nutritional guidelines for critically ill children, but it is unclear how it has affected daily practice in PICUs worldwide.

The critical illness-induced neuro-endocrine alterations in children, possibly modified by the macronutrient deficit in late-PN, are not fully understood yet. Especially the HPT-axis and the HPA-axis in children are of interest, as they could potentially be altered by a macronutrient deficit, and because their normal functioning is necessary for longer-term neurocognitive outcome and growth.

Two years after inclusion, postponing the initiation of supplemental PN in PICU improved and even normalized the executive functioning (inhibition, working memory, metacognition and overall executive functioning), externalizing behavioral problems and visual-motor integration. The biological plausibility of this finding could be explained by the identification of 37 CpG-sites that were differentially methylated by the randomization. Of great importance is to know if these neurocognitive impairments persist or if others appear in the longer term.

Taken together, the quest for answers to these questions could provide more insight in the underlying mechanisms related to the impaired outcome of critically ill children, years after their PICU admission, in order to minimize their long-term footprint.

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CHAPTER 2 - GENERAL HYPOTHESIS AND OBJECTIVES

The **general hypothesis** of this doctoral thesis was that accepting a macronutrient deficit during the first week of critical illness in children affects the neuroendocrine alterations in the acute phase of illness and the neurocognitive development as assessed four years later.

In a **first objective**, we documented neuroendocrine alterations during the acute phase of critical illness in children in relation with short-term patient-centered clinical outcomes, and investigated to what extent these neuroendocrine alterations are modified by accepting an early macronutrient deficit by withholding supplemental PN in the first week in the PICU (late-PN) versus early full feeding with the use of early supplemental PN when enteral nutrition alone was insufficient to reach caloric targets (early-PN). We focused on the alterations in the hypothalamic-pituitary-thyroid axis, described as the non-thyroidal illness syndrome or NTI (**Chapter 3**) and on the alterations in the hypothalamic-pituitary-adrenal axis (**Chapter 4**).

In a **second objective**, we evaluated the long-term impact of late-PN versus early-PN on general health status, growth and neurocognitive development of critically ill children as assessed four years after PICU admission (**Chapter 5**). As the majority of the children in the PEPaNIC RCT were younger than 1 year of age at PICU admission, and since complete neurocognitive testing is only possible from the age of 4 years onwards, a 4-year follow-up study of the PEPaNIC RCT was warranted in addition to the previously performed 2-year follow-up. The impaired neurocognitive development documented 2 years after admission could disappear or persist after 4 years, whereas other problems may emerge. We also investigated whether altered DNA-methylation status could play a role in the biological plausibility of the documented results.

In a **third objective**, we investigated the de-implementation of early initiation of PN at PICUs worldwide (**Chapter 6**). To reach this objective, we conducted a survey among physicians and dieticians, exploring the degree of early de-implementation of initiating PN in the first week in PICU, as well as potential barriers for de-implementation.

CHAPTER 3 - THE NON-THYROIDAL ILLNESS SYNDROME IN CRITICALLY ILL CHILDREN: PROGNOSTIC VALUE AND IMPACT OF NUTRITIONAL MANAGEMENT

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1. ABSTRACT

Introduction: Non-thyroidal illness (NTI), which occurs with fasting and in response to illness, is characterized by thyroid-hormone inactivation with low T_3 and high rT_3, followed by suppressed TSH. Withholding supplemental parenteral nutrition early in pediatric critical illness (late-PN), thus accepting low/no macronutrient intake up to day 8 in the pediatric intensive-care-unit (PICU), accelerated recovery as compared with initiating supplemental parenteral nutrition early (early-PN). Whether NTI is harmful or beneficial in pediatric critical illness, and how it is affected by a macronutrient deficit, remains unclear. This study investigated the prognostic value of NTI, the impact of late-PN on NTI, and whether such impact explains or counteracts the outcome benefit of late-PN in critically ill children.

Methods: This pre-planned secondary-analysis of the PEPaNIC-RCT quantified serum TSH, total T_4, T_3 and rT_3 concentrations in 982 patients upon PICU-admission versus 64 matched healthy children, and in 772 propensity score-matched early-PN and late-PN patients upon-admission and at day 3 or last PICU-day for shorter PICU-stay. Associations between thyroid-hormone concentrations upon-admission and outcome, as well as impact of late-PN on NTI in relation with outcome, were assessed with univariable analysis and multivariable logistic regression, linear regression or Cox proportional hazard analysis, adjusted for baseline risk factors.

Results: Upon PICU-admission, critically ill children revealed lower TSH, T_4, T_3 and T_3/rT_3, and higher rT_3 than healthy children ($P<0.0001$). A more pronounced NTI upon-admission, with low T_4, T_3, T_3/rT_3 and high rT_3, was associated with higher mortality and morbidity. Late-PN further reduced T_4, T_3 and T_3/rT_3, and increased rT_3 ($P\leq0.001$). Statistically, the further T_4 lowering by late-PN reduced the outcome benefit ($P<0.0001$), whereas the further T_3/rT_3 lowering explained part of the outcome benefit of late-PN ($P\leq0.004$). This effect was larger for infants than for older children.

Conclusion: In critically ill children, the peripheral inactivation of thyroid-hormone, characterized by the decrease in T_3/rT_3, which is further accentuated by low/no macronutrient intake, appears beneficial. In contrast, the central component of NTI attributable to suppressed TSH, evidenced by the decrease in T_4, seems to be a harmful response to critical illness. Whether treating the central component with TRH infusion in PICU is beneficial requires further investigation.

2. INTRODUCTION

As in adults, critical illness in children evokes pronounced changes in the thyroid axis (1-7). A low serum concentration of T_3 and a rise in rT_3 are typically observed in the acute phase of critical illness, possibly reflecting an attempt to reduce energy expenditure. Such peripheral inactivation of thyroid hormone is mainly explained by a decrease in type-1 deiodinase (D1) activity in liver and kidney, and an increase in type-3 deiodinase (D3) activity in liver and muscle (8, 9). In the face of these peripheral changes, an acute short-lived rise in TSH (and T_4) has been documented in response to the acute stress of surgery/illness (10). However, when critical illness persists, pulsatile TSH secretion becomes suppressed which results in a lowering of serum T_4 (11-13). Together, these changes are referred to as the "low- T_3" or "non-thyroidal illness" syndrome (NTI). Although the severity of NTI has been associated with adverse clinical outcomes in adults (14, 15) and children (4-7, 16-18), it remains elusive as to what extent the various components of NTI reflect beneficial adaptations to stress, or represent instead a maladaptive and harmful response to illness.

The NTI of critical illness closely mimics the response of the thyroid axis to fasting in healthy individuals. Indeed, fasting also leads to low T_3 and high rT_3 concentrations in the presence of altered deiodinase activity (19). When fasting is prolonged, this is followed by low T_4 without a compensatory rise in TSH. During critical illness, nutrient intake is often insufficient, due to anorexia, a dysfunctional gastrointestinal tract or interruptions of enteral nutrition (20, 21). Hence, a reduced nutrient intake may also play a role in NTI of critical illness.

Recently, our group studied the effect of accepting low or even no macronutrient intake in the early phase of critical illness with delaying the initiation of supplemental parenteral nutrition (PN) until beyond the first week in adults and children admitted to the intensive-care-unit (ICU). The withholding of early supplemental PN reduced the risk of acquiring a new infection and accelerated recovery, as compared with providing early full nutrition by giving PN to supplement any degree of insufficient enteral nutrition (22, 23). The clinical benefit of not using PN up to day 8 in the ICU was greater for critically ill children than for adults. Interestingly, in adult patients, accepting low or no macronutrient intake was found to further aggravate NTI (24). In the adult population, statistical analyses suggested that the more pronounced peripheral inactivation of thyroid hormone with accepting the low/no macronutrient intake in the acute phase of illness reflects a beneficial adaptation to enhance recovery (24). In contrast, the aggravation of the central component of the NTI, with further lowering of T_4, could delay recovery (24). Most nutrition guidelines recommend more aggressive feeding for critically ill children than for adult patients (25). We hypothesized that not using PN up to day 8 in the pediatric ICU (PICU) aggravates NTI even further than in adult patients. We also hypothesized that

more pronounced NTI may explain why the clinical benefits of not using PN up to day 8 in the ICU were also larger for critically ill children than adults.

In this study, we first investigated the prognostic value of NTI upon PICU admission for 90-day mortality, for the time to discharge from PICU, and for the risk of acquiring a new infection in the PICU. Next, we documented the impact of accepting low or no macronutrient intake up to day 8 of critical illness in children on the change of the NTI from admission to day 3 or last day for patients discharged earlier. We also investigated to what extent any such impact offers a statistical explanation for the higher likelihood of an earlier discharge, and the lower risk of acquiring a new infection brought about by not using PN up to day 8 in the PICU.

3. Materials and Methods

Patients

This study was a preplanned secondary analysis of the Early versus Late Parenteral Nutrition in the Pediatric Intensive Care Unit (PEPaNIC) randomized controlled trial (RCT) (23). PEPaNIC investigated the clinical outcomes of withholding supplemental PN up to day 8 in the PICU, further referred to as “late-PN” although for the majority of patients this strategy meant no PN at all, as compared with early supplemental PN whenever enteral nutrition alone was insufficient to reach the caloric target, referred to as “early-PN”, in children admitted to the PICU. All children aged 0-17 years were eligible for inclusion, if a stay of 24 hours or more in the PICU was expected, if they had a moderate or severe risk of malnutrition (score 2 or more on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids)), and if none of the predefined exclusion criteria were met (23). In addition, healthy children, who had never been critically ill and from whom blood was drawn immediately after intravenous catheterization prior to minor elective surgery were included for comparison with the patients. Written informed consent was obtained from parents or legal guardians. The institutional ethical review board at each participating center approved the study protocol and consent forms (ML8052, NL38772.000.12, Pro00038098). The detailed study protocol and primary results have been published elsewhere (23, 26).

The study was conducted in three centers that used early-PN as the standard of care: Leuven (Belgium), Rotterdam (the Netherlands), and Edmonton (Canada). Enteral nutrition was started as soon as possible in both groups. In the early-PN group, supplemental PN was initiated within 24 hours after PICU admission, whereas any PN was withheld up to the morning of day 8 in the PICU for patients of the late-PN group. In the

late-PN group, a mixture of intravenous dextrose (5%) and saline was administered to match the intravenous fluid load given to the early-PN patients. Patients in both groups received intravenous trace elements, minerals and vitamins, and blood glucose control with insulin according to local targets. Blood was systematically sampled upon admission and then daily at 6 a.m. until PICU discharge or death. After clotting and centrifugation, serum was stored at -80°C until analysis.

Serum analyses

Serum TSH concentrations were quantified with a commercially available TSH immunoradiometric assay (TSH IRMA kit; Beckman Coulter, Prague, Czech Republic). Serum total T_4, T_3 and rT_3 concentrations were quantified with commercially available radioimmunoassays (Total T_4 RIA kit and total T_3 RIA kit; Beckman Coulter; RIAZEN Reverse T_3; ZenTech s.a., Liège, Belgium). Free thyroid hormone concentrations were not measured given that the blood samples had been drawn via heparinized lines, which induces artifacts in free hormone quantification (27).

Statistical analyses

Data for univariable analyses are presented as numbers and percentages, means ± standard errors or medians and interquartile ranges. Univariable differences were assessed with the chi-square test for proportions and Mann-Whitney U test for continuous data.

To assess the association between serum concentrations of thyroid hormones upon PICU admission and patient outcomes, we studied death at 90 days, length of PICU stay and risk of acquiring a new infection in PICU. First, we performed univariable analyses for the association of TSH, T_4, T_3, rT_3 and T_3/rT_3 upon PICU admission and outcome. Subsequently, we performed multivariable analyses to assess independent associations of thyroid hormones that were significantly associated with outcome in univariable analysis, where we adjusted for baseline risk factors [treatment center, risk of malnutrition (STRONGkids score (28)), age, diagnosis upon admission, severity of illness (PeLOD reflecting degree of organ failure (29) and PIM2 score estimating risk of death (30))]. Multivariable logistic regression was used to assess associations with 90-day mortality and risk of acquiring a new infection, and multivariable linear regression analysis for length of PICU stay. Effect sizes are reported as odds ratios (OR) and β estimates, respectively, and 95% confidence intervals (CI). Multicollinearity was excluded based on the multicollinearity diagnostics tools in SPSS Statistics 24.0.0.0, with tolerance ≥0.3, variance inflation factor <3.5 and condition index below 30 for all variables entered in the models (31).

To evaluate the effect of late-PN versus early-PN on the early central (TSH, T_4) and peripheral (T_3/rT_3) components of the thyroid axis, we selected a subgroup of patients by propensity score matching (SPSS R-

menu R3.1 (Foundation for Statistical Computing) in IBM SPSS Statistics 23.0.0.0 (SPSS, Chicago, IL)). Logistic regression was used to estimate propensity scores with the baseline risk factors sex, treatment center, stratification group, severity of illness (PeLOD and PIM2 score), risk of malnutrition (STRONGkids score), height and weight as percentiles of population norms, infection upon admission, need for mechanical ventilation, and need for extracorporeal membrane oxygenation (ECMO) or other assist devices as covariates. We hypothesized that a further reduction of the T_3/rT_3 ratio by ±15% would occur in the late-PN group as compared with the early-PN group. This effect size was based upon a similar impact of another metabolic intervention in PICU patients, namely targeting strict age-adjusted fasting blood glucose levels (18). To detect such a difference, with 80% power and 95% certainty, 386 patients per group, were needed. The required number of patients was obtained with use of a caliper of 0.35 for one-to-one nearest-neighbor matching. In this propensity score-matched subset, the changes in hormone concentrations from PICU admission to PICU day 3, or to the last PICU day for patients discharged earlier (denoted as ΔTSH, ΔT_4, ΔT_3, ΔrT_3 and $\Delta T_3/rT_3$), were compared between early-PN and late-PN patients. Differences in these changes from baseline between both groups were analyzed with Mann-Whitney U test and with multivariable linear regression analysis adjusted for the baseline risk factors described above.

To investigate whether any of the observed changes in the central and peripheral components of the thyroid axis (ΔTSH, ΔT_4 and $\Delta T_3/rT_3$) played a role in the beneficial effects of late-PN on time to discharge from PICU (accounting for death as competing risk, by censoring non-surviving patients beyond all survivors at 91 days) and risk of new infection, which were the primary endpoints of the PEPaNIC study, multivariable Cox proportional hazard and logistic regression analyses were performed as described above. In a first step, the model included the baseline risk factors and the randomized intervention. In the second explanatory step, the model was further adjusted for the changes from baseline in the thyroid hormone parameters that were affected by the randomized intervention. A change from baseline of a thyroid hormone would thus statistically explain (part of) the beneficial impact of late-PN on outcome when adjusting for this change would reduce the effect of late-PN on outcome. Vice versa, when adjusting for a change from baseline of a hormone would increase the significant impact of late-PN on outcome, this change could be interpreted as a deleterious side effect of late-PN. Finally, in order to determine whether there were interactions between the effect of late-PN versus early-PN and the age group (infants younger than 1 year versus older children) of the patients, we calculated interaction P values in all multivariable models.

Statistical analyses were performed with JMP Pro-13.0.0 (SAS Institute, Cary, NC). Statistical significance was set at a P value at or below 0.05.

4. RESULTS

Thyroid hormone concentrations upon PICU admission and predictive value for outcomes

PICU admission samples from 982 patients were available for thyroid hormone measurements (**Figure 1**). Baseline characteristics of these patients are described in **Table 1**. In addition, 64 healthy children were studied, matched with the patients for age and sex (**Table 1**).

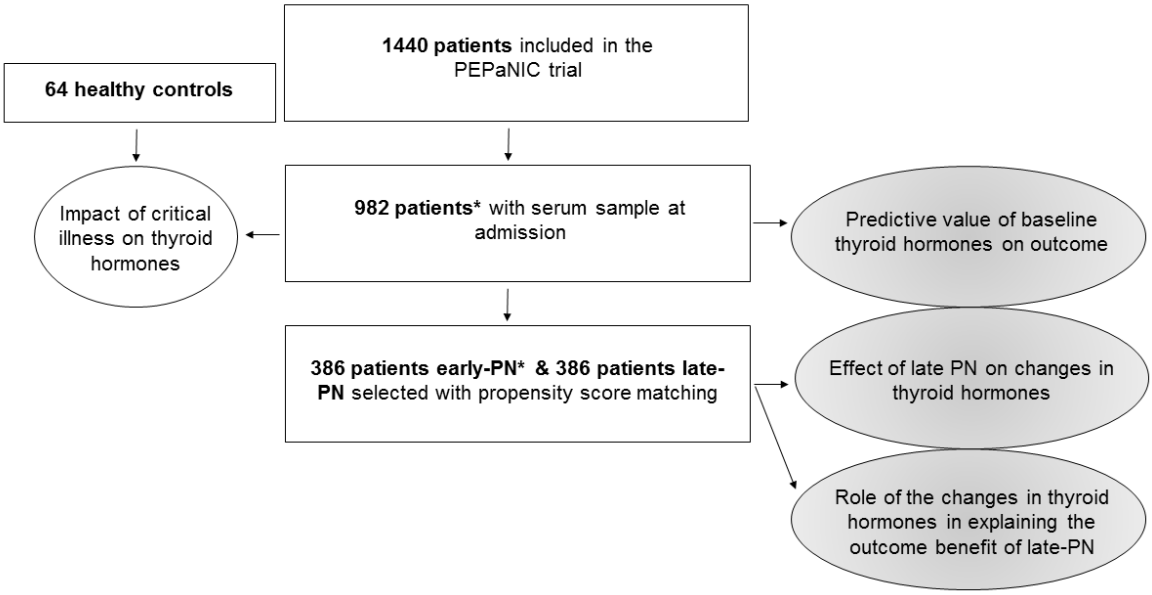

FIGURE 1 DIAGRAM OF THE STUDY DESIGN.
From 982 of the 1440 patients included in the PEPaNIC trial, a PICU admission serum sample was available. Those 982 admission samples were used for the comparison of thyroid hormones in critically ill children with those in healthy control children, and for studying the association of baseline thyroid hormone concentrations with clinical outcome. A subgroup of early-PN and Late-PN patients was selected with propensity score matching to investigate the effect of the randomized intervention on the thyroid axis, as well as the impact of these changes on the outcome benefit of late-PN. *Data were incomplete for one early-PN patient as not enough serum was available to perform the complete analysis. Results of another early-PN patient were retrospectively excluded because of severely aberrant thyroid hormone concentrations after radioactive iodine metaiodobenzoguanidine therapy. PN, parenteral nutrition.

As a group, critically ill children presented upon PICU admission with low serum concentrations of TSH, T_4 and T_3, and high rT_3 as compared with healthy children, resulting in a low T_3/rT_3 ratio (**Table 1**). The critical illness-induced rise in rT_3 was more pronounced among infants and resulted in a lower T_3/rT_3 ratio than among older children (**Figure 2**).

TABLE 1 BASELINE CHARACTERISTICS OF PATIENTS AND HEALTHY CONTROLS USED TO STUDY THE IMPACT OF CRITICAL ILLNESS ON THE THYROID AXIS

Baseline characteristics	**Patients** N=982	**Controls** N=64	***P***
Age (years), median (IQR)	1.92 (0.35-7.57)	2.00 (0.73-6.66)	0.28
Age <1 year, N (%)	402 (40.94)	25 (39.06)	0.76
Male gender, N (%)	567 (57.74)	37 (57.81)	0.99
Weight (kg), median (IQR)	11.75 (5.40-22.00)		
Standard deviation score, median (IQR) *	-0.51 (-1.40-0.46)		
Height (cm), median (IQR)	86 (60-120)		
Standard deviation score, median (IQR) *	-0.30 (-1.38-0.77)		
STRONGkids risk level, N (%)			
Medium	895 (91.14)		
High	87 (8.86)		
PeLOD score, first 24 hours in PICU, median	22 (12-32)		
PIM2 score, median (IQR)	-2.75 (-3.65 – -1.46)		
PIM2-calculated risk of death (%), median	0.06 (0.03-0.19)		
Emergency admission, N (%)	424 (43.18)		
Diagnostic group, N (%)			
Type of illness			
Surgical			
Abdominal	35 (3.56)		
Burns	6 (0.61)		
Cardiac	480 (48.88)		
Neurosurgery-traumatic brain injury	95 (9.67)		
Thoracic	38 (3.87)		
Transplantation	18 (1.83)		
Orthopaedic surgery-trauma	49 (4.99)		
Other	21 (2.14)		
Medical			
Cardiac	32 (3.26)		
Gastrointestinal-hepatic	2 (0.20)		
Oncologic-hematologic	8 (0.81)		
Neurologic	53 (5.40)		
Renal	1 (0.10)		
Respiratory	88 (8.96)		
Other	56 (5.70)		
Condition on admission, N (%)			
Mechanical ventilation required	882 (89.82)		
ECMO or other assist device required	33 (3.36)		
Infection	328 (33.40)		
Outcomes			
90-day mortality, N (%)	65 (6.62)		
Length of PICU stay (days), median (IQR)	3 (2 – 7)		
New infection during PICU stay, N (%)	143 (14.56)		
Thyroid hormones upon PICU admission, median (IQR)			
TSH (mIU/l)	1.74 (0.91-3.26)	2.61 (1.93-3.52)	<0.0001
T_4 (nmol/l)	59.56 (44.67-78.30)	98.02 (85.94-110.33)	<0.0001
T_3 (nmol/l)	1.15 (0.84-1.55)	2.38 (2.04-2.66)	<0.0001
rT_3 (nmol/l)	0.42 (0.23-0.73)	0.24 (0.19-0.30)	<0.0001
T_3/rT_3	2.93 (1.35-5.91)	9.39 (7.47-13.67)	<0.0001

** Age- and gender-specific standard deviation scores were calculated with the use of reference data from the World Health Organization Growth Charts: http://www.bcchildrens.ca/Services/SpecializedPediatrics/EndocrinologyDiabetesUnit/ForProfessionals/AnthropometricCalculators.htm.*
ECMO: extracorporeal membrane oxygenation; IQR: interquartile range; PeLOD: Pediatric Logistic Organ Dysfunction score; PICU: pediatric intensive care unit; PIM2: Pediatric Risk of Mortality 2 score; STRONGkids: Screening Tool for Risk On Nutritional Status and Growth (score of 0 indicating a low risk of malnutrition, a score of 1-3 indicating medium risk, and a score of 4-5 indicating high risk). TSH, thyrotropin; T4, thyroxine; T3, triiodothyronine; rT3, reverse triiodothyronine.

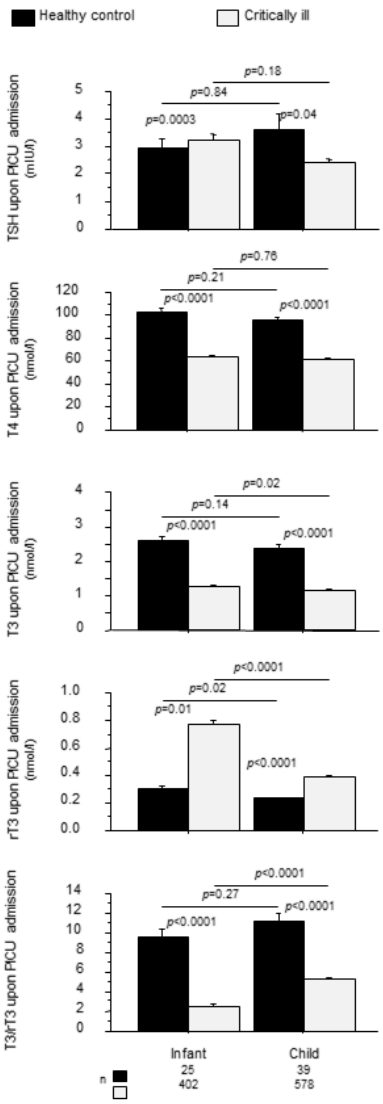

FIGURE 2 IMPACT OF CRITICAL ILLNESS ON THYROID HORMONE CONCENTRATIONS UPON PICU ADMISSION IN INFANTS AND CHILDREN
Infants are younger than 1 year old. Bars represent means and whiskers represent the standard error. The black boxplots represent healthy children and the light gray boxes represent critically ill patients.

In univariable analysis, a lower TSH upon admission was only associated with a PICU stay of longer than the median of 3 days, but no correlation was found with PICU stay as a continuous variable (P=0.79). A lower T_4, T_3, T_3/rT_3 ratio and a higher rT_3 upon admission were associated with a higher mortality at 90 days, a longer PICU stay, and the acquisition of a new infection (**Figure 3**).

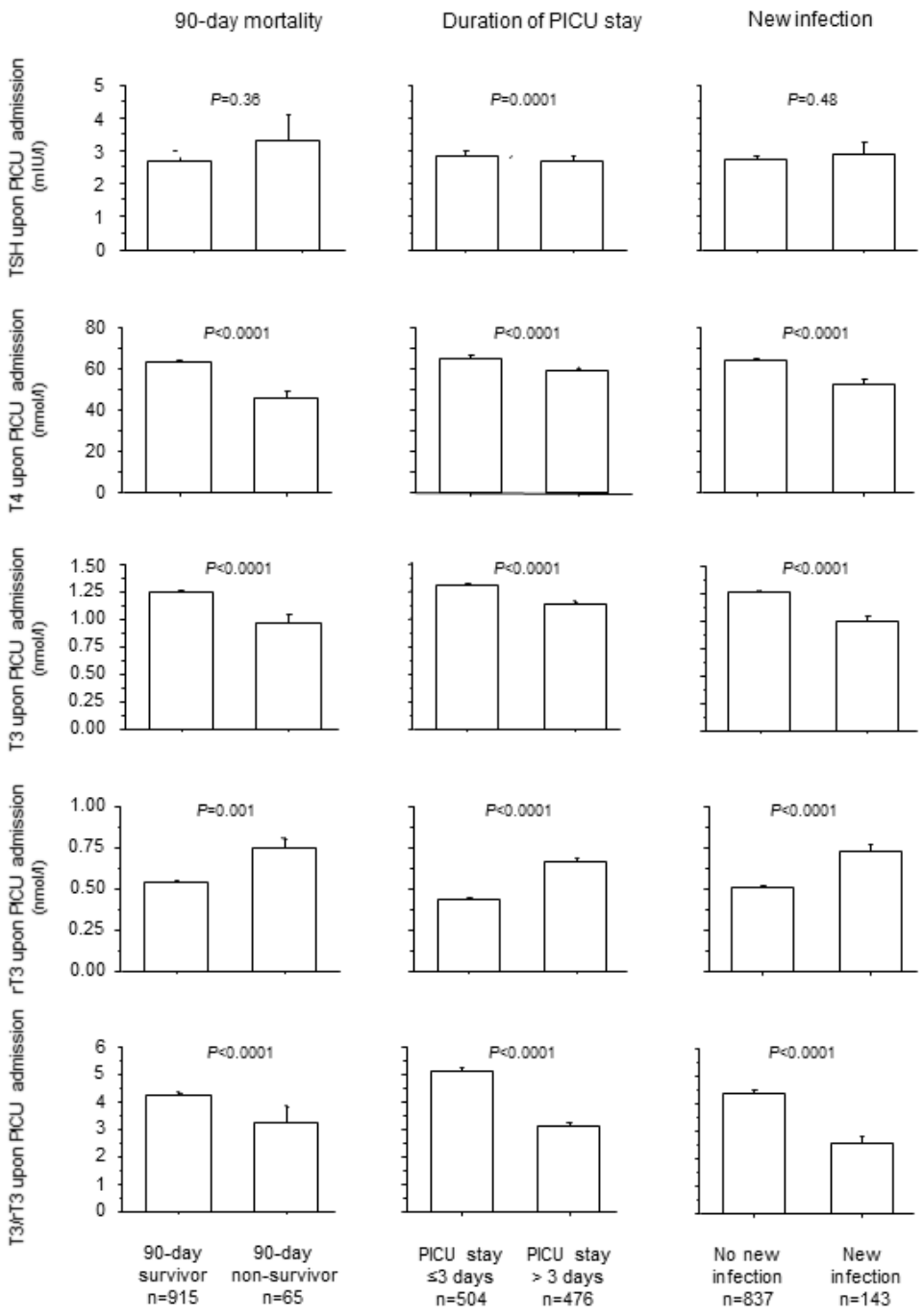

FIGURE 3 UNIVARIABLE ANALYSES FOR THE ASSOCIATION BETWEEN UPON ADMISSION THYROID HORMONES AND OUTCOME
Bars represent means and whiskers represent the standard error.

In multivariable analyses including all thyroid hormones significantly associated with the outcomes in univariable analysis, a lower T_4 and higher rT_3 were systematically associated with unfavorable outcomes, whereas a lower T_3/rT_3 was only associated with a longer PICU stay and a higher risk of acquiring a new infection. The performance of the models adjusted for the thyroid hormones was low as compared with that of the models for the respective outcomes adjusted for the baseline risk factors treatment center, risk of malnutrition, age, type and severity of illness, as reflected by lower R-squares of the former models (**Table 2**). Model performance slightly improved when combining the baseline risk factors with the thyroid

hormones upon PICU admission. Adjusted for baseline risk factors, a lower T_4 upon admission was independently associated with a higher risk of death at 90 days and a higher risk of acquiring a new infection. A lower T_3/rT_3 ratio was independently associated with a longer PICU stay, and a higher risk of a new infection.

TABLE 2 MULTIVARIABLE ANALYSES OF PREDICTIVE VALUE FOR OUTCOME OF THYROID HORMONE CONCENTRATIONS UPON PICU ADMISSION

	90-day mortality		**Length of PICU stay**		**Acquisition of a new infection**	
Model 1: baseline risk factors only	R^2=0.358		R^2=0.081		R^2=0.091	
Model 2: upon admission thyroid hormones only	R^2=0.085		R^2=0.038		R^2=0.065	
	OR (95% CI)	*P*	β estimate (95% CI)	*P*	OR (95% CI)	*P*
Admission T3	1.002 (0.470-2.224)	0.95	9.228 (-2.385-5.143)	0.47	1.197 (0.693-2.066)	0.52
Admission T_4	0.968 (0.953-0.983)	**<0.0001**	-0.049 (-6.995--1.032)	**0.008**	0.983 (0.973-0.993)	**0.0006**
Admission rT3	2.383 (1.384-4.104)	**0.001**	2.347 (0.989-10.529)	**0.01**	1.725 (1.095-2.717)	**0.01**
Admission T3/rT3	1.051 (0.960-1.151)	0.29	-0.298 (-7.052--0.441)	**0.02**	0.898 (0.815-0.989)	**0.01**
Model 3: baseline risk factors and thyroid hormones	R^2=0.398		R^2=0.156		R^2=0.154	
	OR (95% CI)	*P*	β estimate (95% CI)	*P*	OR (95% CI)	*P*
Admission T3	1.847 (0.611-5.584)	0.28	0.865 (-2.428-5.768)	0.42	1.425 (0.736-2.759)	0.29
Admission T_4	0.972 (0.953-0.992)	**0.004**	-0.030 (-5.563-0.679)	0.12	0.987 (0.976-0.998)	**0.02**
Admission rT3	1.528 (0.727-3.213)	0.29	-0.655 (-6.830-3.616)	0.54	1.359 (0.815-2.266)	0.24
Admission T3/rT3	0.885 (0.775-1.011)	0.06	-0.349 (-7.807--0.978)	**0.01**	0.850 (0.763-0.947)	**0.0009**

Baseline risk factors: treatment center, risk of malnutrition (STRONGkids score), age, diagnosis upon admission, severity of illness (PeLOD and PIM2 score). CI: Confidence interval; OR: Odds ratio; PICU: pediatric intensive care unit.

Effect of late-PN versus early-PN on thyroid hormone concentrations

The propensity score-matched subgroups of 386 late-PN and 386 early-PN patients, selected to evaluate the differences between late-PN and early-PN (**Figure 1**), were comparable for baseline characteristics (**Table 3**). According to the study protocol, caloric intake was lower in the late-PN group than in the early-PN group (**Figure 4**).

TABLE 3 BASELINE CHARACTERISTICS OF THE PROPENSITY SCORE-MATCHED PATIENTS SELECTED TO EVALUATE THE EFFECT OF LATE-PN VERSUS EARLY-PN ON THYROID HORMONE CONCENTRATIONS

Baseline characteristics	**Early-PN** N = 386	**Late-PN** N = 386	***P***
Age (years), median (IQR)	2.05 (0.38-7.26)	2.08 (0.38-7.92)	0.63
Age <1 year, N (%)	159 (41.2)	159 (41.2)	>0.99
Age <4 weeks, N (%)	29 (7.51)	31 (8.03)	0.78
Male gender, N (%)	217 (56.2)	230 (59.6)	0.34
Weight (kg), median (IQR)	11.60 (5.61-22.00)	12.00 (5.58-23.13)	0.60
Standard deviation score, median (IQR) *	-0.56 (-1.45 – 0.42)	-0.52 (-1.50 – 0.41)	0.62
Height (cm), median (IQR)	85.50 (61.00-	88.00 (60.75-122.00)	0.54
Standard deviation score, median (IQR) *	-0.37 (-1.59 – 0.57)	-0.34 (-1.28 – 0.67)	0.35
STRONGkids risk level, N (%)			0.55
Medium	364 (94.3)	360 (93.3)	
High	22 (5.7)	26 (6.7)	
PeLOD score, first 24 hours in PICU, median (IQR)	23 (21-32)	31 (21-32)	0.81
PIM2 score, median (IQR)	-2.89 (-3.72 – -1.51)	-2.84 (-3.68 – -1.64)	0.94
PIM2-calculated risk of death (%), median (IQR)	0.05 (0.02-0.18)	0.05 (0.02-0.16)	0.94
Emergency admission, N (%)	134 (34.72)	152 (39.38)	0.17
Diagnostic group, N (%)			0.60
Surgical			
Abdominal	10 (2.6)	8 (2.1)	
Burns	1 (0.3)	2 (0.5)	
Cardiac	215 (55.7)	209 (54.2)	
Neurosurgery-traumatic brain injury	39 (10.1)	32 (8.3)	
Thoracic	14 (3.6)	16 (4.2)	
Transplantation	5 (1.3)	10 (2.6)	
Orthopaedic surgery-trauma	24 (6.2)	20 (5.2)	
Other	7 (1.8)	10 (2.6)	
Medical			
Cardiac	11 (2.9)	12 (3.1)	
Gastrointestinal-hepatic	1 (0.3)	1 (0.3)	
Oncologic-hematologic	0 (0.0)	5 (1.3)	
Neurologic	16 (4.2)	17 (4.4)	
Renal	0 (0)	0 (0)	
Respiratory	24 (6.2)	25 (6.5)	
Other	19 (4.9)	19 (4.9)	
Condition on admission, N (%)			
Mechanical ventilation required	351 (90.9)	346 (89.6)	0.54
ECMO or other assist device required	8 (2.1)	10 (2.6)	0.63
Infection	112 (29.0)	108 (28.0)	0.74
Outcomes			
90-day mortality, N (%)	24 (6.22)	16 (4.15)	0.19
Length of PICU stay (days), median (IQR)	3 (2 – 7)	3 (1.75 – 6)	0.11
New infection during PICU stay, N (%)	56 (14.51)	37 (9.59)	**0.03**
Thyroid hormones upon PICU admission, median (IQR)			
TSH (mIU/l)	1.98 (1.04-3.46)	1.79 (0.94-3.23)	0.21
T_4 (nmol/l)	58.30 (44.32-75.73)	60.98 (45.91-79.55)	0.28
T_3 (nmol/l)	1.18 (0.87-1.58)	1.18 (0.86-1.53)	0.95
rT_3 (nmol/l)	0.39 (0.22-0.67)	0.41 (0.21-0.70)	0.57
$T3/rT_3$ ratio	3.29 (1.53-6.05)	3.01 (1.49-6.19)	0.52

Statistically significant values are shown in bold.

**Age- and sex-specific standard deviation scores were calculated with the use of reference data from the World Health Organization growth charts: www.bcchildrens.ca/Services/SpecializedPediatrics/EndocrinologyDiabetesUnit/ForProfessionals/AnthropometricCalculators.htm.*

PN: parenteral nutrition; ECMO: extracorporeal membrane oxygenation; IQR: interquartile range; PeLOD: Pediatric Logistic Organ Dysfunction score; PICU: pediatric intensive care unit; PIM2: Pediatric Risk of Mortality 2 score; STRONGkids: Screening Tool for Risk On Nutritional Status and Growth (score of 0 indicating a low risk of malnutrition, a score of 1-3 indicating medium risk, and a score of 4-5 indicating high risk).

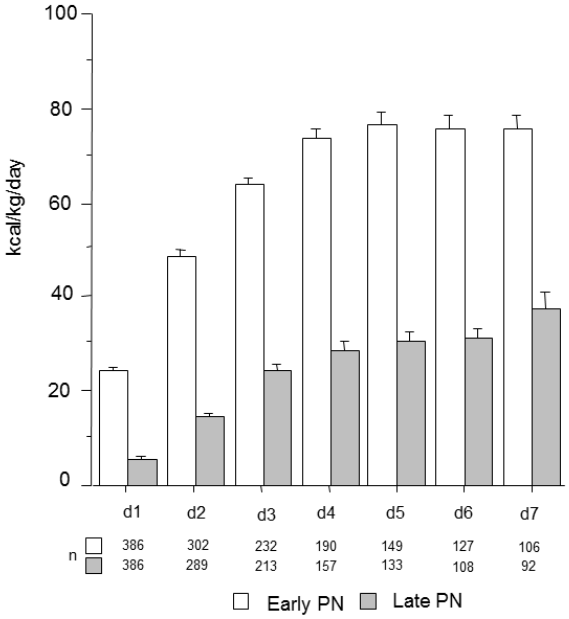

FIGURE 4 TOTAL DAILY CALORIC INTAKE OF THE PATIENTS IN THE PROPENSITY SCORE MATCHED SUBGROUP
Bars show the mean daily amount of energy (kilocalories/kg/day) provided by the combination of the enteral and parenteral route, with whiskers representing the standard error. The open and filled bars represent the patients randomized to the early-PN and late-PN groups, respectively. PN: parenteral nutrition.

The serum concentrations of TSH, T_4, T_3, rT_3 and the T_3/rT_3 ratio determined at admission were comparable to those in the total population and not different for the late-PN and early-PN groups (**Table 3**). In univariable analysis, late-PN further lowered serum TSH concentrations between admission and day 3 or last PICU day as compared with the early-PN group (P=0.04). However, in multivariable analysis adjusting for baseline risk factors, this effect was no longer significant (P=0.18) (**Figure 5**). Late-PN also lowered serum T_4 as compared with the early-PN group, both in univariable analysis (P<0.0001) and after adjustment for risk factors in the multivariable model (P=0.0001). Late-PN also further lowered the T_3/rT_3 ratio (univariable P=0.002; multivariable P=0.001) due to both a lowering of T_3 (univariable and multivariable P<0.0001) and a rise in rT_3 (univariable and multivariable P=0.001). The impact of late-PN versus early-PN on the changes from baseline in T_4, T_3, rT_3 and in the T_3/rT_3 ratio was larger among children than among infants (**Supplemental Figure 1**).

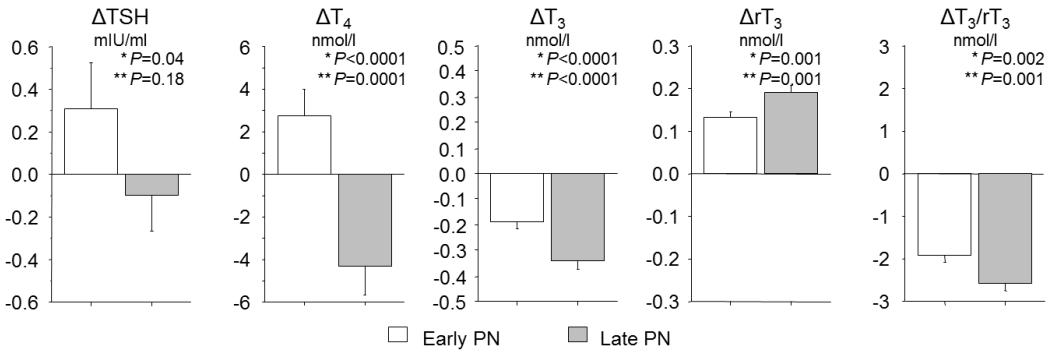

FIGURE 5 EFFECT OF EARLY-PN VERSUS LATE-PN ON THE THYROID AXIS

Bars (mean ± SE) represent the changes (referred to as Δ) from the admission values to day 3 in the PICU (or to the last day for patients with shorter PICU stay) in serum TSH, T_4, T_3, rT_3 and the T_3/rT_3 ratio. The open and filled bars represent the patients randomized to the early-PN and late-PN groups, respectively. Infants are younger than 1 year old. * *P* values obtained with univariable analysis, ** *P* values obtained with multivariable analysis after adjustment for baseline risk factors (treatment center, risk of malnutrition (STRONGkids score), age, diagnosis upon admission, and severity of illness (PeLOD and PIM2 score)). PeLOD: Pediatric Logistic Organ Dysfunction score; PIM2: Pediatric Risk of Mortality 2 score; STRONGkids: Screening Tool for Risk On Nutritional Status and Growth (score of 0 indicating a low risk of malnutrition, a score of 1-3 indicating medium risk, and a score of 4-5 indicating high risk).

Role of the changes in thyroid hormones in explaining the outcome benefit of late-PN

In the propensity score-matched subset, similarly to what has been reported previously for the total PEPaNIC cohort (23), patients in the late-PN group had a higher likelihood of being discharged earlier from PICU than patients in the early-PN group (HR 1.173; 95% CI 1.013-1.357; P=0.03) and had a lower risk of acquiring a new infection (OR 0.571; 95% CI 0.347-0.925; P=0.02) (**Table 4**).

TABLE 4 ROLE OF INDEPENDENT ASSOCIATIONS OF THE CHANGES FROM BASELINE IN THE THYROID HORMONES WITH CLINICAL OUTCOME IN EXPLAINING THE OUTCOME BENEFIT WITH LATE-PN

	HR or OR (95% CI)	*P*
Time to discharge from PICU		
1. Randomization to late-PN versus early-PN	1.173 (1.013-1.357)	**0.03**
2. Randomization to late-PN versus early-PN	1.209 (1.042-1.401)	**0.01**
ΔT_4 (per nmol/l added)	1.010 (1.007-1.013)	**<0.0001**
$\Delta T_3/rT_3$ (per unit added)	0.945 (0.921-0.969)	**<0.0001**
Risk of new infection		
1. Randomization to late-PN versus early-PN	0.571 (0.350-0.930)	**0.02**
2. Randomization to late-PN versus early-PN	0.539 (0.325-0.892)	**0.01**
ΔT_4 (per nmol/l added)	0.978 (0.968-0.989)	**<0.0001**
$\Delta T_3/rT_3$ (per unit added)	1.148 (1.037-1.270)	**0.004**

Model 1: Model adjusted for the baseline risk factors treatment center, risk of malnutrition (STRONGkids score), age, diagnosis upon admission, and severity of illness (PeLOD and PIM2 score). Model 2: The changes from baseline of the thyroid hormones that were significantly affected by late-PN in multivariable analyses were added to model 1 to study their independent association with outcome and to assess any potential implication in explaining the clinical outcome benefit with late-PN. Statistically significant values are shown in bold. CI: Confidence Interval; HR: Hazard Ratio; OR: Odds Ratio; PeLOD: Pediatric Logistic Organ Dysfunction score; PICU: pediatric intensive care unit; PIM2: Pediatric Risk of Mortality 2 score; STRONGkids: Screening Tool for Risk On Nutritional Status and Growth (score of 0 indicating a low risk of malnutrition, a score of 1-3 indicating medium risk, and a score of 4-5 indicating high risk).

To investigate whether the observed acute changes in thyroid hormones by late-PN statistically explained (part of) these benefits, the change from baseline in T_4 (reflecting the impact of late-PN on the central component of the thyroid axis) and in the T_3/rT_3 ratio (reflecting the impact of late-PN on the peripheral conversion) were added to the multivariable Cox proportional hazard and logistic regression models. This somewhat increased the size of the effect of late-PN on the likelihood of an earlier discharge from PICU to a HR of 1.209 (1.042-1.401; P=0.01) (**Table 4**). In the adjusted model, a rise in T_4 from admission to day 3 or last PICU day was independently associated with a higher likelihood of being discharged from PICU (HR 1.010 (1.007-1.013) per nmol/l increase; P<0.0001), whereas a rise in the T_3/rT_3 ratio was independently associated with a lower likelihood (0.945 (0.921-0.969) per unit increase; P<0.0001).

With regard to acquisition of a new infection, addition of the observed changes in thyroid hormones by late-PN to the multivariable model slightly increased the effect size of late-PN on the risk of acquiring a new infection (OR 0.539 (0.323-0.887), P=0.01) (**Table 4**). In the adjusted model, a rise in T_4 was independently associated with a lower risk of infection (0.978 (0.967-0.989) per nmol/l increase; P<0.0001), whereas a rise in the T_3/rT_3 ratio was independently associated with a higher risk of new infection (1.148 (1.042-1.277) per unit increase; P=0.004).

Finally, a significant interaction was found between the change from baseline in the T_3/rT_3 ratio and the age groups (infants and children) in relation to the outcomes affected by late-PN. Indeed, the contribution of a lowered T_3/rT_3 ratio to a higher likelihood of an earlier discharge from PICU (interaction P=0.04) and to a decreased risk of new infection (interaction P=0.01) was larger for infants than for older children [HR per unit T_3/rT_3 ratio increase in infants of 0.873 (95% CI 0.827-0.925) and 0.968 (95% CI 0.940-0.997) in older children for time to PICU discharge; OR per unit increase in T_3/rT_3 ratio of 1.601 (95% CI 1.119-2.346) in infants and 1.057 (0.954-1.183) in older children for acquiring a new infection]. No such interaction with age was observed for the change from baseline in T_4.

5. DISCUSSION

In a large group of critically ill children admitted to the PICU after various life-threatening insults, we first confirmed the development of NTI, with its severity being predictive for adverse clinical outcomes. Second, we demonstrate that the severity of NTI is affected by the nutritional management, since children for whom low or no macronutrient intake was accepted during the first week of critical illness showed a further drop in T_4 and T_3 concentrations and in the T_3/rT_3 ratio, while rT_3 increased further as compared with children who received early full nutrition. The accentuated decrease in T_4, reflecting the central component of NTI, appeared to counteract the outcome benefit of late-PN in terms of early discharge from PICU and risk of new infection. In contrast, the peripheral inactivation of thyroid hormone, reflected in the further decline in the T_3/rT_3 ratio, statistically explained part of the outcome benefit of late-PN. Interestingly, whereas the reduction in the T_3/rT_3 ratio was larger in older children than in infants, the contributory effect on outcome of a decrease in the T_3/rT_3 ratio was more pronounced for critically ill infants than for older children.

The presence of NTI with a decrease in T_4, T_3, and T_3/rT_3, and an increased rT_3, and its association with adverse short-term outcomes in this large cohort of almost 1000 critically ill children confirmed the findings of earlier smaller studies (4-7, 16-18). In multivariable analysis adjusted for baseline risk factors, the association between T_4 and/or T_3/rT_3 at admission remained significant. By adding the thyroid hormones at admission to the baseline risk factors, the performance of the models slightly increased, which suggests a contributing effect of T_4 and/or T_3/rT_3 at admission to the prediction of clinical outcomes, in addition to the other baseline risk factors.

Fasting in healthy individuals decreases serum concentrations of thyroid hormones. A decrease in serum concentrations of leptin and downregulation of hypothalamic TRH neurons are involved, contributing to persistently low serum TSH concentrations (32, 33). Furthermore, reduced D1 activity and increased D3 activity with fasting reduce the conversion of T_4 to active T_3, while favoring formation and hampering clearance of inactive rT_3 (32, 34). Accepting low or no macronutrient intake during the first week in PICU, thus mimicking (virtual) fasting superimposed on the illness, aggravated NTI in critically ill children, reflected by a further drop in T_4, T_3 and T_3/rT_3 and a further rise in rT_3. This is in agreement with findings in critically ill adult patients (24, 35-37). A small observational study of PICU patients, all fed according to a similar enteral/parenteral feeding strategy, could not find an association between the amount of nutrients delivered and the degree of NTI (38). However, the impact of early nutrition on thyroid hormone parameters in critically ill children had not been previously investigated in the context of a randomized controlled study. Interestingly, under early supplemental PN, further accentuation of the peripheral inactivation of thyroid hormone was also observed with tight glycemic control to age-adjusted normal fasting glycemia in critically

ill children, which was found to reduce morbidity and mortality in a large RCT of PICU patients (39). Indeed, this intervention further decreased T_3 and further increased rT_3 during the first days of critical illness, though without an effect on T_4 (18). Hence, this intervention partly mimicked the fasting response of the thyroid axis, an effect that was interpreted as being caused by the fasting levels of blood glucose achieved by tight glycemic control. The low or absent macronutrient intake that was accepted with the late-PN strategy in the present study, combined with lower blood glucose concentrations under glycemic control, had a greater impact, with an additional aggravation of NTI at a central level (i.e. resulting in a further lowering of T_4).

Statistical analysis suggests that the more pronounced suppression of the central component of NTI in the condition of low/absent macronutrient intake, with further lowering of T_4 from baseline, might be deleterious. Indeed, this effect statistically counteracted the outcome benefit of early macronutrient restriction with regard to likelihood of earlier PICU discharge and risk of acquiring a new infection in the PICU. This is in line with the rise in T_4 achieved by TRH infusion during a 5 day period combined with a GH secretagogue in prolonged critically ill adults. This intervention evoked an anabolic/anti-catabolic response in muscle and bone, in which the normalization of T_4 played a key role (40). However, in the sickest individuals of this previous study, the combined secretagogue infusion not only increased T_3 but also rT_3 concentrations, which suggests that the distinction between the central and the peripheral component may not be absolute. In contrast, the peripheral component with more pronounced inactivation of T_3 to rT_3 might be a beneficial adaptation, as it statistically contributed to the outcome benefit of the intervention. This contribution was more pronounced with regard to the lower risk of acquiring a new infection as compared with the higher likelihood of an earlier live PICU discharge. In theory, a lowered energy expenditure with lower T_3 availability brought about by the lack of macronutrients, may have contributed to faster recovery (41). Interestingly, the lower risk of infection associated with a lowered T_3/rT_3 ratio could be explained by optimized bacterial killing capacity mediated by increased D3 activity and elevated rT_3 locally within granulocytes (42). However, recent research also pointed to an anti-inflammatory effect of low intracellular T_3 action in macrophages (43). Hence, one could speculate that the increased rT_3 in particular, rather than the decreased T_3, may have contributed to the lowered infection risk with not using early-PN in the PICU.

Interestingly, as compared with older children, infants showed a more pronounced peripheral inactivation of thyroid hormone upon PICU admission, resulting in a much lower T_3/rT_3 ratio, whereas the further decrease in T_3/rT_3 towards day 3 or last PICU day was less pronounced. However, the contributing effect of this peripheral thyroid hormone inactivation to an earlier discharge from PICU and decreased risk of new infection with late-PN was greater for infants than for older children. These findings might indicate a more appropriate response to the acute stress of critical illness in infants than in older children. Several explanations may be invoked, though remain speculative. Evolutionary, infants may need a stronger fasting

response as part of a survival strategy, since they are entirely dependent on external help for nutrient provision. This is in line with the faster increase in ketone body concentrations and a faster decrease in glucose concentrations in response to fasting in healthy younger children as compared with older children (44). Furthermore, the pronounced peripheral inactivation of T_3 might be related to a more efficient postnatal reactivation of D3 expression during critical illness, considering that fetal and placental D3 levels are physiologically high to protect against maternal thyroid hormone (45). Finally, the targets for age-adjusted normal blood glucose concentrations, particularly in the Leuven center (23), which are lower for infants than for older children (46), or a larger difference in total macronutrient intake between the early-PN and late-PN groups in infants versus older children may theoretically play a role since they might mimic a more pronounced fasting response in infants as compared with older children. The different thyroidal axis responses to critical illness in different age groups stress the importance of this distinction for further research.

Our findings suggest important clinical implications, since the distinction of the changes in the thyroid axis during critical illness as beneficial or harmful is embedded in the controversy about whether or not to treat NTI (47, 48). If NTI is harmful, hormone substitution (central or peripherally) might improve clinical outcomes. However, if NTI reflects a beneficial adaptation of the critically ill body to reduce energy expenditure and to prevent infections, treatment could be deleterious. In several small RCTs, T_3 treatment of critically ill adult patients did not improve short-term outcomes or alter mortality (49-51), nor did treatment with T_4 (52). T_3 infusion in critically ill children undergoing surgery for congenital heart disease appeared to have a slight positive inotropic effect on cardiac function, but did not improve other clinical outcomes either (53-56). The lack of efficacy of T_3 treatment might be due to the iatrogenic suppression of TSH secretion and hereby lowering of the T_4 availability, which could hamper fast normalization of thyroid function upon recovery (52). Moreover, the administration of thyroid hormone is potentially harmful, as excessive levels may lead to coronary ischemia, myocardial infarction, hypertension, arrhythmia, and death (54, 55, 57, 58). Although hypothyroidism in children specifically raises concerns about long-term neurodevelopment and growth (59, 60), prophylactic thyroid hormone therapy given to preterm infants (58, 61) and to children after cardiopulmonary bypass (62) with NTI did not improve neurodevelopmental outcomes. The lack of efficacy of T_3 treatment is in line with the present study's finding that the peripheral conversion of T_3 to rT_3 may be a beneficial adaptation to critical illness in children. Since the present study suggests that the central component of NTI may not be beneficial, TRH infusion as treatment could be considered rather than treatment with thyroid hormone. This treatment could also be safer, as the negative feedback exerted by thyroid hormones on the TSH-producing pituitary cells is maintained, and thereby excessively elevated thyroid hormone levels are avoided (12, 63). However, RCTs evaluating TRH treatment in critically ill adults and children with NTI are needed to ascertain whether this translates to improved clinically relevant outcomes.

Our study has some limitations. First, because blood samples were taken via heparinized lines, we were unable to quantify free hormone concentrations, since these would be distorted by the heparin (27). Second, as accepting low/no macronutrient intake early during critical illness accelerated recovery, we only studied the short-term impact of nutritional management on changes in thyroid hormones in the first few days of critical illness. This was necessary, as otherwise, the findings could have been biased, as no samples were collected from recovered patients after PICU discharge. Finally, although the statistical models point to a potentially harmful central and potentially beneficial peripheral component of NTI in critically ill children, the biological mechanisms for such effects remain to be further investigated.

In conclusion, accepting low or even absent macronutrient intake early during critical illness in children further aggravated NTI as compared with early full feeding. The effect on the peripheral, but not the central, component of NTI with accentuated conversion of T_3 into rT_3 explains part of the outcome benefit of accepting such virtual fasting during critical illness, and thus appears to reflect a beneficial adaptation in this condition.

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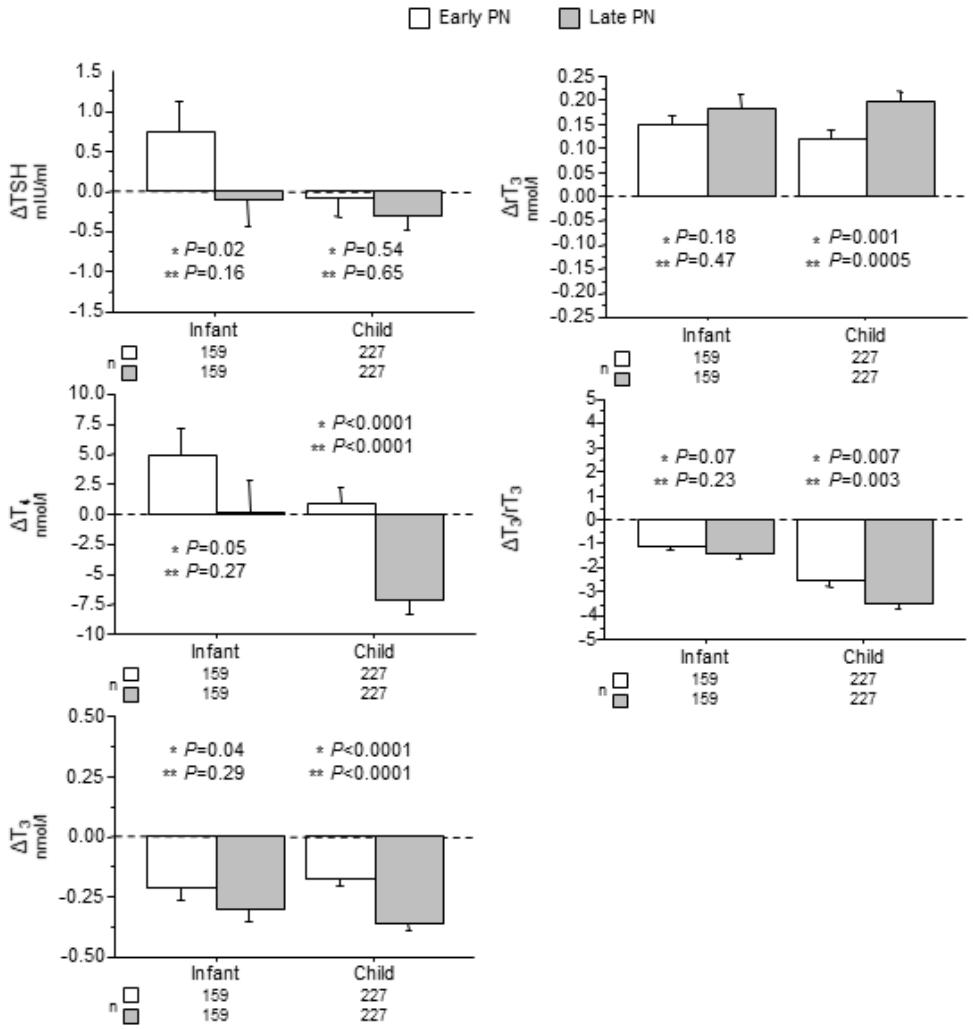
SUPPLEMENTARY MATERIAL

Supplemental Figure



SUPPLEMENTAL FIGURE 1: EFFECT OF EARLY-PN VERSUS LATE-PN ON THE THYROID AXIS BY AGE GROUP

Bars (mean ± SE) represent the changes from the admission values (referred to as Δ) to day 3 in the PICU (or last day for patients with shorter PICU stay) in serum TSH, T_4, T_3, rT_3 and T_3/rT_3 ratio. The open and filled bars represent the patients randomized to the early-PN and late-PN groups, respectively. * P values obtained with univariable analysis, ** P values obtained with multivariable analysis after adjustment for baseline risk factors (treatment center, risk of malnutrition (STRONGkids score), age, diagnosis upon admission, and severity of illness (PeLOD and PIM2 score)). PeLOD: Pediatric Logistic Organ Dysfunction score; PIM2: Pediatric Risk of Mortality 2 score; STRONGkids: Screening Tool for Risk On Nutritional Status and Growth (score of 0 indicating a low risk of malnutrition, a score of 1-3 indicating medium risk, and a score of 4-5 indicating high risk).

CHAPTER 4 -

DYNAMICS AND PROGNOSTIC VALUE OF THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS RESPONSES TO PEDIATRIC CRITICAL ILLNESS AND ASSOCIATION WITH CORTICOSTEROID TREATMENT: A PROSPECTIVE OBSERVATIONAL STUDY

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1. ABSTRACT

Purpose: Increased systemic cortisol availability during adult critical illness is determined by reduced binding-proteins and suppressed breakdown rather than elevated ACTH. Dynamics, drivers and prognostic value of hypercortisolism during pediatric critical illness remain scarcely investigated.

Methods: This preplanned secondary analysis of the PEPaNIC-RCT (N=1440), after excluding 420 children treated with corticosteroids before PICU-admission, documented (a) plasma ACTH, (free)cortisol and cortisol-metabolism at PICU-admission, day-3 and last PICU-day, their prognostic value, and impact of withholding early parenteral nutrition (PN), (b) the association between corticosteroid-treatment and these hormones, and (c) the association between corticosteroid-treatment and outcome.

Results: ACTH was normal upon PICU-admission and low thereafter ($p \leq 0.0004$). Total and free cortisol were only elevated upon PICU-admission ($p \leq 0.0003$) and thereafter became normal despite low binding-proteins ($p < 0.0001$) and persistently suppressed cortisol-metabolism ($p \leq 0.03$). Withholding early-PN did not affect this phenotype. On PICU-day-3, high free cortisol and low ACTH independently predicted worse outcome ($p \leq 0.003$). Also, corticosteroid-treatment initiated in PICU, which further suppressed ACTH ($p < 0.0001$), was independently associated with poor outcomes (earlier live PICU-discharge: $p < 0.0001$, 90-day mortality: $p = 0.02$).

Conclusion: In critically ill children, systemic cortisol availability is elevated only transiently, much lower than in adults, and not driven by elevated ACTH. Further ACTH lowering by corticosteroid-treatment indicates active feedback inhibition at pituitary level. Beyond PICU-admission-day, low ACTH and high cortisol, and corticosteroid-treatment, predicted poor outcome. This suggests that exogenously increasing cortisol availability during acute critical illness in children may be inappropriate. Future studies on corticosteroid-treatment in critically ill children should plan safety analyses, as harm may be possible.

2. INTRODUCTION

Increased systemic cortisol availability is essential in order to cope with the severe physical stress imposed by surgery, trauma or severe illnesses. It has long been assumed that increased systemic cortisol availability in response to critical illnesses requiring admission to an intensive care unit (ICU) is predominantly driven by a sustained activation of the hypothalamus-pituitary-adrenocortical (HPA) axis, with a 6- to 10-fold increase in adrenocortical cortisol synthesis and secretion in response to increased ACTH release from the anterior pituitary. Also fasting, often a consequence of severe illness, is considered a metabolic stressor that can affect the HPA axis [1]. In critically ill adults, high as well as low plasma cortisol concentrations have been associated with adverse outcome [2]. More recently, it has become clear that in most adult patients, throughout the first 4 weeks in the ICU, hypercortisolism is present in the face of low or normal rather than high plasma ACTH concentrations, irrespective of whether or not nutritional goals are targeted early [3-5]. Instead of being determined by increased ACTH-driven cortisol production, the increased systemic cortisol availability during critical illness in adults was shown to be determined by reduced circulating levels of cortisol binding-proteins with suppressed binding affinity and by a suppressed cortisol breakdown in liver and kidney, which, together, via feedback inhibition, at least partially explain the low plasma ACTH [2-4, 6-9].

Although HPA axis alterations have been described in children suffering from meningococcal sepsis [10-12], the dynamics, drivers and prognostic value of systemic cortisol availability in the broader population of critically ill children have only scarcely been investigated [13, 14]. Nevertheless, studies have suggested that a substantial proportion of critically ill children may not be able to increase systemic cortisol availability enough to face the stress of the critical illness, which could cause hemodynamic instability and systemic hyperinflammation with need for vasopressor support [15, 16]. As a consequence, pediatric intensivists often prescribe systemic corticosteroids, often in quite high doses, although adequately powered RCTs investigating its clinical efficacy and safety in children are lacking [15, 16]. It currently remains debated whether or not such treatment improves morbidity and mortality of critically ill children and some studies have suggested possible harm [17-20].

In this study, we aimed at (a) documenting changes over time during the course of pediatric critical illness in plasma ACTH, (free)cortisol and cortisol metabolites, their prognostic value and the impact of withholding early supplemental parenteral nutrition (PN), (b) investigating the association between treatment with systemic corticosteroids and these parameters, and (c) assessing the independent association between treatment with systemic corticosteroids and patient-centered clinical outcomes.

3. MATERIAL AND METHODS

Patients and sample collection

This study was a preplanned secondary analysis of the multicenter PEPaNIC randomized controlled trial (RCT) conducted in Belgium, the Netherlands and Canada (June 2012 – July 2015) [21]. Written informed consent was obtained from parents or legal guardians. The institutional ethical review board at each participating center approved the protocol and consent forms of the study (ML8052, NL38772.000.12, Pro00038098), which was performed in accordance to the 1964 Declaration of Helsinki and later amendments. The detailed study protocol and primary results have been published [21, 22].

The PEPaNIC trial investigated the clinical outcomes of withholding supplemental parenteral nutrition (PN) up to day 8 in the pediatric ICU (PICU), referred to as "late-PN", as compared with early supplemental PN whenever enteral nutrition alone was insufficient to reach the caloric target, referred to as "early-PN". Children aged 0-17 years were eligible for inclusion, if a PICU-stay of 24 hours or more was expected, if they had a moderate or severe risk of malnutrition (score ≥2 on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids)), and if none of the predefined exclusion criteria were met.

Enteral nutrition was started as soon as possible in both groups. In the early-PN group, supplemental PN was initiated within 24 hours after PICU-admission to reach the caloric target (**Supplemental Table 1-2**), whereas PN was withheld up to the morning of PICU day 8 for patients in the late-PN group. Although labeled "late-PN" group, the majority of these patients needed intensive care for less than 8 days and thus did not receive PN. In the late-PN group, a mixture of intravenous dextrose (5%) and saline was administered to match the intravenous fluid load given to the early-PN patients. Patients in both groups equally received intravenous trace elements, minerals and vitamins, and blood glucose control with insulin according to local targets. Blood was sampled upon admission and then daily at 6 a.m. until PICU-discharge or death, within limitations for amount of blood sampling allowed by the institutional ethical review boards per center. For the quantification of hormonal parameters of the HPA axis, blood samples were collected in pre-chilled EDTA tubes and immediately placed on ice, centrifuged at 4°C, with plasma stored at -80°C until assay. Daily urine samples from a 24-h urine collection were stored in Vacuette urine tubes at -80°C until quantification of the cortisol metabolites.

To investigate the time course of the changes in plasma concentrations of ACTH, total cortisol, free cortisol, CBG and albumin during critical illness in children (**Figure 1**, Panel A.1), patients who received corticosteroids within three days before inclusion (including in the emergency room) or prior to the blood sampling day were excluded, as well as those patients who died in PICU on the day of blood sampling to avoid bias evoked by the agonal stress response. Of the remaining patients, we included those 442 patients for whom plasma samples were available upon admission and on day 3 (for 230 long-stay patients treated in PICU for at least 3 days, which is the median duration of PICU stay, **Table 1**) or last PICU day (for 212 short-stay patients treated in PICU for <3 days). For comparison, 64 healthy children, who had never been critically ill and from whom blood was drawn immediately after intravenous catheterization prior to minor elective surgery, age- and gender-matched with the patients, were included (**Figure 1**, Panel A.1). The prognostic value for the clinical outcomes (new infection acquired in the PICU, time to live discharge from PICU and 90-day mortality) of the admission plasma concentrations of ACTH and free cortisol was assessed among these 442 patients (**Figure 1**, Panel A.4). The prognostic value of plasma ACTH and free cortisol on day 3 was also assessed for the 230 long-stay patients (**Figure 1**, Panel A.4). For the estimation of the activity of cortisol-metabolizing enzymes via urinary cortisol metabolites, a representative subset of 76 patients and 25 age- and gender-matched healthy control children was selected (**Figure 1**, Panel A.2).

To investigate whether late-PN, as compared with early-PN, altered the changes in endogenous, illness-induced HPA axis parameters, between PICU admission and day 3 or last PICU day for patients discharged earlier (**Figure 1**, Panel A.3), a propensity score-matched patient subset was selected, given the required exclusion of a substantial number of patients. Propensity scores were estimated with logistic regression based on baseline risk factors as covariates, including treatment center, risk of malnutrition (STRONGkids score), age group, diagnosis upon admission, severity of illness (PeLOD score reflecting degree of organ failure and PIM2 score estimating risk of death) and sepsis upon admission [23-26]. One-to-one nearest neighbor-matching without replacement and a caliper of 0.3 retained 414 patients.

To investigate the association between treatment with corticosteroids in the PICU and plasma concentrations of ACTH, total cortisol, free cortisol, CBG and albumin (**Figure 1**, Panel B), the 33 patients who had a measurement the morning after the first day of corticosteroid-treatment were matched case-by-case to patients who had not been treated with corticosteroids and for whom a measurement was available for the same PICU-stay day (**Figure 1**, Panel B, **Supplemental Methods 1**). Distinction was made between hydrocortisone and synthetic corticosteroids given that the latter are not detected by the assay used for quantifying cortisol.

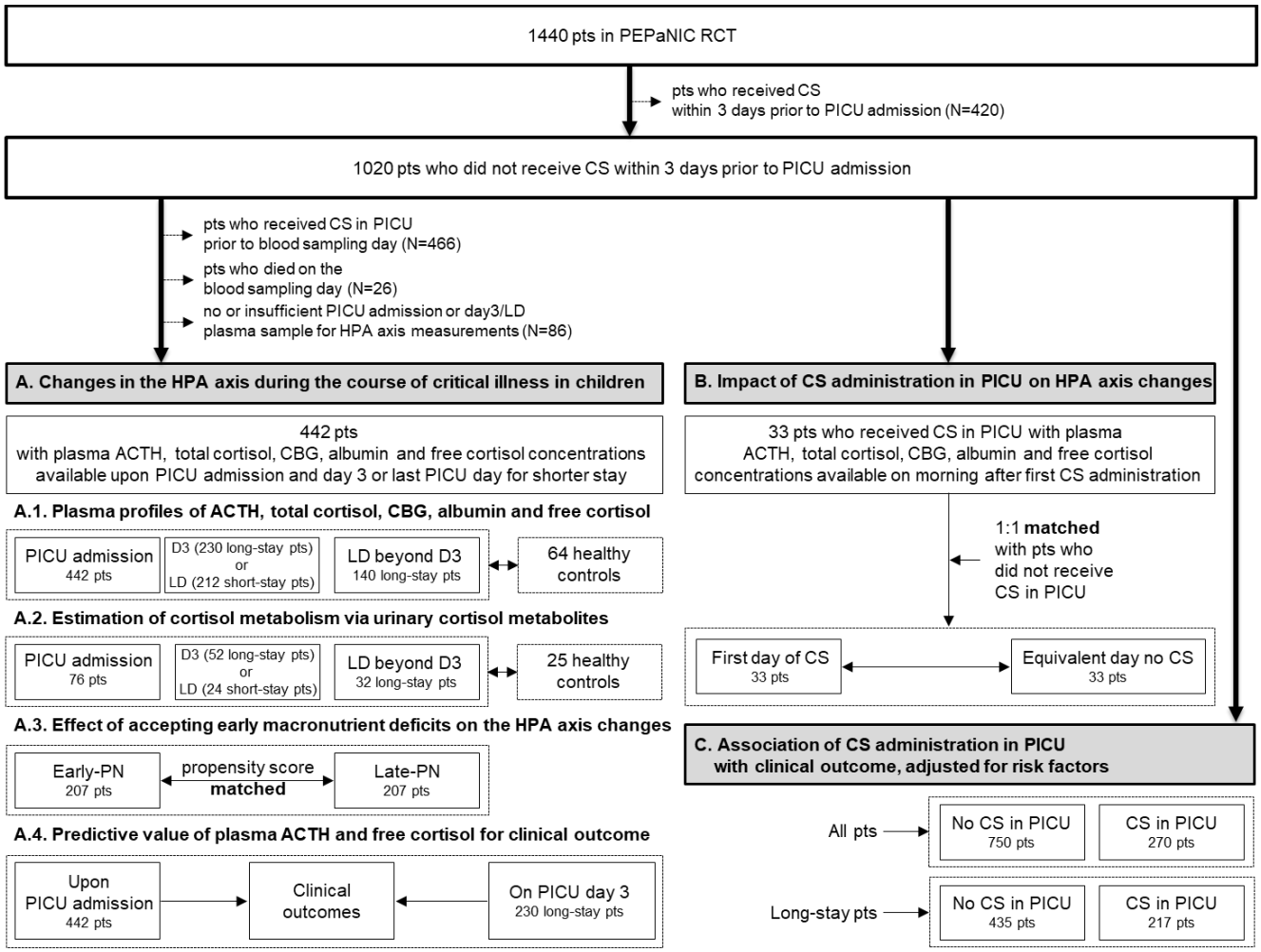

FIGURE 1 DIAGRAM OF THE STUDY DESIGN

ACTH: adrenocorticotropic hormone, CBG: cortisol-binding globulin, CS: corticosteroids, D3: day 3 in PICU, HPA axis: hypothalamus-pituitary-adrenal axis, LD: last day in PICU, PEPaNIC: early versus late parenteral nutrition in the pediatric intensive care unit, PICU: pediatric intensive care unit, PN: parenteral nutrition, RCT: randomized controlled trial. Short-stay patients are patients who needed intensive care for 1 or 2 days, long-stay patients are patients who needed intensive care for at least 3 days.

To investigate the independent association between treatment with corticosteroids initiated in the PICU and patient-centered clinical outcomes (new infection acquired in the PICU, time to live discharge from PICU and 90-day mortality) (**Figure 1**, Panel C), all 1020 patients who had not been treated with corticosteroids before PICU admission were included.

TABLE 1 CHARACTERISTICS OF PICU PATIENTS AND HEALTHY CONTROL CHILDREN

	HPA axis changes			Association of CS administration with HPA axis			Association of CS administration with outcome
Baseline Characteristics	Patients (N=442)	Controls (n=64)	P	No CS (N=33)	CS (N=33)	P	
Age (years), median (IQR)	2.6 (0.4 – 7.6)	2.0 (0.7 – 6.7)	0.68	6.4 (1.1 – 11.5)	3.1 (0.8 – 10.8)	0.37	1.4 (0.3 – 6.7)
Age <1 year, N (%)	167 (37.8)	25 (39.1)	0.84	8 (24.2)	10 (30.3)	0.58	463 (45.4)
Male gender, N (%)	250 (56.5)	37 (57.8)	0.85	21 (63.6)	21 (63.6)	>0.99	593 (58.1)
Weight (SD score), median (IQR)	-0.5 (-1.4 – 0.5)			0.1 (-0.6 – 0.8)	-0.4 (-1.4 – 0.6)	0.12	-0.5 (-1.4 – 0.5)
Height (SD score), median (IQR)	-0.4 (-1.5 – 0.6)			-0.2 (-1.2 – 1.2)	-0.2 (-1.3 – 0.9)	0.63	-0.4 (-1.5 – 0.7)
Treatment center, N (%)						0.55	
Leuven	387 (87.6)			23 (69.7)	19 (57.6)		544 (53.3)
Rotterdam	46 (10.4)			8 (24.2)	12 (36.4)		401 (39.3)
Edmonton	9 (2.0)			2 (6.1)	2 (6.1)		75 (7.4)
STRONGkids risk level, N (%) [a]						0.55	
Medium	415 (93.9)			32 (97.0)	31 (93.9)		907 (88.9)
High	27 (6.1)			1 (3.0)	2 (6.1)		113 (11.1)
PeLOD score, first 24 hrs in PICU,	23 (21 – 32) (10.4)			21 (12 – 32)	23 (10 – 32)	0.60	21 (11 – 31)
PIM2 score, median (IQR) [c]	-2.9 (-3.8 – -1.8)			-2.9 (-4.1 – -1.3)	-3.0 (-3.8 – -1.7)	0.89	-2.8 (-3.7 – -1.6)
PIM2 probability of death, %, median	5.2 (2.3 – 14.5)			4.6 (2.2 – 13.9)	5.4 (2.3 – 15.3)	0.97	5.8 (2.3 – 17.0)
Emergency admission, N (%) [d]	168 (38.0)			17 (51.5)	16 (48.5)	0.80	615 (60.3)
Diagnostic group, N (%)						0.92	
Surgical							
Abdominal	16 (3.6)			0 (0.0)	0 (0.0)		112 (11.0)
Burns	3 (0.7)			0 (0.0)	0 (0.0)		10 (1.0)
Cardiac	222 (50.2)			10 (30.3)	10 (30.3)		294 (28.8)
Neurosurgery-traumatic brain	35 (7.9)			8 (24.2)	8 (24.2)		80 (7.8)
Thoracic	21 (4.8)			1 (3.0)	2 (6.1)		57 (5.6)
Transplantation	0 (0.0)			0 (0.0)	1 (3.0)		8 (0.8)
Orthopedic surgery-trauma	41 (9.3)			3 (9.1)	2 (6.1)		53 (5.2)
Other	11 (2.5)			2 (6.1)	1 (3.0)		39 (3.8)
Medical							
Cardiac	18 (4.1)			2 (6.1)	2 (6.1)		59 (5.8)
Gastrointestinal-Hepatic	2 (0.5)			0 (0.0)	0 (0.0)		4 (0.4)
Oncologic-Hematologic	0 (0.0)			0 (0.0)	1 (3.0)		9 (0.9)
Neurologic	22 (5.0)			1 (3.0)	1 (3.0)		84 (8.2)
Renal	0 (0.0)			0 (0.0)	0 (0.0)		1 (0.1)
Respiratory	28 (6.3)			0 (0.0)	0 (0.0)		141 (13.8)
Other	23 (5.2)			6 (18.2)	5 (15.2)		69 (6.8)
Condition on admission, N (%)							
Mechanical ventilation required	391 (88.5)			28 (84.9)	28 (84.9)	>0.99	875 (85.8)
ECMO or other assist device	7 (1.6)			0 (0.0)	1 (3.0)	0.23	29 (2.8)
Infection	123 (27.8)			12 (36.4)	11 (33.3)	0.79	429 (42.1)
Sepsis	93 (21.0)			11 (33.3)	9 (27.3)	0.59	308 (30.2)
Randomization group, N (%)						0.20	
Early PN	227 (51.4)			15 (45.5)	10 (30.3)		504 (49.4)
Late PN	215 (48.6)			18 (54.6)	23 (69.7)		516 (50.6)
Duration of PICU stay, days, median	3 (1 – 6)			2 (1 – 3)	2 (1 – 3)	>0.99	4 (2 – 8)
Acquisition of a new infection in PICU,	47 (10.6)			1 (3.0)	2 (6.1)	0.55	140 (13.7)
90-day mortality, N (%)	12 (2.7)			2 (6.1)	4 (12.1)	0.39	61 (6.0)

[a] Evaluates risk of malnutrition with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. [b] Scores range from 0 to 71, with higher scores indicating more severe illness. [c] Higher scores indicate a higher risk of mortality. [d] Elective admissions comprise those requiring post-operative intensive care after scheduled surgery (often complex cardiac surgery or surgery on other vital organ systems). Unplanned transfers to the PICU from the general pediatric ward, from the emergency department or from elsewhere are considered emergency admissions. [e] Of whom 270 received CS. CS: corticosteroids, ECMO: extracorporeal membrane oxygenation, HPA axis: hypothalamic-pituitary-adrenal axis. PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU. PICU: pediatric intensive care unit. PIM2: pediatric index of mortality 2 score. PN: parenteral nutrition, STRONGkids: Screening Tool for Risk on Nutritional Status and Growth.

Plasma and urinary analyses

Plasma ACTH concentrations were quantified by double-monoclonal immunoradiometric assay (Brahms Diagnostics) and plasma cortisol (Immunotech) and cortisol-binding-globulin (CBG) (Riazen) by competitive radioimmunoassay. Plasma albumin was quantified by the bromocresol-green colorimetric method (Sigma-Aldrich). Plasma free cortisol was calculated with the Coolens formula adapted for individual albumin and CBG concentrations, which has previously been validated as representative for measured plasma free cortisol concentrations in the ICU context [6, 27]. After deglucoronidation, the absolute urinary concentrations of cortisol (F), 5α-tetrahydrocortisol (allo-THF), 5β-tetrahydrocortisol (THF), cortisone (E), 5α-tetrahydrocortisone (allo-THE) and tetrahydrocortisone (THE) were determined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as reported previously [4].

Statistical analyses

For research question A and C (**Figure 1**), sample size was determined by availability. For research question B (**Figure 1**), given lack of data for children in the PICU, sample size was determined based on published data regarding the impact of exogenous corticosteroids on plasma ACTH outside the context of critical illness [28, 29]. To detect a lowering in plasma ACTH in this order of magnitude, with 80% power and 95% certainty, 33 patients per group were required.

Continuous data and proportions were compared with Wilcoxon Rank Sum and Chi-square tests, respectively. Data are presented as medians and interquartile ranges or numbers and percentages.

Independent associations of plasma concentrations of ACTH and free cortisol, or of corticosteroid-treatment, with patient-centered clinical outcomes were analyzed with multivariable logistic regression and proportional hazard analyses, adjusted for baseline risk factors [treatment center, risk of malnutrition (STRONGkids score), age group, diagnosis upon admission, severity of illness (PeLOD and PIM2), sepsis upon admission] [23-26]. Effect sizes are reported as odds ratios (OR) and hazard ratios (HR), respectively, with 95% confidence intervals (CI).

Propensity score matching was performed with SPSS R-menu R3.1 (Foundation for Statistical Computing) in IBM SPSS Statistics 25.0.0.0 (SPSS, Chicago, IL). All analyses were performed with JMP®Pro14.0.0 (SAS Institute Inc, Cary, NC). Two-sided p-values <0.05 were considered to indicate statistical significance.

4. RESULTS

For the 3 main research questions, depicted in **Figure 1**, comparisons of the patient characteristics are provided in **Table 1** and **Supplemental Table 3-4**.

Time course of the changes in plasma concentrations of ACTH, total cortisol, free cortisol, CBG, and albumin and estimations of cortisol metabolism during critical illness, prognostic value and impact of late-PN versus early-PN

Upon PICU-admission, plasma ACTH concentrations were not significantly different from those in the matched healthy children and became low thereafter throughout PICU stay (**Figure 2A**). Plasma total cortisol concentrations were only above normal at PICU-admission and became normal thereafter (**Figure 2B**). Also, plasma free cortisol concentrations were only higher than in healthy children upon PICU-admission. Thereafter, plasma free cortisol levels were no longer elevated (**Figure 2C**), despite plasma CBG and albumin concentrations that were mostly low (**Figure 2D,E**) and despite the observation that the estimated activities of cortisol-metabolizing enzymes, 11β-hydroxysteroid-dehydrogenase-2 and the A-ring reductases 5α-reductase and 5β-reductase, remained low throughout PICU stay (**Figure 2F-I).**

The use of late-PN, as compared with early-PN, did not affect the changes over time in plasma concentrations of ACTH, (free)cortisol, CBG, or albumin (**Supplemental Figure 1**).

Adjusted for baseline risk factors, including type and severity of illness among others, upon PICU-admission plasma concentrations of ACTH and free cortisol were not independently associated with the clinical outcomes (**Supplemental Table 5**). However, among long-stay patients, higher plasma ACTH concentrations on day 3 in the PICU were independently indicative of a higher likelihood of earlier live discharge from PICU, whereas higher plasma free cortisol concentrations on that day were independently indicative of a lower likelihood of earlier live discharge from PICU (**Table 2**). There were no independent associations between plasma ACTH or free cortisol on day 3 and the risk of new infections or the risk of 90-day mortality.

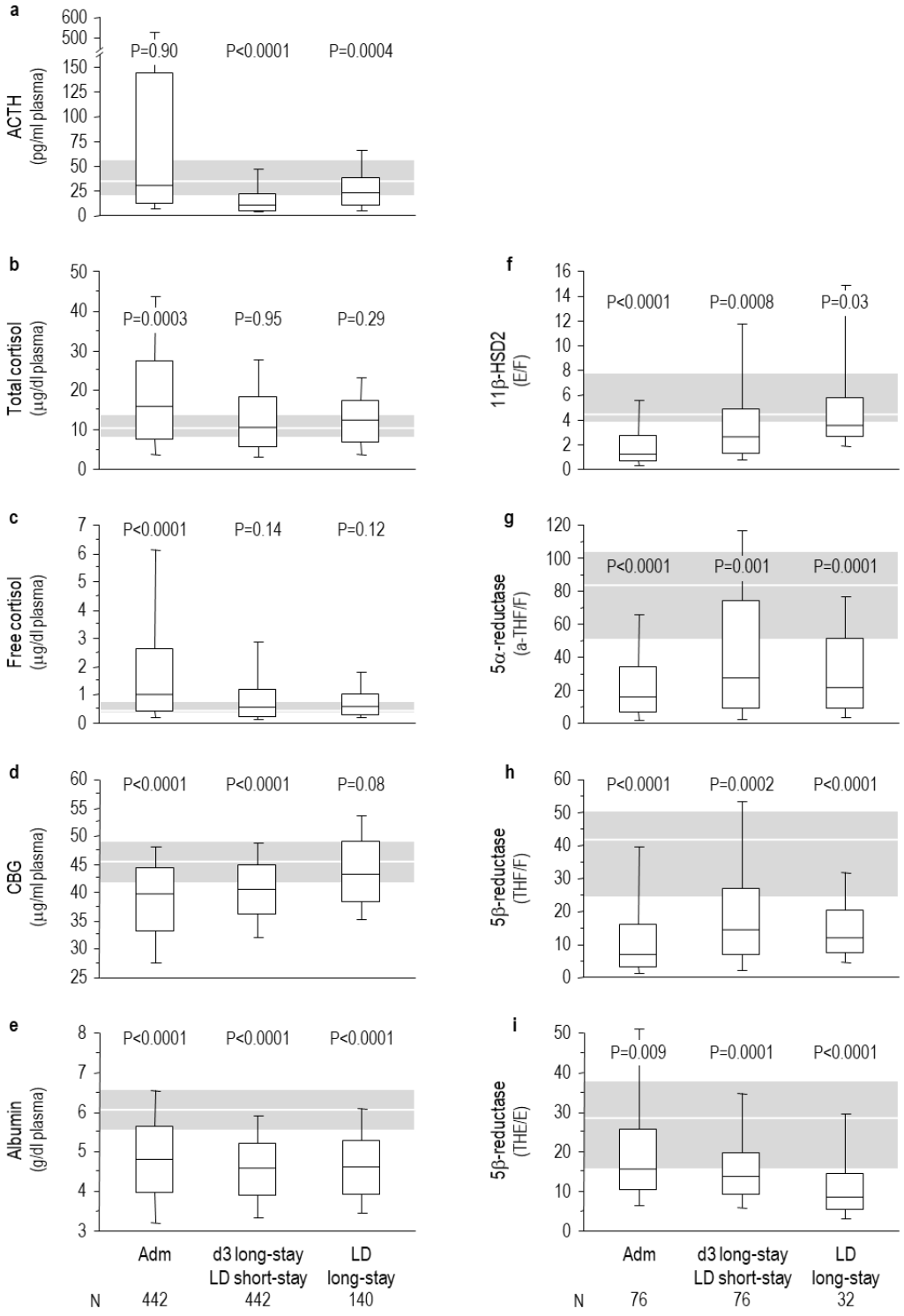

FIGURE 2 TIME COURSE OF THE CHANGES IN PLASMA CONCENTRATIONS OF ACTH, TOTAL CORTISOL, FREE CORTISOL, CBG AND ALBUMIN, AND ESTIMATIONS OF CORTISOL METABOLISM DURING CRITICAL ILLNESS

Plasma concentrations of ACTH (a), total cortisol (b), free cortisol (c), CBG (d) and albumin (e) were quantified, and activities of the cortisol metabolizing enzymes 11β-HSD2 (f), 5α-reductase (g) and 5β-reductase (h, i) were estimated upon PICU admission, on PICU day 3 or last PICU day for patients with a shorter PICU stay, and on the last PICU day beyond day 3 if still in PICU beyond day 3. Results overall apply irrespective of the presence of sepsis, and irrespective of whether patients were admitted after cardiac surgery or for other reasons. Box plots show medians, interquartile ranges, and 10th and 90th percentiles. The grey bars represent the interquartile ranges obtained for the healthy control children, with the white lines indicating the medians. ACTH: adrenocorticotropic hormone, aTHF: allo-THF (5α-tetrahydrocortisol), 11β-HSD2: 11β-hydroxysteroid-dehydrogenase type 2, CBG: cortisol-binding globulin, D3: day 3 in PICU, E: cortisone, F: cortisol, LD short-stay: last day in PICU for patients who needed intensive care for 1 or 2 days, LD long-stay: last day in PICU beyond day 3 for long-stay patients who needed intensive care for at least 3 days, PICU: pediatric intensive care unit, THE: tetrahydrocortisone, THF: 5β-tetrahydrocortisol.

TABLE 2 PROGNOSTIC VALUE OF THE CHANGES IN THE HPA AXIS FOR PATIENT-CENTERED CLINICAL OUTCOMES AND INDEPENDENT ASSOCIATION BETWEEN TREATMENT WITH CORTICOSTEROIDS IN THE PICU AND THOSE OUTCOMES

	Prognostic value of HPA axis changes in long-stay patients [a] N=230		Association of CS administration in PICU with outcome of long-stay patients [a] N=652	
	OR/HR (95%CI)	**P**	**OR/HR (95%CI)**	**P**
Risk of new infection [b]				
Infant vs child [c]	1.082 (0.427-2.743)	0.86	1.143 (0.680-1.922)	0.61
Center - Rotterdam vs Leuven	3.567 (1.014-12.547)	0.04	1.563 (0.796-3.072)	0.19
Center - Edmonton vs Leuven	0.000 (0.000-inf)	0.99	2.192 (0.944-5.089)	0.06
High vs medium risk of malnutrition [d]	2.241 (0.601-8.357)	0.22	1.934 (1.053-3.554)	0.03
PeLOD score (per unit added) [e]	1.046 (0.991-1.103)	0.09	1.033 (1.006-1.060)	0.01
PIM2 score (per unit added) [f]	1.518 (1.080-2.135)	0.01	1.306 (1.095-1.558)	0.003
Diagnostic group		0.004		0.94
Sepsis vs no sepsis upon admission	0.218 (0.068-0.700)	0.01	0.411 (0.236-0.714)	0.001
Randomization to late-PN vs early-PN	0.540 (0.242-1.202)	0.13	0.507 (0.320-0.802)	0.003
ACTH (per pg/ml added)	0.987 (0.967-1.007)	0.15		
Free cortisol (per µg/ml added)	1.082 (0.861-1.359)	0.52		
CS therapy vs no CS therapy in PICU			1.057 (0.648-1.724)	0.82
Likelihood of earlier live PICU discharge				
Infant vs child [c]	0.709 (0.513-0.983)	0.03	0.788 (0.652-0.953)	0.01
Center - Rotterdam vs Leuven	0.830 (0.516-1.311)	0.42	0.752 (0.594-0.954)	0.01
Center - Edmonton vs Leuven	1.191 (0.406-2.789)	0.72	0.928 (0.672-1.264)	0.63
High vs medium risk of malnutrition [d]	1.076 (0.561-1.894)	0.81	0.832 (0.641-1.066)	0.14
PeLOD score (per unit added) [e]	0.998 (0.980-1.017)	0.85	0.990 (0.981-1.000)	0.04
PIM2 score (per unit added) [f]	0.719 (0.633-0.813)	<0.0001	0.785 (0.732-0.842)	<0.0001
Diagnostic group		0.11		0.15
Sepsis vs no sepsis upon admission	1.038 (0.715-1.508)	0.84	0.925 (0.765-1.118)	0.42
Randomization to late-PN vs early-PN	1.006 (0.758-1.337)	0.96	1.336 (1.132-1.578)	0.0006
ACTH (per pg/ml added)	**1.009 (1.003-1.013)**	**0.0003**		
Free cortisol (per µg/ml added)	**0.906 (0.840-0.964)**	**0.003**		
CS therapy vs no CS therapy in PICU			**0.677 (0.563-0.814)**	**<0.0001**
Risk of 90-day mortality				
Infant vs child [c]	1.871 (0.290-12.058)	0.51	1.602 (0.727-3.529)	0.24
Center - Rotterdam vs Leuven	0.134 (0.007-2.478)	0.17	1.139 (0.442-2.939)	0.78
Center - Edmonton vs Leuven	0.000 (0.000-inf)	0.99	0.000 (0.000-inf)	0.99
High vs medium risk of malnutrition [d]	2.387 (0.223-25.502)	0.47	0.693 (0.220-2.185)	0.53
PeLOD score (per unit added) [e]	0.943 (0.845-1.052)	0.27	1.011 (0.973-1.051)	0.57
PIM2 score (per unit added) [f]	2.722 (1.435-5.165)	0.0004	1.860 (1.435-2.411)	<0.0001
Diagnostic group		0.61		0.79
Sepsis vs no sepsis upon admission	0.966 (0.141-6.613)	0.97	1.322 (0.571-3.058)	0.51
Randomization to late-PN vs early-PN	0.285 (0.051-1.586)	0.15	0.498 (0.235-1.057)	0.06
ACTH (per pg/ml added)	0.979 (0.937-1.023)	0.30		
Free cortisol (per µg/ml added)	1.190 (0.775-1.827)	0.48		
CS therapy vs no CS therapy in PICU			**2.307 (1.125-4.731)**	**0.02**

[a] Long-stay patients are patients who needed intensive care for at least 3 days. Patients who received corticosteroids within the three days prior to PICU admission were excluded from these analyses. [b] For the model on the association of CS administration in PICU with new infections, only infections occurring beyond the first day of CS administration were considered ("left-truncation"). [c] Infants are patients younger than 1 year. [d] Evaluated with the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. [e] Scores range from 0 to 71, with higher scores indicating more severe illness. [f] Higher scores indicate a higher risk of mortality. ACTH: adrenocorticotropic hormone, CI: confidence interval, CS: corticosteroids, HPA axis: hypothalamus-pituitary-adrenal axis, HR: hazard ratio, OR: odds ratio, PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU, PICU: pediatric intensive care unit, PIM2: pediatric index of mortality 2 score, PN: parenteral nutrition.

Association between treatment with corticosteroids initiated in the PICU and plasma concentrations of ACTH, total cortisol, free cortisol, CBG and albumin

Among the 33 selected patients who received corticosteroids on median day 2 (IQR 1-3), 28 received synthetic corticosteroids and 5 received hydrocortisone. Corticosteroid-treatment further suppressed plasma concentrations of ACTH as compared with the 33 matched patients who did not receive corticosteroids and were also assessed on median day 2 (IQR 1-3) (**Figure 3**). Expectedly, the use of synthetic corticosteroids lowered plasma total and free cortisol concentrations (**Figure 3**), whereas hydrocortisone increased plasma total and free cortisol. There was no association with the plasma concentrations of CBG or albumin.

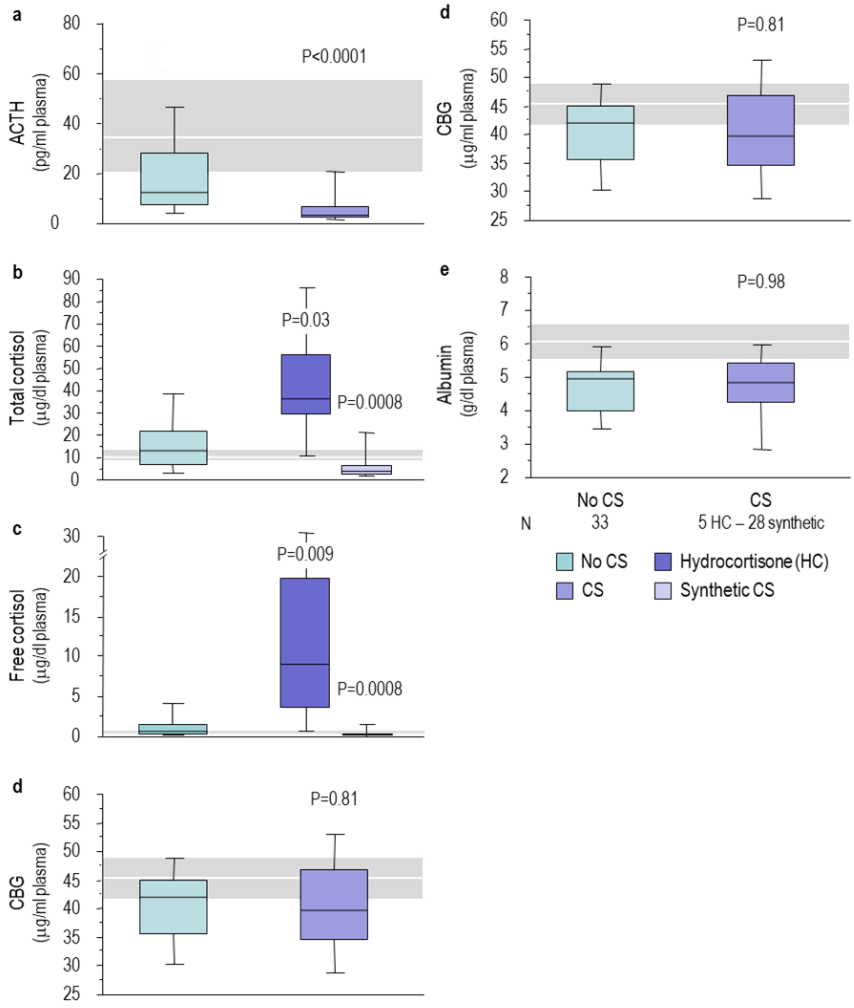

FIGURE 3 ASSOCIATION BETWEEN TREATMENT WITH CORTICOSTEROIDS INITIATED IN THE PICU AND PLASMA CONCENTRATIONS OF ACTH, TOTAL CORTISOL, FREE CORTISOL, CBG, AND ALBUMIN

Plasma concentrations of ACTH (a), total cortisol (b), free cortisol (c), CBG (d) and albumin (e) were quantified on the morning after the first day of CS administration and on equivalent days for patients who did not receive corticosteroids, manually matched 1:1 with the patients who received CS. To evaluate the association between CS administration and total and free cortisol, a distinction was made between hydrocortisone and synthetic CS, since the radioimmunoassay for cortisol does not measure synthetic CS. Box plots show medians, interquartile ranges, and 10th and 90th percentiles. The grey bars represent the interquartile range obtained for the healthy control children, with the white lines indicating the medians. P-values represent the comparison of CS (or type of CS) versus no CS administration. ACTH: adrenocorticotropic hormone, CBG: cortisol-binding globulin, CS: corticosteroids, PICU: pediatric intensive care unit, PN: parenteral nutrition.

Independent association between treatment with corticosteroids initiated in the PICU and patient-centered clinical outcomes

Of the 1020 patients who had not been treated with corticosteroids prior to PICU admission, 270 (26.5%) received corticosteroids initiated on median day 2 (IQR 1-5) after admission to the PICU. Among the 652 long-stay patients in PICU for at least 3 days, 217 (33.3%) received corticosteroids initiated on median day 3 (IQR 1-6) after PICU admission. Adjusted for baseline risk factors, the use of corticosteroids during PICU stay was independently associated with a lower likelihood of an earlier live discharge from ICU and with a higher risk of 90-day mortality for all patients (**Supplemental Table 6**) and for long-stay patients alike (**Table 2**). There was no independent association between use of corticosteroids and the risk of new infections.

5. DISCUSSION

In this study of critically ill children admitted to the PICU, plasma ACTH concentrations were either normal or low and were further lowered by the use of exogenous corticosteroids. Total and free plasma cortisol levels were elevated only briefly, thereafter lowering to normal levels despite persistently low circulating levels of cortisol binding-proteins and despite suppressed cortisol metabolism throughout PICU stay. Withholding supplemental PN during the first week in PICU did not affect this phenotype. Adjusted for risk factors, upon admission plasma concentrations of ACTH or free cortisol did not hold prognostic value for patient-centered clinical outcomes whereas on day 3 in the PICU, a high free cortisol and a low plasma ACTH level independently predicted worse outcome. Also, treatment with systemic corticosteroids initiated during the first few days in the PICU, which further suppressed ACTH, was found to independently associate with poor outcomes, adjusted for other risk factors.

Systemic cortisol availability in critically ill children treated in the PICU was found to be elevated only transiently and to a much lesser extent than was previously reported for adults [3, 5, 30]. The finding that plasma ACTH in patients was never significantly higher than in matched healthy children, not even in the presence of elevated plasma cortisol upon PICU-admission, was in line with previous adult data [3, 5, 30]. These findings suggest that peripheral mechanisms, such as low plasma binding and suppressed cortisol breakdown, are active to increase and maintain cortisol availability also in the pediatric critically ill population [3, 9]. The observation that exogenous corticosteroids further suppressed plasma ACTH suggests that ACTH is not fully endogenously suppressed during pediatric critical illness and thus that central activators balance against suppressors in exogenous corticosteroid-naive patients but that pituitary feedback inhibition remains responsive to a further increase of glucocorticoid signaling.

The low plasma ACTH observed beyond the PICU-admission day in the critically ill children cannot be brought about by feedback-inhibition through free cortisol, given that free cortisol was no longer elevated, unless the set-point for feedback inhibition would be lower in this context. A lower set-point for feedback inhibition by thyroid hormones has also been suggested as an explanation for the low T_3 syndrome of critical illness [31-33]. Absence of elevated free cortisol concentrations after a few days in the PICU, while cortisol binding-proteins were low and cortisol metabolism was suppressed, suggests that the concomitantly low ACTH must have played a role. Endogenous or iatrogenic suppressors of ACTH may be involved. Candidate endogenous suppressors of ACTH are elevated levels of bile acids as these have shown to be able to cross the blood-brain barrier and act as ligands for the glucocorticoid receptor, hereby potentially suppressing corticotropin-releasing hormone and hereby suppressing ACTH [34-36]. The use of opioids and other drugs in the PICU may have added suppressive effects on the HPA axis [30, 37, 38].

Admission levels of plasma ACTH and cortisol did not hold prognostic value for clinical outcome when adjusted for type and severity of illness and other risk factors. Hence, they appeared to be merely a correlate of the type and severity of illness. However, it was striking to observe that among long-stay patients, after extensive adjustment for risk factors including type and severity of illness, a high plasma free cortisol and a low plasma ACTH on day 3, a time point where both cortisol and ACTH levels were lowered as compared with PICU admission, together were independently predictive of poor outcome. Also, exogenous steroid treatment initiated during the first few days in the PICU, which further lowered plasma ACTH, was predictive of poor outcome after extensive adjustment for risk factors. Together these findings may suggest that the amount of cortisol availability required for an effective struggle for survival and recovery from critical illness in children may not be as high as previously assumed and lower than in adults. Excessive amounts of cortisol could indeed also be harmful [18, 19]. Alternatively, a direct role of endogenous and/or exogenous ACTH suppressors in mediating the independent association between low plasma ACTH and adverse outcome is possible and cannot be excluded from this study.

The strengths of this study are the large sample size of a heterogeneous cohort of critically ill children and the use of samples and data from a well-documented RCT which allowed to carefully adjust for prospectively registered confounders and which allowed case-by-case matching. A weakness of the study is that data were predominantly gathered during the first week in the PICU so that no conclusions can be drawn on a more prolonged phase of pediatric critical illness. Another weakness is the observational nature of our analyses of the associations with patient-centered clinical outcomes, which (although adjusted for baseline risk factors) cannot fully take into account potential confounding factors such as disease course. Lastly, free cortisol concentrations were not directly measured but calculated.

In conclusion, cortisol availability during pediatric critical illness is only briefly elevated, driven by peripheral rather than central mechanisms, and is followed by low ACTH and normal free cortisol levels until PICU-discharge. Low ACTH is further lowered by treatment with corticosteroids suggesting active feedback inhibition at the pituitary level. Beyond PICU-admission day, a high circulating cortisol as well as the administration of systemic corticosteroids appeared independent predictors of adverse outcome. These findings raise the hypothesis that increasing cortisol availability during the first days after onset of critical illness in children may be inappropriate. Studies investigating the effect of treatment with corticosteroids in critically ill children should thus carefully plan interim safety analyses to avoid possible harm.

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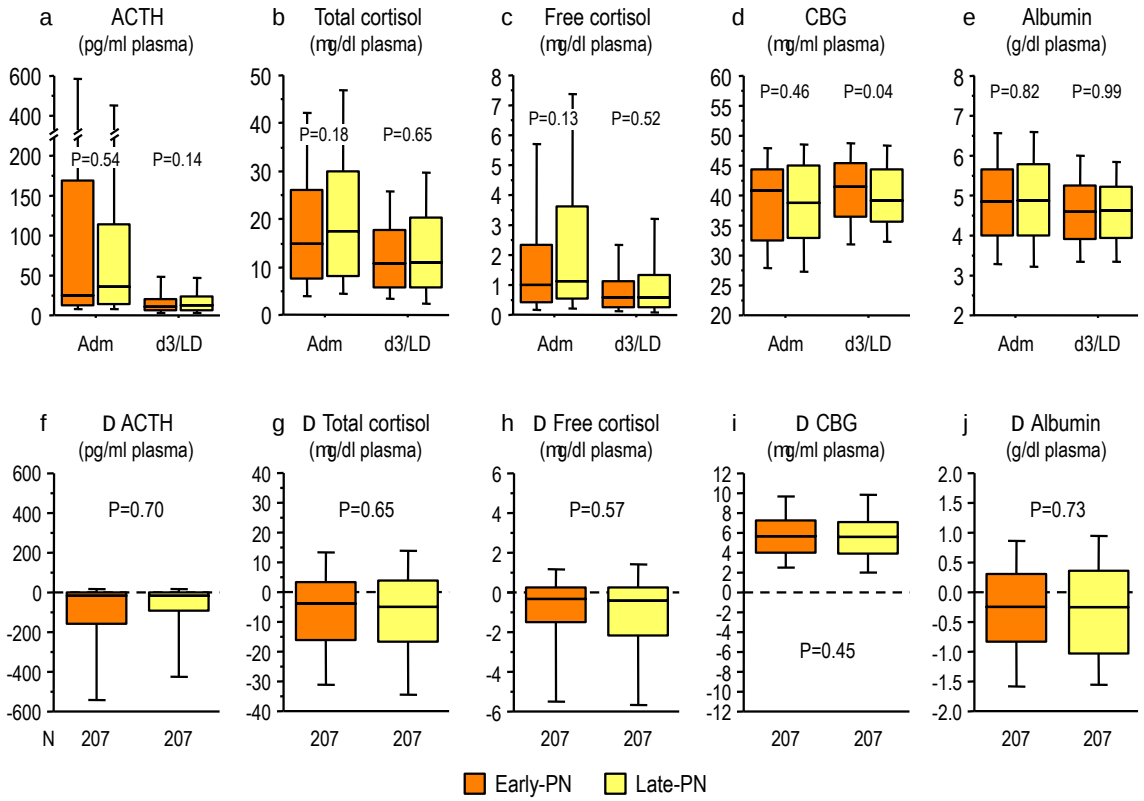
SUPPLEMENTARY MATERIAL

Supplemental Methods

Supplemental Methods 1: Selection of patients to investigate the association between treatment with corticosteroids in the PICU and plasma concentrations of ACTH, total cortisol, free cortisol, CBG and albumin

The 33 patients who had a measurement the morning after the first day of corticosteroid-treatment were matched case-by-case to patients who had not been treated with corticosteroids and for whom a measurement was available for the same PICU-stay day (**Figure 1**, Panel B). The patients were matched for baseline characteristics (randomization, center, age, gender, diagnostic category, STRONGkids score, PIM2 score, PeLOD score, elective versus emergency admission, mechanical ventilation upon admission, assist device upon admission, infection upon admission, height and weight) and for one post-admission characteristic (length of PICU stay). Distinction was made between hydrocortisone and synthetic corticosteroids given that the latter are not detected by the assay used for quantifying cortisol.

Supplemental Figure



SUPPLEMENTAL FIGURE 1 IMPACT OF LATE-PN VERSUS EARLY-PN ON THE TIME COURSE OF THE CHANGES IN PLASMA CONCENTRATIONS OF ACTH, TOTAL CORTISOL, FREE CORTISOL CBG, AND ALBUMIN

The plasma concentrations upon PICU admission and on PICU day 3 or last PICU day for patients with a shorter PICU stay, and the changes from PICU admission in the plasma concentrations on PICU day 3 or last PICU day for patients with a shorter PICU stay (indicated as Δ) of ACTH (a, f), total cortisol (b, g), free cortisol (c, h), CBG (d, i) and albumin (e, j) are shown and compared for patients randomized to early-PN or late-PN. Box plots show medians, interquartile ranges, and 10th and 90th percentiles. ACTH: adrenocorticotropic hormone, CBG: cortisol-binding globulin, d3/LD: day 3 or last PICU day for patients with a shorter PICU stay, PICU: pediatric intensive care unit, PN: parenteral nutrition.

Supplemental Tables

SUPPLEMENTAL TABLE 1 LOCAL PROTOCOLS FOR INITIATION OF PN IN THE EARLY-PN GROUP

Center	On admission	Day 2	Day 3	Subsequent stay
Leuven, Belgium	Mixture of glucose 30% and Vaminolact® (Fresenius)	Addition of lipids (SMOFlipid® Fresenius)	All replaced by mixture of glucose 50% and SMOFlipid® with Vaminolact® or Vamin 18®	Glucose 50% and SMOFlipid® with Vaminolact® or Vamin 18®
Rotterdam, The Netherlands	Continuous glucose infusion with glucose intake 4-6 mg/kg/min (< 30 kg) or 2-4 mg/kg/min (> 30 kg)	Pharmacy-made PN: mixture of glucose, Primene® (Baxter) and Intralipid® (Baxter, 50% of final dose). Children >30 kg Olimel® (Baxter, N5 or N4 depending on central or peripheral line)	Increase of lipids to 100%	Pharmacy-made PN: mixture of glucose, Primene® (Baxter) and Intralipid® (Baxter, 100% of final dose). Children >30 kg Olimel® (Baxter, N5 or N4 depending on central or peripheral line)
Edmonton, Canada	Continuous glucose infusion with glucose intake 3-4 mg/kg/min	Addition of 20% IV lipids (50% of final dose)	Increase of lipids to 100% Mixture amino acids and concentrated glucose	Mixture amino acids, concentrated glucose and 20% IV lipids

In all participating centers enteral nutrition was attempted as soon as possible. Supplemental PN was initiated within 24 hrs in the early-PN group if enteral nutrition alone was insufficient to reach caloric targets. IV: intravenous, PN: parenteral nutrition.

SUPPLEMENTAL TABLE 2 CALORIC TARGETS PER CENTER

Center	First day	Subsequent stay
Leuven, Belgium	First 10 kg: 100 kcal/kg 10-20 kg: + 50 kcal/kg >20 kg: + 20 kcal/kg (adjusted downward when fluid restriction required)	
Rotterdam, The Netherlands	EN: basal metabolic rate by Schofield-weight [a] PN: ESPGHAN [b]	EN: Recommended Dietary Allowances [c] PN: ESPGHAN [b]
Edmonton, Canada	Resting energy expenditure by indirect calorimetry If indirect calorimetry impossible: 65% of basal metabolic rate (FAO-WHO [d])	Adjusted by the dietitian based on clinical information

[a] Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39 Suppl 1:5-41.[15] [b] Koletzko B, Goulet O, Hunt J, et al. 1. Guidelines on Pediatric Parenteral Nutrition of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Pediatric Research (ESPR). J Pediatr Gastroenterol Nutr 2005;41 Suppl 2:S1-87.[16] [c] Dietary Reference Intake: energy, protein and digestible carbohydrates, . Health Council of the Netherlands: The Hague2001.[17] [d] Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. World Health Organ Tech Rep Ser 1985;724:1-206.[18] EN: enteral nutrition, PN: parenteral nutrition.

SUPPLEMENTAL TABLE 3 CHARACTERISTICS OF THE PICU PATIENTS AND HEALTHY CONTROLS INCLUDED FOR THE INVESTIGATION OF THE URINARY CORTISOL METABOLITES

Baseline characteristics	Patients N=76	Controls N=25	P
Age (years), median (IQR)	2.38 (0.32 – 7.53)	2.49 (1.08 – 4.81)	0.76
Age <1 year, N (%)	30 (39.47)	6 (24.00)	0.15
Male gender, N (%)	32 (42.11)	11 (44.00)	0.86
Weight (SD score), median (IQR)	-0.60 (-1.38 – 0.21)		
Height (SD score), median (IQR)	-0.50 (-1.81 – 0.62)		
Treatment center, N (%)			
Leuven	73 (96.05)		
Rotterdam	3 (3.95)		
Edmonton	0 (0)		
STRONGkids risk level, N (%) [a]			
Medium	69 (90.79)		
High	7 (9.21)		
PeLOD score, first 24 hrs in PICU, median (IQR) [b]	31 (22 – 32)		
PIM2 score, median (IQR) [c]	-2.68 (-3.75 – -1.33)		
PIM2 probability of death, %, median (IQR)	5.79 (2.48 – 17.60)		
Emergency admission, N (%)	26 (34.21)		
Diagnostic group, N (%)			
Surgical			
Cardiac	45 (59.21)		
Neurosurgery-traumatic brain injury	3 (3.95)		
Thoracic	4 (5.26)		
Orthopedic surgery-trauma	7 (9.21)		
Other	3 (3.95)		
Medical			
Cardiac	3 (3.95)		
Neurologic	1 (1.32)		
Respiratory	4 (5.26)		
Other	6 (3.95)		
Condition on admission, N (%)			
Mechanical ventilation required	71 (93.42)		
ECMO or other assist device required	3 (3.95)		
Infection	26 (34.21)		
Sepsis	22 (28.95)		
Randomization group, N (%)			
Early PN	42 (55.26)		
Late PN	34 (44.74)		
Short-stay patients, N (%)	24 (31.58)		
Duration of PICU stay, days, median (IQR)	3 (2 – 5)		
Acquisition of a new infection, N (%)	5 (6.6)		
90-day mortality, N (%)	1 (1.3)		

[a] Evaluates risk of malnutrition with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. [b] Scores range from 0 to 71, with higher scores indicating more severe illness. [c] Higher scores indicate a higher risk of mortality. ECMO: extracorporeal membrane oxygenation. PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU. PICU: pediatric intensive care unit. PIM2: pediatric index of mortality 2 score. PN: parenteral nutrition, STRONGkids: Screening Tool for Risk on Nutritional Status and Growth.

SUPPLEMENTAL TABLE 4 CHARACTERISTICS OF PICU PATIENTS IN THE PROPENSITY SCORE-MATCHED SUBSET TO ADDRESS THE IMPACT OF EARLY-PN VERSUS LATE-PN ON THE TIME COURSE OF THE CHANGES IN PLASMA CONCENTRATIONS OF ACTH, TOTAL CORTISOL, FREE CORTISOL CBG, AND ALBUMIN

Baseline characteristics	Early-PN N=207	Late-PN N=207	P
Age (years), median (IQR)	2.5 (0.5 – 7.1)	2.4 (0.4 – 8.3)	0.93
Age <1 year, N (%)	78 (37.7)	82 (39.6)	0.68
Male gender, N (%)	116 (56.0)	118 (57.0)	0.84
Weight (SD score), median (IQR)	-0.5 (-1.3 – 0.4)	-0.5 (-1.6 – 0.5)	0.89
Height (SD score), median (IQR)	-0.4 (-1.7 – 0.5)	-0.2 (-1.2 – 0.8)	0.10
Treatment center, N (%)			0.88
Leuven	185 (89.4)	182 (87.9)	
Rotterdam	19 (9.2)	22 (10.6)	
Edmonton	3 (1.5)	3 (1.5)	
STRONGkids risk level, N (%) [a]			0.83
Medium	195 (94.2)	194 (93.7)	
High	12 (5.8)	13 (6.3)	
PeLOD score, first 24 hrs in PICU, median (IQR) [b]	23 (21 – 32)	23 (21 – 32)	0.31
PIM2 score, median (IQR) [c]	-3.0 (-3.8 – -1.8)	-2.9 (-3.8 – -1.7)	0.97
Emergency admission, N (%)	70 (33.8)	84 (40.6)	0.15
Diagnostic group, N (%)			0.99
Surgical			
Abdominal	9 (4.4)	6 (2.9)	
Burns	1 (0.5)	2 (1.0)	
Cardiac	107 (51.7)	105 (50.7)	
Neurosurgery-traumatic brain injury	17 (8.2)	16 (7.7)	
Thoracic	11 (5.3)	9 (4.4)	
Transplantation	0 (0.0)	0 (0.0)	
Orthopedic surgery-trauma	17 (8.2)	19 (9.2)	
Other	5 (2.4)	5 (2.4)	
Medical			
Cardiac	7 (3.4)	9 (4.4)	
Gastrointestinal-Hepatic	1 (0.5)	1 (0.5)	
Oncologic-Hematologic	0 (0.0)	0 (0.0)	
Neurologic	10 (4.8)	11 (5.3)	
Renal	0 (0.0)	0 (0.0)	
Respiratory	12 (5.8)	13 (6.3)	
Other	10 (4.8)	11 (5.3)	
Condition on admission, N (%)			
Mechanical ventilation required	187 (90.3)	180 (87.0)	0.27
ECMO or other assist device required	3 (1.5)	4 (1.9)	0.70
Infection	55 (26.6)	58 (28.0)	0.74
Sepsis	43 (20.8)	41 (19.8)	0.80
Short-stay patients, N (%) [d]	102 (49.3)	99 (47.8)	0.76
Days of PICU stay, median (IQR) [e]	3 (1 – 6)	3 (1 – 6)	0.66
Acquisition of a new infection, N (%)	28 (13.5)	18 (8.7)	0.11
90-day mortality, N (%)	8 (3.9)	3 (1.5)	0.12

[a] Evaluates risk of malnutrition with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. [b] Scores range from 0 to 71, with higher scores indicating more severe illness. [c] Higher scores indicate a higher risk of mortality. [d] Patients with a PICU stay shorter than 3 days. [e] Exclusion of a number of patients who died and/or who did not have a sample available for analysis (potentially biased by length of PICU stay) hampers investigation of the impact of late-PN versus early-PN on time to live PICU discharge. ACTH: adrenocorticotropic hormone, CS: corticosteroids, ECMO: extracorporeal membrane oxygenation, HPA axis: hypothalamic-pituitary-adrenal axis. PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU. PICU: pediatric intensive care unit. PIM2: pediatric index of mortality 2 score. PN: parenteral nutrition, STRONGkids: Screening Tool for Risk on Nutritional Status and Growth. CBG: cortisol binding globulin.

SUPPLEMENTAL TABLE 5 PROGNOSTIC VALUE OF THE CHANGES IN THE HPA AXIS FOR PATIENT-CENTERED CLINICAL OUTCOMES

	HPA axis at PICU admission short- and long-stay patients N=442		HPA axis at PICU day 3 long-stay patients [a] N=230	
	OR/HR (95%CI)	**P**	**OR/HR (95%CI)**	**P**
Risk of new infection				
Infant vs child [b]	1.557 (0.663-3.655)	0.30	1.082 (0.427-2.743)	0.86
Center - Rotterdam vs Leuven	4.105 (1.231-13.684)	0.02	3.567 (1.014-12.547)	0.04
Center - Edmonton vs Leuven	0.000 (0.000-inf)	0.99	0.000 (0.000-inf)	0.99
High vs medium risk of malnutrition [c]	2.885 (0.888-9.369)	0.07	2.241 (0.601-8.357)	0.22
PeLOD score (per unit added) [d]	1.065 (1.012-1.121)	0.01	1.046 (0.991-1.103)	0.09
PIM2 score (per unit added) [e]	1.925 (1.414-2.622)	<0.0001	1.518 (1.080-2.135)	0.01
Diagnostic group		0.0003		0.004
Sepsis vs no sepsis upon admission	0.540 (0.190-1.537)	0.24	0.218 (0.068-0.700)	0.01
Randomization to late-PN vs early-PN	0.550 (0.261-1.155)	0.11	0.540 (0.242-1.202)	0.13
ACTH (per pg/ml added)	1.000 (0.999-1.001)	0.58	0.987 (0.967-1.007)	0.15
Free cortisol (per µg/ml added)	0.978 (0.916-1.044)	0.48	1.082 (0.861-1.359)	0.52
Likelihood of earlier live PICU discharge				
Infant vs child [b]	0.601 (0.479-0.752)	<0.0001	0.709 (0.513-0.983)	0.03
Center - Rotterdam vs Leuven	0.728 (0.489-1.067)	0.10	0.830 (0.516-1.311)	0.42
Center - Edmonton vs Leuven	1.039 (0.483-1.965)	0.91	1.191 (0.406-2.789)	0.72
High vs medium risk of malnutrition [c]	0.962 (0.614-1.443)	0.85	1.076 (0.561-1.894)	0.81
PeLOD score (per unit added) [d]	0.992 (0.980-1.005)	0.22	0.998 (0.980-1.017)	0.85
PIM2 score (per unit added) [e]	0.691 (0.630-0.759)	<0.0001	0.719 (0.633-0.813)	<0.0001
Diagnostic group		0.03		0.11
Sepsis vs no sepsis upon admission	0.602 (0.448-0.809)	0.0008	1.038 (0.715-1.508)	0.84
Randomization to late-PN vs early-PN	1.012 (0.833-1.230)	0.90	1.006 (0.758-1.337)	0.96
ACTH (per pg/ml added)	1.000 (1.000-1.000)	0.88	**1.009 (1.003-1.013)**	**0.0003**
Free cortisol (per µg/ml added)	0.998 (0.974-1.023)	0.86	**0.906 (0.840-0.964)**	**0.003**
Risk of 90-day mortality				
Infant vs child [b]	1.776 (0.277-11.393)	0.54	1.871 (0.290-12.058)	0.51
Center - Rotterdam vs Leuven	0.167 (0.008-3.296)	0.23	0.134 (0.007-2.478)	0.17
Center - Edmonton vs Leuven	0.000 (0.000-inf)	0.99	0.000 (0.000-inf)	0.99
High vs medium risk of malnutrition [c]	1.485 (0.143-15.453)	0.74	2.387 (0.223-25.502)	0.47
PeLOD score (per unit added) [d]	0.964 (0.883-1.053)	0.41	0.943 (0.845-1.052)	0.27
PIM2 score (per unit added) [e]	3.229 (1.776-5.872)	<0.0001	2.722 (1.435-5.165)	0.0004
Diagnostic group		0.36		0.61
Sepsis vs no sepsis upon admission	1.347 (0.200-9.054)	0.75	0.966 (0.141-6.613)	0.97
Randomization to late-PN vs early-PN	0.512 (0.121-2.171)	0.36	0.285 (0.051-1.586)	0.15
ACTH (per pg/ml added)	0.999 (0.996-1.002)	0.59	0.979 (0.937-1.023)	0.30
Free cortisol (per µg/ml added)	1.004 (0.910-1.107)	0.93	1.190 (0.775-1.827)	0.48

[a] Long-stay patients are patients who needed intensive care for at least 3 days. [b] Infants are patients younger than 1 year. [c] Evaluated with the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. [d] Scores range from 0 to 71, with higher scores indicating more severe illness. [e] Higher scores indicate a higher risk of mortality. CI: confidence interval, ACTH: adrenocorticotropic hormone, HPA axis: hypothalamus-pituitary-adrenal axis, HR: hazard ratio, OR: odds ratio, PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU, PICU: pediatric intensive care unit, PIM2: pediatric index of mortality 2 score, PN: parenteral nutrition.

SUPPLEMENTAL TABLE 6 INDEPENDENT ASSOCIATION BETWEEN TREATMENT WITH CORTICOSTEROIDS INITIATED IN THE PICU AND PATIENT-CENTERED CLINICAL OUTCOMES

	Short- and long-stay patients N=1020		Long-stay patients [a] N=652	
	OR/HR (95%CI)	**P**	**OR/HR (95%CI)**	**P**
Risk of new infection [b]				
Infant vs child [c]	1.440 (0.897-2.312)	0.13	1.143 (0.680-1.922)	0.61
Center - Rotterdam vs Leuven	1.558 (0.805-3.015)	0.18	1.563 (0.796-3.072)	0.19
Center - Edmonton vs Leuven	2.605 (1.159-5.857)	0.02	2.192 (0.944-5.089)	0.06
High vs medium risk of malnutrition [d]	2.478 (1.388-4.422)	0.002	1.934 (1.053-3.554)	0.03
PeLOD score (per unit added) [e]	1.033 (1.007-1.059)	0.01	1.033 (1.006-1.060)	0.01
PIM2 score (per unit added) [f]	1.263 (1.082-1.475)	0.003	1.306 (1.095-1.558)	0.003
Diagnostic group		0.93		0.94
Sepsis vs no sepsis upon admission	0.569 (0.328-0.987)	0.04	0.411 (0.236-0.714)	0.001
Randomization to late-PN vs early-PN	0.517 (0.333-0.804)	0.003	0.507 (0.320-0.802)	0.003
CS therapy vs no CS therapy in PICU	1.324 (0.815-2.149)	0.25	1.057 (0.648-1.724)	0.82
Likelihood of earlier live PICU discharge				
Infant vs child [c]	0.662 (0.572-0.766)	<0.0001	0.788 (0.652-0.953)	0.01
Center - Rotterdam vs Leuven	0.667 (0.549-0.810)	<0.0001	0.752 (0.594-0.954)	0.01
Center - Edmonton vs Leuven	0.774 (0.588-1.007)	0.05	0.928 (0.672-1.264)	0.63
High vs medium risk of malnutrition [d]	0.833 (0.667-1.030)	0.09	0.832 (0.641-1.066)	0.14
PeLOD score (per unit added) [e]	0.990 (0.983-0.998)	0.01	0.990 (0.981-1.000)	0.04
PIM2 score (per unit added) [f]	0.758 (0.716-0.801)	<0.0001	0.785 (0.732-0.842)	<0.0001
Diagnostic group		0.01		0.15
Sepsis vs no sepsis upon admission	0.704 (0.598-0.828)	<0.0001	0.925 (0.765-1.118)	0.42
Randomization to late-PN vs early-PN	1.186 (1.042-1.351)	0.01	1.336 (1.132-1.578)	0.0006
CS therapy vs no CS therapy in PICU	**0.586 (0.500-0.687)**	**<0.0001**	**0.677 (0.563-0.814)**	**<0.0001**
Risk of 90-day mortality				
Infant vs child [c]	1.765 (0.854-3.650)	0.12	1.602 (0.727-3.529)	0.24
Center - Rotterdam vs Leuven	1.140 (0.477-2.725)	0.76	1.139 (0.442-2.939)	0.78
Center - Edmonton vs Leuven	0.000 (0.000-inf)	0.99	0.000 (0.000-inf)	0.99
High vs medium risk of malnutrition [d]	0.403 (0.122-1.329)	0.13	0.693 (0.220-2.185)	0.53
PeLOD score (per unit added) [e]	1.007 (0.972-1.043)	0.70	1.011 (0.973-1.051)	0.57
PIM2 score (per unit added) [f]	2.326 (1.825-2.965)	<0.0001	1.860 (1.435-2.411)	<0.0001
Diagnostic group		0.68		0.79
Sepsis vs no sepsis upon admission	1.490 (0.680-3.264)	0.31	1.322 (0.571-3.058)	0.51
Randomization to late-PN vs early-PN	0.516 (0.257-1.037)	0.06	0.498 (0.235-1.057)	0.06
CS therapy vs no CS therapy in PICU	**2.621 (1.326-5.180)**	**0.005**	**2.307 (1.125-4.731)**	**0.02**

Patients who received corticosteroids within the three days prior to PICU admission were excluded from these analyses. a Long-stay patients are patients who needed intensive care for at least 3 days. b Only infections occurring beyond the first day of CS administration were considered ("left-truncation"). c Infants are patients younger than 1 year. d Evaluated with the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) with scores ranging from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk. e Scores range from 0 to 71, with higher scores indicating more severe illness. f Higher scores indicate a higher risk of mortality. CI: confidence interval, CS: corticosteroids, HR: hazard ratio, OR: odds ratio, PeLOD: pediatric logistic organ dysfunction score first 24 hrs in PICU, PICU: pediatric intensive care unit, PIM2: pediatric index of mortality 2 score, PN: parenteral nutrition.

CHAPTER 5 - LONG-TERM DEVELOPMENTAL IMPACT OF WITHHOLDING PARENTERAL NUTRITION IN PEDIATRIC-ICU: A 4-YEAR FOLLOW-UP OF THE PEPANIC RANDOMIZED CONTROLLED TRIAL

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1. ABSTRACT

Background: The PEPaNIC-RCT (Leuven-Rotterdam-Edmonton, N=1440 recruited from 2012-2015) showed that withholding parenteral nutrition for 1 week in critically ill children (late-PN), as compared with early supplemental PN (early-PN), prevented infections, accelerated recovery and -mediated by prevention of abnormal DNA-methylation of 37 CpG-sites- improved neurocognitive development assessed 2-years later. As several neurocognitive domains can only be thoroughly assessed from 4-years of age onwards, we determined the impact of late-PN versus early-PN on physical, neurocognitive, and emotional/behavioral development 4-years post-randomization.

Methods: This is a pre-planned, blinded, 4-year follow-up study of PEPaNIC patients and of matched healthy children (ClinicalTrials.gov-NCT01536275). Studied outcomes were anthropometrics, health status, parent/caregiver-reported executive functions and emotional/behavioral problems, and clinical tests for intelligence, visual-motor integration, alertness, motor coordination and memory. Via multivariable linear and logistic regression analyses, after imputation for missing values (≤30%) and adjustment for risk factors, we investigated the impact of early-PN versus late-PN hereon and assessed whether altered DNA-methylation status played a mediating role.

Findings: Patients testable for neurocognitive development (356 late-PN, 328 early-PN) revealed worse anthropometric, health status, neurocognitive and emotional/behavioral developmental outcomes than 369 healthy controls. Outcomes of late-PN patients were never worse than those of early-PN patients. In contrast, late-PN patients had fewer internalizing (P=0.042), externalizing (P=0.046), and total emotional/behavioral problems (P=0.007) than early-PN patients, which were normalized by late-PN. Avoiding early-PN-induced abnormal DNA-methylation explained these findings.

Interpretation: Omitting early-PN use for critically ill children protected against emotional/behavioral problems 4-years post-randomization, mediated by avoiding abnormal DNA-methylation. This further supports de-implementation of early-PN.

2. INTRODUCTION

Critical illness in children is associated with impaired physical, neurocognitive and emotional/behavioral development, which often persists years after discharge from the pediatric intensive care unit (PICU) and hospital.[1,2] In the last decade, avoidable intensive care-related factors contributing to parts of this legacy have been identified. These include hyperglycemia, phthalates leaching into the blood from indwelling medical devices, and the early use of parenteral nutrition (PN) in the PICU.[3-5] The multicenter randomized clinical "Pediatric Early versus Late Parenteral Nutrition in Critical Illness – PEPaNIC" trial showed that withholding PN for one week in the PICU (late-PN), as compared with initiating PN within 24 h after admission to supplement insufficient enteral nutrition (Early-PN), not only improved intensive care outcomes but also executive functioning, externalizing behavioral problems and visual-motor integration, as assessed two years later.[5,6] This was found to be mediated by the prevention of adversely altered DNA-methylation status evoked by early-PN, in particular of 37 CpG-sites related to genes involved in brain development.[7]

A methodological limitation of the 2-year follow-up study of the PEPaNIC-RCT was the large proportion of patients included who were younger than 4 years when tested neurocognitively.[5] Indeed, the brain of children matures further during the first years of life and as a consequence assessment of most neurocognitive domains is only possible from 4 years of age onwards.[8,9] Also, during development, impairments in physical or neurocognitive domains that were observed at 2 years follow-up may persist or disappear, whereas other problems may emerge. Taken together, this warrants a physical, neurocognitive and emotional/behavioral assessment at a later time point after critical illness. We therefore performed a 4-year follow-up study of the PEPaNIC-RCT to assess health status, parent/caregiver-reported and clinically observed neurocognitive and emotional/behavioral outcomes of patients in comparison with matched healthy children and to investigate the impact hereon of late-PN as compared with early-PN. Furthermore, we assessed whether altered DNA-methylation status could offer a biological basis hereof.

3. METHODS

Study design and participants

In the PEPaNIC-RCT, 1440 critically ill infants and children admitted to the participating PICUs (University Hospitals Leuven, Belgium; Erasmus-MC Sophia Children's Hospital, Rotterdam, The Netherlands; Stollery Children's Hospital, Edmonton, Alberta, Canada) had been enrolled from 2012 to 2015.[6] The study protocol has been published.[10] The current study represents the pre-planned 4 years follow-up of this RCT.[6]

As described previously,[5] during PICU-admission, parents or legal guardians of the patients provided consent to contact them for long-term follow-up testing. First, survival status was assessed by reviewing hospital notes, via the National Register or via contact with the general practitioner or referring pediatrician. After receiving a standardized information letter, PICU-survivors and parents/caregivers were contacted by phone to obtain consent for scheduling an appointment for the medical and neurocognitive assessment, either at the hospital or at the patient's home. For patients who could not be reached by phone, survival status was reassessed at the end of the study.

For comparison, 369 healthy control children, demographically matched to the patients for age and gender, were recruited to undergo identical medical and neurocognitive assessment. Apart from unrelated children, also siblings and relatives of the patients were included, to control as much as possible for genetic and socio-economic/environmental background. Healthy control children were only included if they were not previously admitted to a neonatal ICU or PICU, or admitted to the hospital for 7 days or more with need for an intravenous line. History of inborn chronic metabolic diseases requiring a specific diet, such as diabetes, and conditions that require home parenteral nutrition such as short bowel syndrome were additional exclusion criteria.

Parents or legal guardians, and when applicable also adolescents, gave written informed consent according to local regulations. The institutional review boards at each participating site approved this follow-up study (ML8052; NL49708.078; Pro00038098).

Procedures, randomization and masking

After having obtained informed consent, children who were admitted to the PICU during the PEPaNIC-RCT were randomly allocated (1:1) to receive "early-PN", with PN initiated within 24 h after PICU-admission to supplement enteral nutrition whenever 80% of targeted calories per age and weight categories was not yet reached, or "late-PN", which meant that all PN was withheld for up to 1 week in PICU. For the late-PN group, this corresponded to no PN in the majority of children. When enteral nutrition covered ≥80% of calculated targets, supplemental PN was discontinued. Total macronutrient doses administered on each of the first seven days in PICU are shown in **Supplemental Figure 1**. After one week, for both groups equally, PN could be administered if necessary. Enteral nutrition was initiated early for both groups equally, and all patients received intravenous micronutrients until fully enterally fed.

Outcome assessors of the 4-year follow-up study were physicians and experienced pediatric psychologists who had not been involved in the management of the patients during their stay in the PICU and who were strictly blinded for treatment allocation. Parents had not been masked during the time the child was treated in the PICU and were not actively informed about the initial PEPaNIC study results.[6]

Outcomes

As performed in the previous 2-year follow-up study,[5] at 4-year follow-up, head circumference, body weight and height were measured. A clinical neurological examination was performed to assess gross neurological abnormalities. Via a structured interview with the parents/caregivers, it was assessed whether the child had been diagnosed with a somatic or psychiatric illness, and/or had been admitted to a hospital for medical or surgical reasons during the past four years for healthy control children and during the four years following the index PICU-admission for patients. Neurocognitive testability was determined based on screening of the medical file or based upon clinical judgement prior to the start of the neurocognitive assessment by the physician/psychologist and confirmed by the parents/caregivers.

To score performance for a broad range of neurocognitive functions, validated, internationally recognized questionnaires and clinical tests with adequate normative data were used. Parent-reported questionnaires that were used included the Behavior Rating Inventory of Executive Function[11,12] (executive functioning, T-scores, with mean 50 and SD 10) and the Child Behavior Checklist[13,14] (emotional and behavioral problems, T-scores, with mean 50 and SD 10). On both questionnaires, higher scores indicate more problems. Clinical tests consisted of the age-appropriate versions of the Wechsler Intelligence Quotient Scale[15-17] (intelligence,

standard scores, with mean 100 and SD 15), the Beery Developmental Test of Visual-Motor Integration18 (visuomotor integration, scaled score, with mean 10 and SD 3), tasks of the Amsterdam Neuropsychological Task Battery[9] (ANT for children aged 4 years or older), and the Children's Memory Scale8 (CMS for children aged 5 to 16 years). Tasks of the ANT consisted of ANT-Baseline Speed (alertness, reaction time) and ANT-Tapping (motor coordination, number of taps,). Tasks of the CMS that were used were CMS-Numbers (verbal short-term memory and working memory, scaled scores with mean 10 and SD 3), CMS-Word Pairs (short-term and long-term verbal memory, and recognition, proportion of correct responses ranging from 0 to 1), CMS-Picture Locations (short-term visual memory, proportion of correct responses), and CMS-Dot Locations (short-term and long-term visual memory proportion of correct responses). The CMS-Learning index represents learning abilities of the child (standard score, with mean 100 and SD 15). For the clinical tests, a higher score indicates better functioning, with the exception of ANT-Baseline Speed. The extended description of the questionnaires and of the clinical/neuropsychological test battery is available in **Supplemental Methods 1**.

Statistical analyses

For patients who had been included in the PEPaNIC-RCT, and who were alive and testable 4 years later, we estimated a loss to follow-up of the PICU-survivors of about 30%, based on previous trials.[3,5] With this sample size, we calculated to have >80% statistical power to detect, with a certainty of >95%, clinically relevant differences between the two randomization arms, in the same order of magnitude as previously reported.[3,5] For the healthy control group, a sample size of 369 allows to detect similar differences between healthy control children and patients with a power of >80% and certainty of >95%.

Inability to fully complete the neurocognitive test battery may indicate poor neurocognitive function and thus introduce bias. Similar to what was done for the earlier 2-year follow-up study,[5] missing values were therefore imputed by chained equations, with use of all available data per individual (**Supplemental Methods 2, Supplemental Figures 2-4**).[19] Imputation of data for age-specific tests was only performed within the respective age group. Bias and instability of the imputation model was minimized by only including outcomes with ≤30% missing data.[19] The number of imputation models was set at 31 to avoid loss of statistical power (**Supplemental Methods 2, Supplemental Figures 2-4**).[19]

Univariable comparison of the pooled data from the imputed models was performed with use of Fisher-Exact test, Student t-test or Wilcoxon rank-sum test as appropriate. Multivariable linear and logistic regression analyses were performed on the 31 imputed datasets with the pooled beta-estimates or odds ratios reported

to investigate the differences in outcomes between patients and healthy control children, and to analyze the differences between patients randomly allocated to late-PN or early-PN during the PEPaNIC-RCT.[5] All multivariable analyses were adjusted for baseline risk factors including age, center, gender, race, geographic origin, language, hand preference, history of malignancy, a predefined "syndrome" (**Supplemental Method 3**), and the educational and occupational status of the parents/caregivers (**Supplemental Method 4**). Additional adjustment for admission diagnosis, severity of illness upon PICU-admission (PIM3 and PeLOD), risk of malnutrition (STRONGkids), and parental smoking behavior prior to PICU-admission was performed for the comparison between the late-PN and early-PN groups. Acute effects of the randomization on acquisition of new infections and on the duration of hypoglycemia, ventilatory support and stay in the PICU, could potentially mediate any long-term effect and thus further adjustment here for was done in the multivariable models. In addition, further adjustment was performed for other post-randomization treatments that could theoretically play a role (duration of hemodynamic support, treatment with antibiotics, corticosteroids, opioids, benzodiazepines, hypnotics and α-2-agonists).

In analogy with the earlier 2-year follow-up study, a mediation analysis was performed to investigate any role of the altered leukocyte DNA-methylation status of the 37 CpG-sites, shown to be evoked by early-PN, in statistically explaining any impact of the randomized intervention on the 4-year follow-up outcomes (**Supplemental Method 5**).[7] To this end, we performed multivariable non-linear regression analyses, adjusted for baseline risk factors and further for the methylation status of those 37 CpG-sites. Models were built with and without addition of the randomized intervention. Covariate significance levels obtained via permutation importance were used.[7] The robustness of the results was evaluated by 100 bootstrapped replicates and the fold-change in optimism-corrected R^2 between models with and without addition of the CpG-sites differentially methylated by early-PN as compared with late-PN as covariates was calculated.[7] Heatmaps with dendrograms were used to visualize and summarize the findings.

Data are presented as beta-estimates and odds ratios with 95% confidence intervals (CI), means and standard deviations (SD), or numbers and proportions, as appropriate.

Statistical analyses were performed with use of R version 3.5.3, MICE versions 3.4.0 and 3.6.0, and JMP© version 14.0.0 (SAS Institute, Inc, Cary, NC). Two-sided P-values at or below 0.05 were considered statistically significant. As the studied developmental outcomes are not independent (**Supplemental Method 6, Supplemental Figure 5**), correction for multiple comparisons was not performed.[7,20]

This trial is registered with ClinicalTrials.gov, NCT01536275.

4. RESULTS

Sixty-six (9.2%) late-PN patients and 71 (9.8%) early-PN patients did not survive to 4-years follow-up (p=0.69) and for 18 patients, survival status was unknown (**Figure 1**). A total of 222 late-PN patients and 247 early-PN patients survived but declined participation or were not contactable (p=0.47). Hence, loss to follow-up was 33.8% (487/1440). At follow-up, 59 (8.2%) late-PN patients and 73 (10.1%) early-PN patients were too disabled for neurocognitive testing (p=0.21) and were excluded from the analyses. For transparency, any available clinical data and/or questionnaire results for these patients are provided in **Supplemental Table 1**. Between March 8th 2016 and November 8th 2019, a total of 684 patients and 369 healthy controls underwent long-term neurocognitive testing and were included in the imputation models for subsequent multivariable analyses. Neurocognitive testing was performed at the hospital for 442 (64.6%) patients and 301 (81.6%) healthy controls (p<0.0001), with no differences in place of assessment between late-PN and early-PN patients (p=0.99). Demographics and medical characteristics of patients and healthy control children are shown in **Table 1**. Randomization allocation and primary and secondary intensive care outcomes of patients who were tested at 4-year follow-up were overall comparable with the initial PEPaNIC study population.

In univariable and multivariable comparison, patients at 4-year follow-up had worse outcomes for height, weight and head circumference, for health status, clinically assessed neurological functioning, parent/caregiver-reported executive functioning and emotional and behavioral problems, clinical tests for intelligence, visual-motor integration, alertness, motor-coordination, and memory than healthy control children (**Table 2, Table 3**).

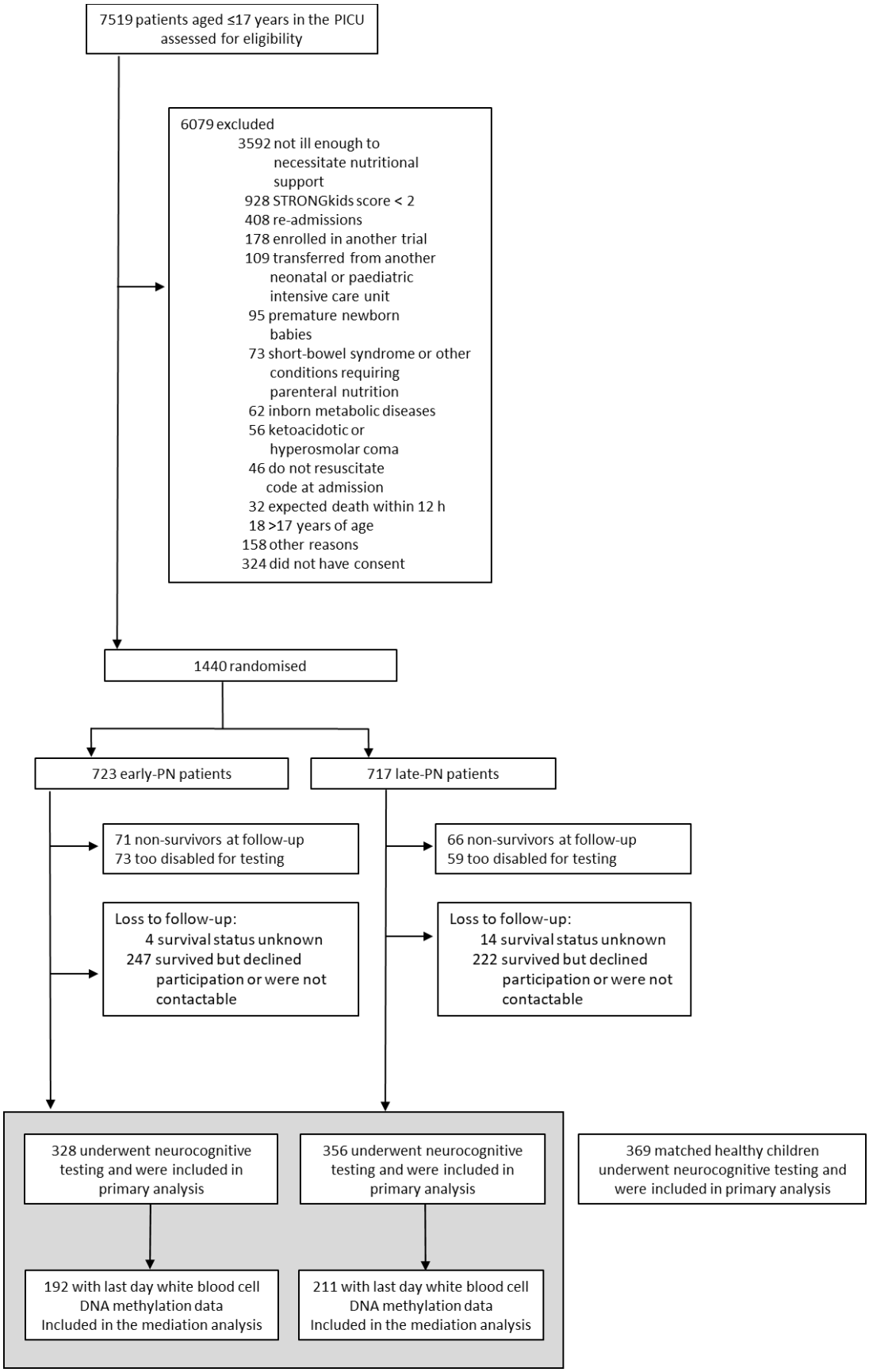

FIGURE 1 CONSORT DIAGRAM OF THE STUDY PARTICIPANTS

TABLE 1 DEMOGRAPHICS, POST-RANDOMISATION TREATMENTS IN THE PICU, AND ACUTE OUTCOMES OF PATIENTS AND HEALTHY CONTROL CHILDREN

	Tested populations		Total PICU population		Tested PICU population	
	Healthy control children N=369	Patients N=684	Early-PN N=723	Late-PN N=717	Early-PN N=328	Late-PN N=356
Demographics						
Age at 4-years' follow-up - yr	7·5 (4·3)	7·3 (4·3)	NA	NA	7·4 (4·3)	7·2 (4·2)
Sex						
Male	202 (54·7%)	393 (57·5%)	415 (57·4%)	412 (52·5%)	187 (57·0%)	206 (57·9%)
Female	167 (45·3%)	291 (42·5%)	308 (42·6%)	305 (42·5%)	141 (43·0%)	150 (42·1%)
Known non-Caucasian race[a]	27 (7·3%)	53 (7·8%)	50 (6·9%)	33 (4·6%)	33 (10·1%)	20 (5·6%)
Known non-European origin[a]	45 (12·2%)	129 (18·9%)	161 (22·3%)	128 (17·9%)	73 (22·3%)	56 (15·7%)
Known not exclusive Dutch or English language	71 (19·2%)	158 (23·1%)	122 (16·9%)	106 (14·8%)	78 (23·8%)	80 (22·5%)
Socioeconomic status						
Educational level parents[b]						
Educational level 1	12 (3·3%)	30 (4·4%)	NA	NA	10 (3·1%)	20 (5·6%)
Educational level 1·5	13 (3·5%)	51 (7·5%)	NA	NA	29 (8·5%)	22 (6·2%)
Educational level 2	47 (12·7%)	157 (23·0%)	NA	NA	75 (22·9%)	82 (23·0%)
Educational level 2·5	68 (18·4%)	116 (17·0%)	NA	NA	53 (16·2%)	63 (17·7%)
Educational level 3	207 (56·1%)	183 (26·8%)	NA	NA	86 (26·2%)	97 (27·3%)
Educational level unknown	22 (6·0%)	147 (21·5%)	NA	NA	75 (22·9%)	72 (20·2%)
Occupational level parents[c]						
Occupational level 1	2 (0·5%)	7 (1·0%)	NA	NA	1 (0·3%)	6 (1·7%)
Occupational level 1·5	20 (5·4%)	63 (9·2%)	NA	NA	23 (7·0%)	40 (11·2%)
Occupational level 2	42 (11·4%)	108 (15·8%)	NA	NA	50 (15·2%)	58 (16·3%)
Occupational level 2·5	25 (6·8%)	69 (10·1%)	NA	NA	39 (11·9%)	30 (8·4%)
Occupational level 3	80 (21·7%)	118 (17·3%)	NA	NA	52 (15·9%)	66 (18·5%)
Occupational level 3·5	40 (10·8%)	53 (7·8%)	NA	NA	30 (9·2%)	23 (6·5%)
Occupational level 4	117 (31·7%)	102 (14·9%)	NA	NA	44 (13·4%)	58 (16·3%)
Occupational level unknown	43 (11·7%)	164 (24·0%)	NA	NA	89 (27·1%)	75 (21·1%)
Patient characteristics upon PICU admission						
Infant (age<1y) at randomisation	NA	331 (48·4%)	328 (45·4%)	325 (45·3%)	153 (46·7%)	178 (50·0%)
STRONGkids risk level[d]						
Medium	NA	613 (89·6%)	644 (89·1%)	644 (89·8%)	291 (88·7%)	322 (90·5%)
High	NA	71 (10·4%)	79 (10·9%)	73 (10·2%)	37 (11·3%)	34 (10·0%)
PeLOD score, first 24h in PICU [e]	NA	20·0 (11·6)	19·7 (12·0)	20·1 (12·3)	19·4 (11·6)	20·5 (11·5)
PIM3 score[f]	NA	-3·5 (1·4)	-3·2 (1·6)	-3·2 (1·7)	-3·4 (1·4)	-3·5 (1·3)
PIM3 probability of death - % [g]	NA	6·6 (11·7)	9·4 (15·9)	9·1 (17·4)	6·9 (11·9)	6·4 (11·7)
Diagnostic category						
Surgical						
Abdominal	NA	68 (9·9%)	53 (7·3%)	60 (8·4%)	34 (10·4%)	34 (10·0%)
Burns	NA	3 (0·4%)	5 (0·7%)	5 (0·7%)	2 (0·6%)	1 (0·3%)
Cardiac	NA	291 (42·5%)	279 (38·6%)	268 (37·4%)	137 (41·8%)	154 (43·3%)

Neurosurgery-Traumatic brain injury	NA	58 (8·5%)	63 (8·7%)	53 (7·4%)	31 (9·5%)	27 (7·6%)
Thoracic	NA	38 (5·6%)	34 (4·7%)	27 (3·8%)	21 (6·4%)	17 (4·8%)
Transplantation	NA	11 (1·6%)	7 (1·0%)	17 (2·4%)	3 (0·9%)	8 (2·3%)
Orthopaedic surgery-Trauma	NA	19 (2·8%)	28 (3·9%)	26 (3·6%)	12 (3·7%)	7 (2·0%)
Other	NA	25 (3·7%)	21 (2·9%)	27 (3·8%)	11 (3·4%)	14 (3·9%)
Medical						
Cardiac	NA	23 (3·4%)	30 (4·2%)	31 (4·3%)	8 (2·4%)	15 (4·2%)
Gastrointestinal-Hepatic	NA	2 (0·3%)	2 (0·3%)	4 (0·6%)	1 (0·3%)	1 (0·3%)
Oncologic-Haematologic	NA	6 (0·9%)	8 (1·1%)	7 (1·0%)	2 (0·6%)	4 (1·1%)
Neurologic	NA	42 (6·1%)	51 (7·1%)	52 (7·3%)	19 (5·8%)	23 (6·5%)
Renal	NA	0 (0·0%)	1 (0·1%)	1 (0·1%)	0 (0·0%)	0 (0·0%)
Respiratory	NA	70 (10·2%)	99 (13·7%)	96 (13·4%)	33 (10·1%)	37 (10·4%)
Other	NA	28 (4·1%)	42 (5·8%)	43 (6·0%)	14 (4·3%)	14 (3·9%)
Malignancy	0 (0·0%)	38 (5·6%)	51 (7·1%)	33 (4·6%)	22 (6·7%)	16 (4·5%)
Diabetes	0 (0·0%)	0 (0·0%)	3 (0·4%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Syndrome[h]	2 (0·5%)	63 (9·2%)	123 (17·0%)	118 (16·5%)	26 (7·9%)	37 (10·4%)
Known parental smoking between birth and PICU admission	NA	151 (22·1%)	NA	NA	69 (23·1%)	82 (24·5%)
Acute effects of randomisation in PICU						
Duration of stay in the PICU – days	NA	7·8 (16·0)	9·2 (21·3)	6·5 (10·0)	9·3 (19·8)	6·5 (11·2)
Patients who acquired a new infection in PICU	NA	96 (14·0%)	134 (18·5%)	77 (10·7%)	59 (18·0%)	37 (10·4%)
Duration of mechanical ventilatory support – days	NA	5·0 (11·7)	6·4 (18·6)	4·4 (7·3)	6·0 (15·0)	4·0 (7·4)
Number of days with hypoglycaemia <40 mg/dl – days	NA	0·1 (0·5)	0·1 (0·6)	0·2 (0·6)	0·1 (0·5)	0·2 (0·6)
Post-randomisation treatments effects						
Duration of antibiotic treatment – days	NA	5·4 (14·2)	6·7 (19·0)	4·6 (8·7)	6·6 (17·7)	4·4 (9·8)
Duration of haemodynamic support – days	NA	2·7 (7·7)	3·0 (7·4)	2·4 (6·2)	2·9 (8·2)	2·5 (7·3)
Duration of treatment with opioids – days	NA	5·0 (9·3)	6·1 (16·5)	4·1 (6·2)	5·8 (11·5)	4·2 (6·5)
Duration of treatment with benzodiazepines – days	NA	4·4 (10·2)	5·4 (16·7)	4·0 (8·8)	4·9 (10·5)	4·0 (10·0)
Duration of treatment with hypnotics – days	NA	1·5 (6·0)	1·8 (6·3)	1·3 (3·1)	1·8 (8·1)	1·1 (3·0)
Duration of treatment with alpha-2-agonists – days	NA	1·1 (6·8)	1·1 (8·7)	1·0 (6·0)	1·1 (6·4)	1·1 (7·1)
Duration of treatment with corticosteroids - days	NA	1·2 (3·9)	1·6 (4·3)	1·3 (3·9)	1·4 (4·5)	1·1 (3·3)

Data are n (%) or mean (SD).

[a] Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.

*[b] The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Methods 4**).*

*[c] The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Methods 4**).*

[d] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.

[e] Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.

[f] Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.

[g] Pediatric Index of Mortality 3 (PIM3) probability of death, ranging from 0% to 100%, with higher percentages indicating a higher probability of death in PICU.

*[h] A pre-randomization syndrome or illness a priori defined as affecting or possibly affecting neurocognitive development (**Supplemental Methods 3)**.*

Abbreviations: BMI, body mass index; NA, not applicable (values only known when the patients were seen at follow-up, or not applicable for healthy control children); PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition; SD, standard deviation.

TABLE 2 POOLED UNIVARIABLE ANALYSES OF THE DIFFERENCES IN THE OUTCOMES ASSESSED AT 4 YEARS' FOLLOW-UP BETWEEN PATIENTS AND HEALTHY CONTROL CHILDREN AND BETWEEN LATE-PN AND EARLY-PN PATIENT GROUPS

		Tested populations			Tested PICU population		
	No. (%) available data per outcome prior to imputation	**Healthy control children**	**Patients**	**P-value**	**Early-PN**	**Late-PN**	**P-value**
	N=1053						
Outcomes assessed at 4 years' follow-up[a]		**N=369**	**N=684**		**N=328**	**N=356**	
Height - cm	1012 (96·1%)	124·7 (23·4)	121·1 (23·2)	**0·02**	122·1 (23·1)	120·8 (23·2)	0·26
Z-score[b]	1012 (96·1%)	0·40 (0·99)	-0·03 (1·23)	**<0·0001**	0·04 (1·22)	-0·09 (1·25)	0·16
Weight - kg	1004 (95·3%)	28·0 (16·5)	27·0 (17·1)	0·33	27·2 (16·5)	26·7 (17·5)	0·70
Z-score[b]	1004 (95·3%)	0·32 (0·87)	0·12 (1·17)	**0·005**	0·17 (1·18)	0·08 (1·17)	0·55
BMI - kg/m^2	1003 (95·3%)	16·68 (2·94)	16·86 (3·33)	0·69	16·84 (3·13)	16·89 (3·50)	0·56
Z-score[b]	1003 (95·3%)	0·12 (1·00)	0·21 (1·17)	0·25	0·17 (1·43)	0·09 (1·26)	0·19
Head circumference - cm	1008 (95·7%)	52·5 (2·3)	52·0 (2·7)	**0·001**	52·1 (2·8)	51·8 (2·6)	0·27
Z-score[b]	1008 (95·7%)	0·49 (1·08)	0·13 (1·34)	**<0·0001**	0·17 (1·43)	0·09 (1·26)	0·19
Diagnosed with a somatic illness	840 (79·8%)	120 (32·4)	370 (54·0)	**<0·0001**	180 (54·6)	190 (53·5)	0·70
Diagnosed with a psychiatric illness	960 (91·2%)	16 (4·3)	63 (9·2)	**0·005**	32 (9·5)	31 (8·9)	0·81
Admitted to hospital for a medical or surgical reason	1011 (96·0%)	101 (27·3)	453 (66·2)	**<0·0001**	230 (70·0)	223 (62·7)	**0·05**
Clinical neurological evaluation score (range, 0-8)[a]	970 (92·1%)	0·2 (0·6)	0·6 (1·3)	**<0·0001**	0·7 (1·4)	0·5 (1·2)	0·09
Executive functioning as reported by parents/caregivers - T-score [a c]							
Inhibition	918 (89·9%)	45·7 (9·8)	49·8 (13·2)	**<0·0001**	50·8 (13·3)	49·0 (12·6)	0·07
Flexibility	919 (90·0%)	45·7 (8·5)	49·3 (11·8)	**<0·0001**	50·2 (12·2)	48·5 (11·2)	**0·05**
Emotional control	919 (90·0%)	46·2 (9·4)	48·9 (11·2)	**<0·0001**	49·5 (11·3)	48·4 (11·0)	0·18
Working memory	918 (89·9%)	46·4 (9·6)	51·9 (13·5)	**<0·0001**	52·7 (13·7)	51·1 (12·6)	0·12
Planning and organisation	917 (89·8%)	46·3 (9·5)	50·4 (12·8)	**<0·0001**	50·6 (12·8)	50·2 (12·0)	0·60
Meta-cognition index	916 (89·7%)	45·6 (9·8)	50·6 (13·2)	**<0·0001**	50·9 (13·5)	50·2 (12·4)	0·51
Total score	915 (89·6%)	44·8 (9·8)	49·9 (13·2)	**<0·0001**	50·5 (13·3)	49·2 (12·5)	0·18
Emotional and behavioural problems as reported by parents/caregivers - T-score [a c]							
Internalising problems	940 (92·1%)	46·7 (10·5)	51·0 (12·3)	**<0·0001**	52·1 (12·1)	50·0 (12·2)	**0·02**
Externalising problems	940 (92·1%)	45·6 (9·7)	48·8 (11·2)	**<0·0001**	49·7 (11·0)	47·9 (11·1)	**0·03**
Total problems	940 (92·1%)	45·4 (9·9)	50·1 (11·9)	**<0·0001**	51·5 (11·6)	48·8 (11·9)	**0·003**
Intelligence (range, 45-155) [a]							
Total IQ	940 (92·1%)	105·7 (13·4)	93·1 (18·2)	**<0·0001**	93·2 (17·0)	93·0 (18·2)	0·89
Verbal IQ	940 (92·1%)	107·5 (14·4)	95·2 (19·0)	**<0·0001**	93·2 (16·0)	92·5 (16·2)	0·56
Performal IQ	940 (92·1%)	102·7 (13·2)	92·9 (16·2)	**<0·0001**	94·8 (18·3)	95·6 (18·6)	0·56
Visual-motor integration (range, 0·9-20) [a]	1025 (97·3%)	10·0 (2·1)	8·7 (3·1)	**<0·0001**	8·7 (3·1)	8·7 (2·7)	0·88
Alertness and motor coordination [a c]							
Alertness [a c d]							
Reaction time right hand – Z-score	739 (72·0%)	0·8 (4·3)	1·7 (12·6)	**0·03**	1·7 (8·9)	1·7 (9·4)	0·65
Within subject SD of repeated tests – Z-score	739 (72·0%)	1·1 (3·4)	2·0 (8·5)	**<0·0001**	2·0 (6·1)	2·0 (6·4)	0·68

Reaction time left hand – Z-score	752 (73·3%)	0·3 (2·5)	1·0 (5·8)	**<0·0001**	1·0 (4·3)	1·1 (4·5)	0·64
Within subject SD of repeated tests – Z-score	752 (73·3%)	1·0 (2·5)	1·7 (4·0)	**<0·0001**	1·6 (3·3)	1·7 (3·2)	0·59
Motor coordination (No of taps in 10s) [a c]							
No of unimanual taps							
Right hand	816 (79·5%)	34·6 (29·6)	32·6 (52·3)	0·12	32·7 (40·0)	32·5 (37·0)	0·76
Left hand	816 (79·5%)	30·5 (32·3)	28·9 (60·4)	0·18	29·1 (46·0)	28·7 (41·7)	0·65
No of valid alternating taps	742 (72·3%)	22·9 (30·0)	19·7 (56·8)	**0·05**	19·6 (43·8)	19·9 (40·7)	0·71
No of valid synchronous taps	785 (76·5%)	16·5 (18·3)	13·2 (27·9)	**<0·0001**	12·9 (21·9)	13·5 (20·5)	0·47
Memory [a c]							
Verbal-auditory							
Numbers (range, 1-19)							
Memory span (forward)	418 (85·1%)	9·9 (3·1)	8·7 (4·3)	**<0·0001**	9·0 (4·0)	8·5 (3·6)	0·18
Working memory (backward)	394 (80·2%)	10·3 (3·1)	9·5 (5·3)	**0·01**	9·7 (4·5)	9·3 (4·3)	0·24
Word pairs (proportion of correct responses)							
Learning	350 (71·2%)	0·5 (0·2)	0·4 (0·4)	**<0·0001**	0·4 (0·4)	0·4 (0·3)	0·67
Immediate memory	346 (70·5%)	0·4 (0·5)	0·4 (1·3)	0·07	0·4 (1·0)	0·4 (0·9)	0·55
Delayed memory	343 (69·9%)	0·4 (0·7)	0·4 (1·6)	0·12	0·4 (1·3)	0·4 (1·1)	0·43
Recognition	343 (69·9%)	0·9 (0·5)	0·9 (1·3)	0·15	0·9 (0·9)	0·9 (0·9)	0·46
Non-verbal, visual-spatial							
Pictures (proportion of correct responses)	404 (82·2%)	0·8 (0·1)	0·8 (0·2)	**<0·0001**	0·8 (0·2)	0·8 (0·2)	0·74
Dots (proportion of correct responses)							
Learning	370 (75·4%)	0·9 (0·2)	0·8 (0·4)	**0·001**	0·8 (0·4)	0·8 (0·3)	0·26
Immediate memory	367 (74·7%)	0·9 (0·3)	0·8 (0·7)	**0·01**	0·8 (0·5)	0·8 (0·5)	0·27
Delayed memory	361 (73·5%)	0·8 (0·4)	0·7 (1·1)	**0·004**	0·7 (0·8)	0·7 (0·8)	0·66
Learning index (range, 50-150)	341 (69·5%)	101·0 (22·6)	88·1 (33·2)	**<0·0001**	88·5 (27·4)	87·7 (25·8)	0·65

Results are presented in numbers with proportions (%) or mean (SD) from the 31 datasets combined generated by multiple data imputation by chained equations under a 'missing at random' assumption for the 684 post-PICU patients and 369 healthy control children.

[a] For the clinical neurological evaluation score, higher scores reflect worse performance. For parent-reported executive functioning and emotional and behavioural problems, higher scores reflect worse performance. For intelligence and visual-motor integration, higher scores reflect better performance. For reaction time alertness and within-subject SD of repeated tests, higher scores reflect worse performance. For motor coordination, higher scores reflect better performance. For memory tests, higher scores reflect better performance.

[b] Age- and gender-adjusted Z-scores, were calculated with the use of reference data from the World Health Organisation Growth Charts: http://www.bcchildrens.ca/Services/SpecializedPediatrics/EndocrinologyDiabetesUnit/ForProfessionals/AnthropometricCalculators.htm.

[c] For alertness, motor coordination, executive functions, emotional and behavioral problems and memory, applicable imputation was limited to relevant age-ranges.

[d] For alertness, age adjusted Z-scores were calculated and imputed in the dataset

Abbreviations: BMI, body mass index; IQ, intelligence quotient; PICU, pediatric intensive care unit; PN, parenteral nutrition; SD, standard deviation.

TABLE 3 MULTIVARIABLE LINEAR AND LOGISTIC REGRESSION ANALYSES OF THE DIFFERENCES IN THE OUTCOMES ASSESSED AT 4 YEARS' FOLLOW-UP BETWEEN PATIENTS AND HEALTHY CONTROL CHILDREN AND BETWEEN LATE-PN AND EARLY-PN PATIENT GROUPS

Outcomes assessed at 4 years' follow-up[a]	No. (%) available data per outcome prior to imputation N=1053	Beta-estimate or odds ratio (95% CI) for the comparison patients vs. controls, adjusted for risk factors[d]	P-value	Beta-estimate or odds ratio (95% CI) for the comparison late PN vs. early PN, adjusted for risk factors[f]	P-value
Height – cm	1012 (96·1%)	-2·108 (-3·152 to -1·063)	**<0·0001**	-0·814 (-3·448 to 1·820)	0·54
Weight – kg	1004 (95·3%)	-0·091 (-0·966 to 0·785)	0·83	0·129 (-2·047 to 2·304)	0·91
Head circumference – cm	1008 (95·7%)	-0·421 (-0·665 to -0·176)	**0·0007**	-0·113 (-0·461 to 0·234)	0·52
Diagnosed with a somatic illness	840 (79·8%)	2·232 (1·635 to 3·047) [e]	**<0·0001**	0·974 (0·683 to 1·390) [e]	0·88
Diagnosed with a psychiatric illness	960 (91·2%)	2·465 (1·248 to 4·871) [e]	**0·009**	1·035 (0·562 to 1·905) [e]	0·91
Admitted to hospital for a medical or surgical reason	1011 (96·0%)	4·269 (3·120 to 5·842) [e]	**<0·0001**	0·715 (0·501 to 1·020) [e]	0·06
Clinical neurological evaluation score (range, 0-8)[a]	970 (92·1%)	0·237 (0·098 to 0·376)	**0·0008**	-0·098 (-0·275 to 0·079)	0·28
Executive functioning as reported by parents/caregivers - T-score [a b]					
Inhibition	918 (89·9%)	2·685 (1·059 to 4·310)	**0·001**	-1·665 (-3·643 to 0·313)	0·10
Flexibility	919 (90·0%)	2·706 (1·259 to 4·153)	**0·0002**	-1·487 (-3·283 to 0·309)	0·10
Emotional control	919 (90·0%)	2·061 (0·601 to 3·520)	**0·005**	-1·189 (-2·938 to 0·560)	0·18
Working memory	918 (89·9%)	3·695 (2·096 to 5·293)	**<0·0001**	-1·375 (-3·328 to 0·577)	0·17
Planning and organisation	917 (89·8%)	2·866 (1·327 to 4·406)	**0·0002**	-0·380 (-2·270 to 1·511)	0·69
Meta-cognition index	916 (89·7%)	3·334 (1·714 to 4·954)	**<0·0001**	-0·610 (-2·580 to 1·359)	0·54
Total score	915 (89·6%)	3·566 (1·950 to 5·183)	**<0·0001**	-1·266 (-3·246 to 0·714)	0·21
Emotional and behavioural problems as reported by parents/caregivers – T-score [a b]					
Internalising problems	940 (92·1%)	2·730 (1·185 to 4·275)	**0·0005**	-1·880 (-3·690 to -0·071)	**0·042**
Externalising problems	940 (92·1%)	1·631 (0·185 to 3·076)	**0·02**	-1·731 (-3·433 to -0·028)	**0·046**
Total problems	940 (92·1%)	2·951 (1·443 to 4·459)	**0·0001**	-2·442 (-4·215 to -0·668)	**0·007**
Intelligence (range, 45-155) [a]					
Total IQ	937 (89·0%)	-7·349 (-9·311 to -5·387)	**<0·0001**	-1·100 (-3·399 to 1·198)	0·35
Verbal IQ	931 (88·4%)	-6·955 (-8·986 to -4·924)	**<0·0001**	-0·126 (-2·493 to 2·241)	0·92
Performal IQ	943 (89·6%)	-5·968 (-7·905 to -4·030)	**<0·0001**	-1·645 (-3·902 to 0·612)	0·15
Visual-motor integration (range, 0·9-20) [a]	1025 (97·3%)	-0·888 (-1·202 to -0·574)	**<0·0001**	-0·081 (-0·448 to 0·286)	0·66
Alertness and motor coordination [a b]					
Alertness [a b c]					
Reaction time right hand – Z-score	739 (72·0%)	0·668 (0·186 to 1·150)	**0·007**	0·077 (-0·334 to 0·489)	0·71
Within subject SD of repeated tests – Z-score	739 (72·0%)	0·663 (0·254 to 1·071)	**0·001**	0·020 (-0·393 to 0·434)	0·92
Reaction time left hand – Z-score	752 (73·3%)	0·498 (0·177 to 0·819)	**0·002**	0·141 (-0·221 to 0·502)	0·44
Within subject SD of repeated tests – Z-score	752 (73·3%)	0·476 (0·168 to 0·784)	**0·002**	0·173 (-0·166 to 0·512)	0·32
Motor coordination (No of taps in 10s) [a b]					

No of unimanual taps					
Right hand	816 (79·5%)	-1·762 (-3·448 to -0·076)	**0·04**	0·240 (-1·844 to 2·325)	0·82
Left hand	816 (79·5%)	-1·720 (-3·415 to -0·024)	**0·04**	0·094 (-1·893 to 2·081)	0·93
No of valid alternating taps	742 (72·3%)	-2·412 (-4·848 to 0·023)	**0·05**	0·503 (-2·202 to 3·209)	0·71
No of valid synchronous taps	785 (76·5%)	-2·066 (-3·348 to -0·783)	**0·001**	0·354 (-1·192 to 1·901)	0·65
Memory [a b]					
Verbal-auditory					
Numbers (range, 1-19)					
Memory span (forward)	418 (85·1%)	-0·644 (-1·270 to -0·019)	**0·04**	-0·601 (-1·371 to 0·168)	0·12
Working memory (backward)	394 (80·2%)	-0·165 (-0·781 to 0·450)	0·59	-0·323 (-1·047 to 0·400)	0·38
Word pairs (proportion of correct responses)					
Learning	350 (71·3%)	-0·081 (-0·122 to -0·040)	**0·0001**	-0·021 (-0·060 to 0·019)	0·30
Immediate memory	346 (70·5%)	-0·040 (-0·101 to 0·021)	0·19	-0·030 (-0·089 to 0·026)	0·31
Delayed memory	343 (70·0%)	-0·034 (-0·098 to 0·029)	0·28	-0·012 (-0·088 to 0·064)	0·76
Recognition	434 (70·0%)	-0·033 (-0·084 to 0·018)	0·20	-0·010 (-0·048 to 0·027)	0·58
Non-verbal, visual-spatial					
Pictures (proportion of correct responses)	404 (82·3%)	-0·029 (-0·056 to -0·003)	**0·02**	0·008 (-0·028 to 0·044)	0·68
Dots (proportion of correct responses)					
Learning	370 (75·4%)	-0·046 (-0·080 to -0·012)	**0·007**	0·007 (-0·040 to 0·054)	0·77
Immediate memory	367 (74·7%)	-0·053 (-0·102 to -0·003)	**0·03**	-0·012 (-0·073 to 0·050)	0·70
Delayed memory	361 (73·5%)	-0·078 (-0·148 to -0·007)	**0·03**	0·005 (-0·071 to 0·080)	0·90
Learning index (range, 50-150)	341 (70·0%)	-10·216 (-13·883 to -6·549)	**<0·0001**	-1·383 (-5·351 to 2·585)	0·49

Results are the combined beta-estimates and odds ratios from 31 datasets generated by multiple data imputation by chained equations under a 'missing at random' assumption for the 684 patients and 369 healthy control children.

[a] For the clinical neurological evaluation score, higher scores reflect worse performance. For parent-reported executive functioning and emotional and behavioural problems, higher scores reflect worse performance. For intelligence and visual-motor integration, higher scores reflect better performance. For reaction time alertness and within-subject SD of repeated tests, higher scores reflect worse performance. For motor coordination, higher scores reflect better performance. For memory tests, higher scores reflect better performance.

[b] For alertness, motor coordination, executive functions, emotional and behavioral problems and memory, applicable imputation was limited to relevant age-ranges.

[c] For alertness, age adjusted Z-scores were calculated and imputed in the dataset

[d] Estimates and odds ratios were adjusted for the following risk factors: age, center, race, gender, geographic origin, language, hand preference, history of malignancy, a predefined "syndrome", and the educational and occupational status of parents.

[e] These values are odds ratios.

[f] Estimates and odds ratios were adjusted for the following risk factors: age, center, race, gender, geographic origin, language, hand preference, history of malignancy, a predefined "syndrome", the educational and occupational status of parents, PIM3 score and PeLOD score upon PICU admission, STRONGkids risk category, and parental smoking behavior prior to PICU admission.

Abbreviations: IQ, intelligence quotient; PeLOD score, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3 score, pediatric index of mortality 3 score; PN, parenteral nutrition; SD, standard deviation; STRONGkids, Screening Tool Risk On Nutritional Status and Growth.

Sensitivity analyses to the "missing at random' assumption and with imputing worst test-scores for the severely disabled and thus non-testable children, as specified in the Methods S2, further supported the robustness of these results.

As compared with patients who had been allocated to early-PN, late-PN patients were comparable for height, weight, body mass index, and head circumference, and for clinically assessed neurological functioning in univariable and multivariable analysis (**Table 2, Table 3**). In univariable analyses, as compared with early-PN patients, fewer late-PN patients were admitted to hospital and parents/caregivers of late-PN patients reported significantly fewer internalizing, externalizing and total emotional and behavioral problems and problems regarding flexibility **(Table 2, Figure 2A**). After adjustment for risk factors, internalizing, externalizing and total emotional and behavioral problems remained significantly less present in late-PN than in early-PN patients (**Table 3, Supplemental Table 2**). For internalizing and externalizing problems as well as total emotional and behavioral problems, late-PN patients were not different from healthy control children (**Supplemental Table 3**).

Differences in intensive care outcomes of the randomized intervention and other post-randomization factors overall did not explain the observed differences at 4-year follow-up (**Supplemental Table 2**). Interestingly, treatment with benzodiazepines was independently associated with worse outcome, whereas α2-agonist use was associated with better outcome.

DNA-methylation data were available for 403 of the 684 tested patients (192 early-PN and 211 late-PN patients, **Figure 1, Supplemental Table 4**). The mediation analysis revealed that the prevention by late-PN of the early-PN-induced adversely altered DNA-methylation of 37 CpG-sites,[7] statistically explained its beneficial impact on the three behavioral outcomes (**Figure 2B**). When adding the 37 CpG-sites that are differentially methylated by early-PN to the multivariable regression models adjusted for baseline risk factors for the 3 behavioral outcomes, the explanatory power (optimism-corrected R^2) improved with a 1.710 to 1.851-fold increase. We refer to **Supplemental Table 5** for details on the added explanatory power for the 3 long-term outcomes provided by the 37 differentially methylated CpG-sites. The clustering revealed distinct patterns of association of CpG-site methylation status with internalizing versus externalizing behavior (**Figure 2B**). In addition, the most important "mediating" CpG-sites for the three behavioral outcomes were intergenic or non-coding CpG-sites or CpG-sites related to genes involved in mood regulation and behavior, attention deficit hyperactivity disorder, and bipolar disorder, but also in several cognitive functions (intellectual disability, memory, visual learning, and flexibility), in nervous system development and function (cerebral cortex development, neuronal development, plasticity, survival and neurotransmission, neuronal network formation and information processing), and in neuroimmune/neuroinflammatory processes, apoptosis, and lipid metabolism.

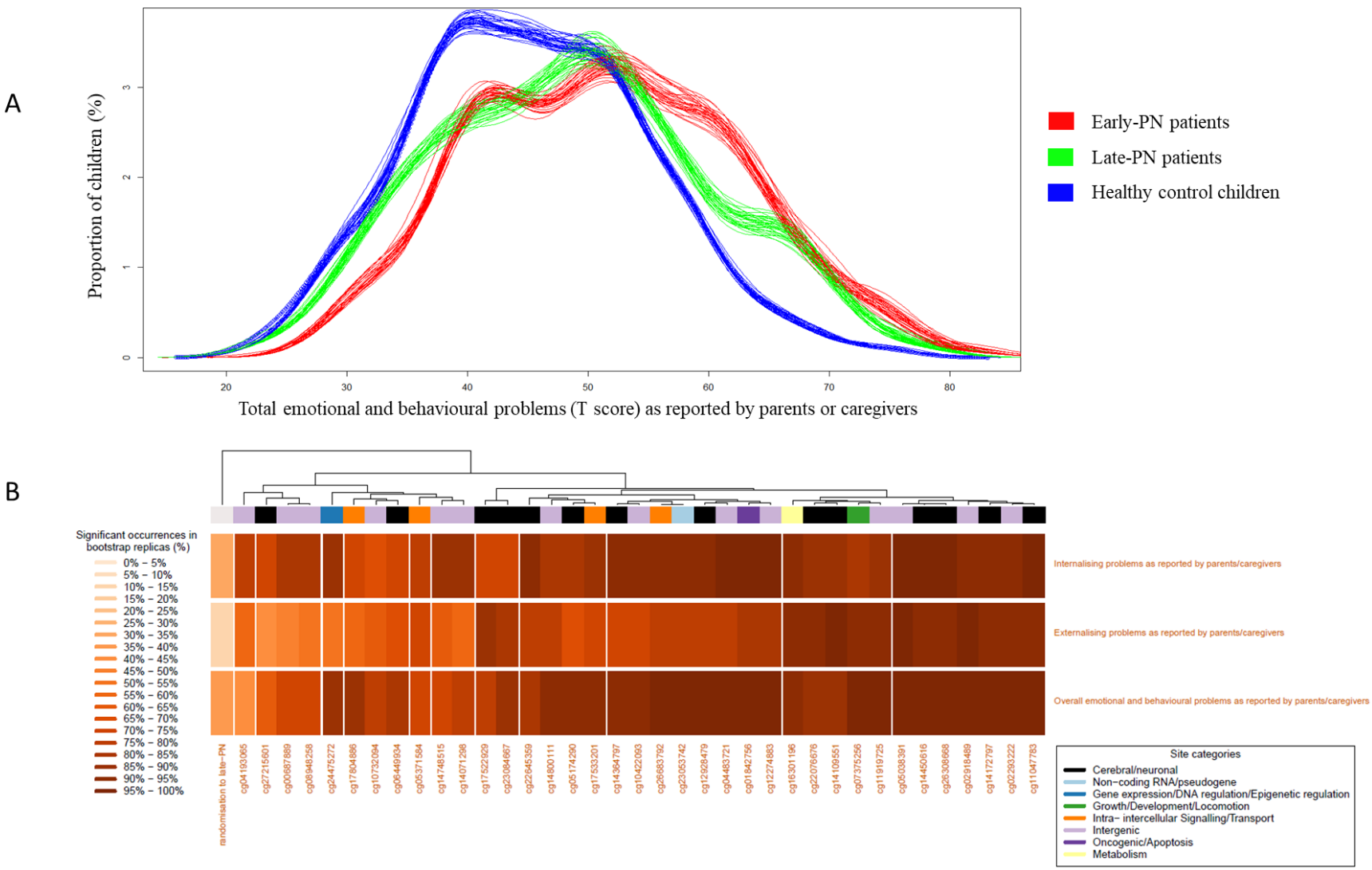

FIGURE 4 IMPACT OF LATE-PN VERSUS EARLY-PN IN PATIENTS ON LONG-TERM EMOTIONAL/BEHAVIOURAL PROBLEMS AND VISUALISATION OF THE MEDIATING ROLE OF ALTERED DNA-METHYLATION.
Panel A represents the density estimates for total behavioral and emotional problems reported by parents or caregivers. Each line corresponds to an imputed dataset. Densities, which correspond to the proportions of children with a certain score (equivalent to a smoothed histogram), are shown separately for early-PN patients (red), late-PN patients (green) and healthy controls (blue). Higher scores indicate more total behavioral and emotional problems. Panel B summarizes the results of the mediation analyses that were performed to investigate any role of avoiding early-PN-induced adversely altered leukocyte DNA-methylation of 37 CpG-sites[7] in statistically explaining the beneficial impact of late-PN on internalizing, externalizing and total behavioral problems identified at 4-year follow-up. All multivariable models resulted from random forest machine learning, with covariate significance levels obtained via permutation importance,[21,22] including the baseline risk factors age, center, race, gender, geographic origin, language, history of malignancy, a predefined "syndrome" (**Supplemental Methods 3**), diagnosis and severity of illness (PIM3 and PeLOD), risk of malnutrition (STRONGkids), the randomized intervention, and the 37 differentially methylated CpG-sites evoked by early-PN.[7] The robustness of these results was evaluated in 100 bootstrapped replicates.[23,24] Each row corresponds to

the 100 bootstrap replicates of the multivariable non-linear models for the internalizing, externalizing and total behavioral problems. Columns correspond to the 37 CpG-sites that were differentially methylated by early-PN. Color intensity of the boxes reflects the frequency with which a CpG-site was found to be independently and significantly (P<0·05) associated with the behavioral outcomes in the 100 bootstrapped replicated analyses, with darker orange colors corresponding to a higher frequency. Outcomes and CpG-sites were clustered based on these frequencies, with the clustering hierarchy shown in the dendrograms. The base of the column dendrogram is color-coded according to CpG-site functional classes "Cerebral/neuronal", "Growth/Development/Locomotion", "Metabolism", "Gene expression/DNA regulation/Epigenetic regulation", "Intra/intercellular Signaling/Transport", "Non-coding RNA/pseudogene", "Oncogenic/Apoptosis", or "Intergenic", as previously described.[7]

The CpG-sites that were most often retained in the bootstrapped non-linear models as independently associated with internalizing, externalizing or total behavioral problems were cg14172797 in PRKCA (involved in memory, mood regulation and behavior), cg11047783 in KAT6B (involved in cerebral cortex development, attention deficit hyperactivity disorder (ADHD) and intellectual disability), cg14109551 in CEP85L (involved in brain tumors, ADHD and bipolar disorder), cg14450616 in PLD3 (involved in neuronal development, survival and neurotransmission, visual learning, memory and flexibility), cg26308668 in srGAP1 (involved in neuronal development and migration, linked to mental retardation), cg14364797 in FNBP1 (involved in neuronal network formation and information processing), cg22645359 in TCF7L2 (involved in Wnt signalling and related to neurodevelopment and plasticity of mature neurons), cg12928479 in NLRC5 (involved in neuroimmune and neuroinflammatory processes), cg22076676 in THADA (involved in apoptosis, neuroinflammation and multiple sclerosis), cg01842756 in RNF217 (involved in apoptosis), cg16301196 in PLA2G15 (involved in lipid metabolism), cg07375256 in ZSCAN25 (transcriptional regulation (DNA binding and protein-protein interactions); genetic variation in ZSCAN25 has been associated with body weight, hip and brachial circumference), cg26683792 in SLC35E1 (unknown, putative transporter), and several intergenic and non-coding CpG-sites. Similar results were obtained when the randomized intervention was excluded from the models.

5. DISCUSSION

Four years after critical illness, children were found to still suffer from an important legacy characterized by broad abnormalities in all investigated developmental domains such as growth, health status, and neurocognitive and emotional/behavioral functioning, a finding that confirmed earlier observations.[3] The omission of supplemental PN throughout the first week in the PICU did not harm physical and neurocognitive development and protected patients specifically against emotional/behavioral problems that were present in patients who received early-PN.

A first important finding was that the 4-year legacy of critical illness was still spanning all developmental domains. To what extent these abnormalities are acquired during PICU stay remains debated.[25] However, the developmental legacy documented 4 years after critical illness was found to remain present after adjustment for all known baseline risk factors upon PICU admission. The documented developmental abnormalities are relevant, as they are known to have direct implications for daily life and to hamper future societal perspectives.[2,26,27] Moreover, the developmental impairment after pediatric critical illness is at least as pronounced as what has been reported for children who survived cancer[28-30] and for children suffering from chronic diseases.[31,32]

Interestingly, part of this legacy, more specifically the emotional and behavioral problems at 4-year follow-up, was found preventable by omitting the use of early-PN in the PICU. These emotional and behavioral problems comprised internalizing, externalizing and other problems. Internalizing problems are evidenced by anxious and depressive symptoms, and by social withdrawal.[13,14] These are the consequences of over-controlling behavior. Externalizing problems become apparent in aggressive and norm-deviant behavior, and are the consequence of under-controlling behavior that results in conflicts with others and in violation of social norms. In the total score for the emotional and behavioral problems, not only internalizing and externalizing behavioral problems, but also sleep problems for younger children and social, thinking and attention problems for older children are included. Such emotional and behavioral problems are thought to be in part a consequence of poor development of the executive functions, such as poor inhibitory control.[33,34] This could explain why, at 2-year follow-up, we found that not being exposed to early-PN predominantly reduced abnormal inhibitory control[5] whereas 2 years later, the impact on the emotional and behavioral problems became more apparent.

These behavioral problems attributed to the use of early-PN were found to be at least partially mediated by adversely altered DNA-methylation by early-PN, which thus provides a biological basis hereof. Interestingly, many of the CpG-sites of which DNA-methylation appeared explanatory for the behavioral problems at 4-year follow-up were the same as the ones that explained the impaired inhibitory control by early-PN observed at 2-year follow-up.[7] This further supports the above interpretation that emotional and behavioral problems could be a consequence of poor development of the executive functions. Also, other studies have found that emotional and behavioral problems following adverse early life events can be mediated by altered DNA-methylation.[35,36]

The developing brain of children thus appears vulnerable to metabolic insults during periods of critical illness. We previously showed that tight glycemic control in PICU prevented impaired motor coordination 4 years after admission,[3] an impairment that was less apparent in the patients of the current study, who had received at least some form of blood glucose control in the PICU. In addition to avoiding pronounced hyperglycemia, omitting parenteral nutrition early during critical illness was here found to further protect the normal development of other neurobiological pathways that coordinate emotions and behavior. This indicates that the neurocognitive legacy of pediatric critical illness is multifactorial, and improvement can only be expected by a stepwise elimination of various causal factors.

This study has limitations to highlight. First, for the clinical tests that assess inhibition and flexibility, missing data for >30% of the population did not allow imputation and thus no information on differences between groups could be provided. Second, neuroimaging studies were not performed due to ethical and practical considerations. The strengths of the study comprise the limited loss to follow-up as compared with other long-term follow-up studies of PICU patients[37,38] and the broad assessment of the physical, neurocognitive and emotional/behavioral development of patients and matched healthy control children.

In conclusion, also 4 years after critical illness, an important physical, neurocognitive and emotional/behavioral legacy was documented. The omission of the use of early-PN in the PICU did not harm any of the developmental domains and specifically protected patients against emotional/behavioral problems that were no longer overrepresented in late-PN patients as compared with healthy controls. Altered DNA-methylation was found to be a potential biological mediator hereof. These data further support de-implementation of the use of PN early during critical illness in infants and children. The findings also open perspectives for future identification of other modifiable risk factors related to the intensive care management.

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SUPPLEMENTARY MATERIAL

Supplemental Methods

Supplemental Method 1. Detailed description of outcome measures

Medical assessment

Anthropometric data

At the beginning of the follow-up visit, height (in cm), body weight (in kg) and head circumference (in cm) were measured.

Health status

In an interview with the parents, the need for medical support of all kind during the past two years for healthy control children and during the 4 years following the index PICU admission for patients, was recorded. The hospital admissions because of surgery or a medical reason, and the occurrence of a psychiatric diagnosis were documented.

Clinical neurological examination

In order to assess whether there were gross neurological abnormalities, during a structured clinical neurological examination, signs of major neurologic dysfunction were detected in the following domains: interaction/language skills, gross motor function, involuntary movements, reflexes, coordination and balance, fine motor function, cranial nerves, and special senses (sensory, visual, and auditory function). These were all scored normal or abnormal. An abnormal result for each of these domains was given 1 point and the sum was made of all the abnormal results, with a range of 0-8.

Neurocognitive testing

A broad range of neurocognitive functions, including general intellectual functioning, visual-motor integration, alertness, motor coordination, verbal and visual-spatial learning, and memory were evaluated, as previously reported.[1]

Patient/Parents-reported outcomes (PROs)

Executive functioning was assessed with the Behavior Rating Inventory of Executive Function in children aged 3 years 6 months - 5 years 11 months with BRIEF-P, and in children 6 years – 17 years 11 months with BRIEF, filled out by the parents/caregivers of the child. Overlapping scales and indices of both questionnaires (Inhibition, Flexibility, Emotional Control, Working Memory, Planning and Organization, Meta-cognition) and a Total Score were analyzed (T-scores, with mean 50 and SD 10).[2,3]

Emotional and behavioral problems were assessed by the parent/caregiver with the Child behavior Checklist (CBCL 1.5-5 years or CBCL 6-18 years).[4,5] Internalizing, externalizing, and total problems were analyzed (T-scores, with mean 50 and SD 10).[4,5]

Intelligence

General intellectual ability was assessed with use of age-appropriate versions of the Wechsler Intelligence Quotient (IQ) tests. The Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL)[6] was used for children aged 3 years 6 months – 5 years 11 months (one version for age range 3 years 6 months – 3 years 11 months, and another version for age range 4 years – 5 years 11 months), the Wechsler Intelligence Scale for Children (WISC-III-NL)[7] was used for children aged 6 years – 16 years 11 months, and the Wechsler Adult Intelligence Scale (WAIS-IV-NL)[8] for adolescents who were 17 years or older. For all these tests Total IQ, Verbal IQ, and Performance IQ scores (standard scores, with mean 100, SD 15) were computed.

Visual-motor integration
We used the Beery Developmental Test of Visual-Motor Integration, 6th Edition (VMI) to assess the ability to integrate visual and motor functions (scaled score, with mean 10 and SD 3). This involves eye-hand coordination.[9]

Alertness and motor-coordination
To measure alertness and motor coordination, the validated Amsterdam Neuropsychological Tasks (ANT) program was used.[10] The ANT is a computerized assessment battery of reaction time (RT) tasks that allows for the systematic evaluation of information processing capacities.

Children aged 4 years and older performed ANT-Baseline Speed (BS) and ANT-Tapping (TP). The ANT-BS evaluated alertness by measuring simple RT to visual stimuli (Z-scores of mean RT and SD of RT with mean 0 and SD 1 were obtained for the right and left hand separately). The ANT-TP assessed motor coordination for the right hand, left hand, bimanual alternating, and bimanual synchronous (number of taps,).

Memory
Auditory/verbal memory and Visual-spatial/non-verbal memory were assessed with use of four tests from the Children's Memory Scale (CMS) for children aged between 5 and 16 years 11 months.[11]
As to verbal memory, CMS-Numbers assessed short-term verbal memory span (forward digit recall) and verbal working memory load (backward digit recall). The CMS-Word Pairs (recall a list of word pairs) assessed short-term and long-term verbal memory, and recognition.

As to non-verbal memory, CMS-Picture Locations (remembering and recall of pictures in various locations) assessed short-term visual memory. CMS-Dot Locations (remembering and recall of the location of dots) assessed short-term and long-term visual memory.

For CMS-Numbers, scaled scores (with mean 10 and SD 3) for verbal memory span, CMS-numbers forward, and verbal working memory load, CMS-numbers backward were reported. For CMS-Word Pairs, CMS-Picture Locations, and CMS-Dot Locations, proportional scores were analyzed (proportion of correct responses ranging from 0 to 1, with higher scores reflecting better performance).

The CMS-Learning index is a standardized score of the sum of the three learning trials of the CMS-Word Pairs and the three learning trials of the CMS-Dot Locations subtests. The range of the score is 50-150, with a higher score representing a better learning ability.

Supplemental Method 2. Imputation
Missing data (excluding the deceased and the severely disabled whereby non-testable children) were handled by ***multiple data imputation with chained equations under a 'missing at random' assumption***. There were no missing data in the baseline variables. Predictors for missing values included all covariates listed below, and were retained in the predictor models with a minimum correlation of 0·1 with the prediction target. Predictive mean matching[12] was used for numeric variables except for factors with two levels (which were imputed based on logistic regression) and factors with more than two levels (for which polytomous (unordered) regression was used). A monotonous visiting scheme was used such that variables for imputation were visited in increasing order of the number of missing data. Imputation convergence was assessed visually and set at 100 iterations (**Supplemental Figure 2**). 31 complete imputed datasets were used in the analyses,[13] and pooled results were obtained across datasets using Rubin's rules.[14]

Plausibility of the imputations was assessed visually via the densities of the observed data and that resulting from the imputed values (**Supplemental Figure 3)** ***Sensitivity of results to the 'missing at random' assumption*** was assessed with use of pattern mixture models[14-16] assuming the original imputed values were either too high by a factor of 0·07 or too low by a factor of 0·1 for the main result of total emotional and behavioral problems as reported by parents/caregivers. Under this assumption, the obtained beta-estimates and P-values for randomization to late-PN vs. early-PN for the multivariable linear regression analyses performed to determine significant and independent associations between risk factors and total

emotional/behavioral problems as reported by the parents/caregivers at 4 years' follow-up within the tested patient population ranged from -1·98 (P=0·05) to -1·84 (P=0·04). The effect-sizes thus remained of the same order of magnitude, sign, and statistical significance as observed for the original imputed datasets, which suggested that the analyses were robust against the investigated 'missing at random' violation.

To further evaluate the robustness of the main findings, the analyses were repeated after imputing a penalized test result for all severely disabled and thus non-testable patients, defined as the worst result in the observed patients or controls, plus or minus one, as appropriate for each test. In this case, the obtained beta-estimates (P-values) for randomization to late-PN vs. early-PN for the multivariable linear regression analyses were respectively: A) -1·80 (P=0·05) for internalizing emotional/behavioral problems as reported by the parents/caregivers B) -1·62 (P=0·06) for externalizing emotional/behavioral problems as reported by the parents/caregivers and C) -2·36 (P=0·01) for total emotional/behavioral problems as reported by the parents/caregivers. These sensitivity analyses corresponded closely to the primary results as reported in **Table 3** of the main manuscript.
All multiple data imputation analyses were performed with R version 3·5·3 and MICE versions 3·4·0 and 3·6·0.

List of variables used for multiple data imputation by chained equations

- **Demographics of patients and control children and patient characteristics upon PICU admission**

Centre, randomization for late-PN or early-PN, patient vs. controls, race, gender, geographic origin, language, hand preference, history of malignancy, history of diabetes, a predefined "syndrome", educational and occupational status of parents, diagnosis, PIM3 and PeLOD scores upon PICU admission, risk of malnutrition (STRONGkids category), parental smoking before, during and after pregnancy, age at randomization, age group at randomization.

- **Acute effects of randomization and post-randomization treatments in PICU**

Acquisition of new PICU infections, duration of PICU stay, duration of mechanical ventilatory support, hypoglycemia, duration of treatment with hemodynamic support, antibiotics, corticosteroids, opioids, benzodiazepines, hypnotics and alpha-2-agonists.

- **At 4-years' follow-up**

Age, test location, height, weight, head circumference, composite endpoint "diagnosed with a somatic illness", composite endpoint "diagnosed with a psychiatric illness", composite endpoint "admitted to hospital for a medical or surgical reason", clinical neurological examination, verbal IQ, performance IQ, total IQ, visual motor integration, Z-score reaction time left hand, Z-score reaction time right hand, Z-score within subject SD of reaction time left hand, Z-score within subject SD of reaction time left hand, number of unimanual taps right hand, number of unimanual taps left hand, number of valid alternating taps, number of valid synchronous taps, numbers memory span forward, numbers working memory backward, word pairs learning, word pairs immediate memory, word pairs delayed memory, word pairs recognition, pictures, dots learning, dots immediate memory, dots delayed memory, learning index, executive functioning as reported by parents/caregivers (inhibition, flexibility, emotional control, working memory, planning and organization, meta-cognition index, and total score), emotional and behavioral problems as reported by parents/caregivers (internalizing problems, externalizing problems, and total problems).

Supplemental Method 3. Definition of "Syndrome"

A pre-randomization syndrome or illness *a priori* defined as affecting or possibly affecting neurocognitive development, and which is subdivided in the following categories:[17]

- Genetically confirmed syndrome or pathogenic chromosomal abnormality
- Clearly defined syndrome, association or malformation without (identified) genetic aberration
- Polymalformative syndrome of unknown etiology
- Clear auditory or visual impairment without specified syndrome
- Congenital hypothyroidism due to thyroid agenesis

- Brain tumor or tumor with intracranial metastatic disease
- Pediatric psychiatric disorder (e.g. autism spectrum disorder, (treatment for) attention deficit hyperactivity disorder)
- Severe medical disorder, not primarily neurologic, but suspected to alter psychomotor and/or mental performance
- Severe neonatal problem (e.g. severe asphyxia)
- Severe craniocerebral trauma or near-drowning
- Severe infectious encephalitis or drug-induced encephalopathy
- Infectious meningitis, encephalitis or Guillain-Barré
- Resuscitation and/or need for extracorporeal membrane oxygenation prior to randomization
- Severe convulsions or stroke prior to randomization

Supplemental Method 4. Definition of educational and occupational level of parents

Educational level of parents[17]

The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level.

Occupational level of parents[17]

The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions.[18] In case one of the parents filled in two jobs in the questionnaire, the highest Isco code level was used. In case “unemployed”, “disabled”, “student”, or “housewife/houseman” was filled in, an Isco code level of 1 was given to that parent. When the parents described their profession as “employee”, “worker”, “liberal profession”, or “retired”, they were given an Isco code level of 2.

Supplemental Method 5. Mediation analyses for altered DNA methylation by early-PN versus late-PN

Extraction and processing of leukocyte DNA and stepwise identification of CpG-sites that became differentially methylated by early-PN versus late-PN during PICU stay have been described previously,[19] and are summarized below together with the subsequent mediation analysis.

DNA extraction, bisulfite conversion, genome-wide DNA methylation analysis, and quality assessment

Leukocyte DNA was extracted with the Maxwell®RSC instrument and the corresponding blood DNA purification kit (Promega Benelux b.v., Leiden, The Netherlands). DNA concentrations were quantified with the Qubit® 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA). Bisulfite conversion of the DNA (400 ng) was performed with the EZ and EZ-96 DNA Methylation-Direct® Kits (Zymo Research, Irvine, CA). A genome-wide DNA methylation analysis was subsequently performed with the Infinium® HumanMethylation EPIC BeadChip, interrogating >850000 CpG-sites and spanning >99% of genes in the Reference Sequence (RefSeq) database, according to the manufacturer’s instructions (Illumina Inc., San Diego, CA, processed by Genomics Core, KU Leuven).[20] Investigators were blinded to participants' characteristics and randomisation.

Methylation β-values, ranging from 0 (no methylation) to 1 (full methylation), and M-values, which are the log2 ratios of the intensities of methylated probes versus unmethylated probes,[21] were obtained after functional normalization of the raw intensities with use of Partek Genomics Suite® 7.0 (Partek, St. Louis, MO).[22] Quality of the DNA methylation data was assessed with evaluation of the bi-peak curve of the M-value distribution among the samples in the low- and high-end range, and with principal component analysis (PCA) that assessed the variance in the dataset. Probes with a mean detection P-value >0·01 were excluded, to ensure that signals were expressed above the background defined by negative control probes, as were probes on X and Y chromosomes and probes spanning single nucleotide polymorphisms.[23]

Of the extracted leukocyte DNA samples, 814 last PICU day samples, 694 PICU admission samples, and 351 samples from healthy children passed all quality controls.[19]

Identification of CpG-sites that became differentially methylated during PICU stay
In order to investigate whether DNA methylation was altered between PICU admission and discharge it was first necessary (to avoid bias) to identify all CpG-sites that were differentially methylated in patients upon PICU admission as compared with healthy children, as these differences reflect pre-morbid conditions and illness-induced alterations in leukocyte composition.[24-28] To identify these CpG-sites, the corresponding M-values were compared between patients upon admission and controls with ANOVA, applying a false discovery rate (FDR) <0·05 according to the Benjamini & Hochberg "Step-up" procedure to account for multiple testing inherent to epigenome-wide analyses.[29] All CpG-sites that were differentially methylated between patients and controls were discarded from further analyses.

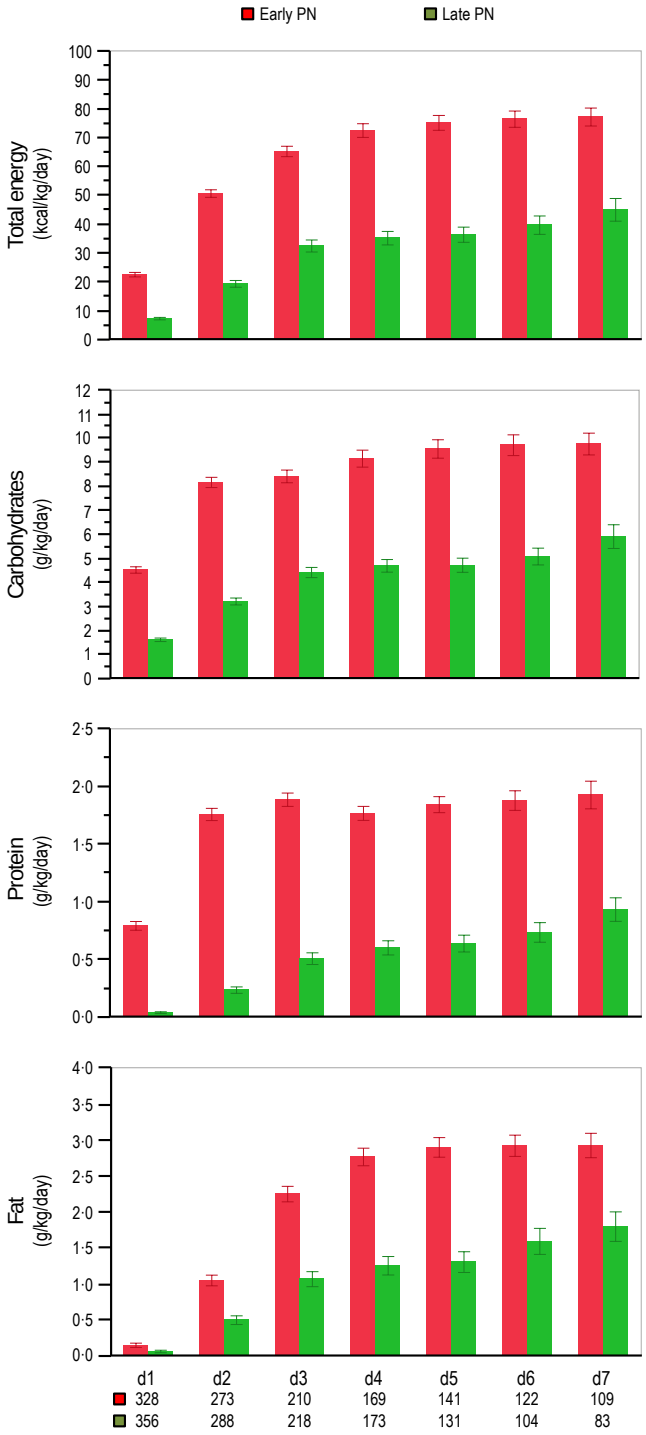
To subsequently investigate whether *de novo* DNA methylation changes occurred during critical illness, the degree of DNA methylation in the remaining CpG-sites was compared with ANOVA and FDR<0·05, between the last PICU day patient samples and samples from healthy children with similar age and gender distribution. This analysis identified 159 CpG-sites as methylated differently in patients on the last PICU day than in matched controls.[19]

Identification of de novo differential methylation of CpG-sites by early-PN versus late-PN
To investigate which of the identified 159 CpG-sites became differentially methylated by early-PN versus late-PN on the last PICU day in patients, multivariable linear regression analyses were performed adjusting for baseline risk factors [age, center, race, gender, geographic origin, language, history of malignancy, diabetes, predefined “syndrome” (**Supplemental Method 3**), diagnosis and severity of illness (PIM3 and PeLOD), and risk of malnutrition (STRONGkids)]. The analysis was repeated in 100 bootstrap samples and the result declared “robust” if the association (P<0·05) was present in at least 50.[30,31] These analyses showed that early-PN, as compared with late-PN, contributed to the abnormal methylation status of 37 of the 159 CpG-sites.[19]

Mediation analysis for altered DNA methylation by early-PN versus late-PN
In analogy with the earlier 2-year follow-up study, a mediation analysis was performed to investigate any role of the altered DNA methylation status of the 37 CpG sites, shown to be evoked by early-PN, in statistically explaining any impact of the randomized intervention on the 4-year follow-up outcomes.[19] To this end, we performed multivariable non-linear regression analyses, adjusted for baseline risk factors and further for the methylation status of those 37 CpG-sites. Models were built with and without addition of the randomized intervention. Covariate significance levels obtained via permutation importance were used.[30] The robustness of the results was evaluated by 100 bootstrapped replicates and the fold-change in optimism-corrected R^2 between models with and without addition of the CpG-sites differentially methylated by early-PN as compared with late-PN as covariates was calculated.[32] Heatmaps with dendrograms were used to visualize and summarize the findings.

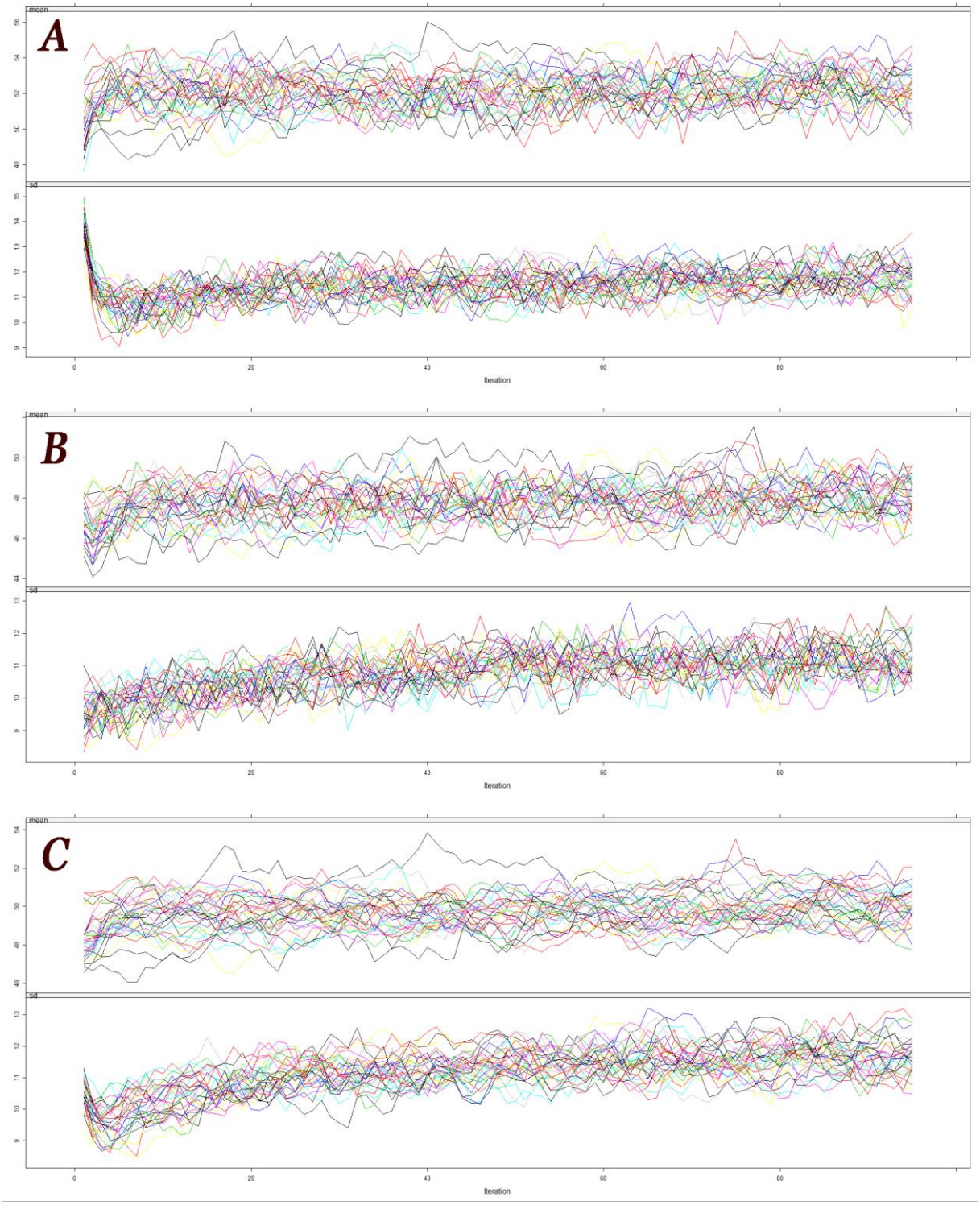
Supplemental Method 6. Correlation of physical, neurocognitive and psychosocial outcomes
We computed a correlation matrix to investigate the univariate association between all pairwise combinations of the physical, neurocognitive and psychosocial outcomes evaluated at 4-year follow up. In all cases we used a Pearson correlation of pairwise complete observations. This correlation matrix was then visualized directly with a color-code indicating the sign and strength of the correlation. This analysis was performed with the “Corrr” package version 0.4.0. for R version 3.5.3.

Supplemental figures



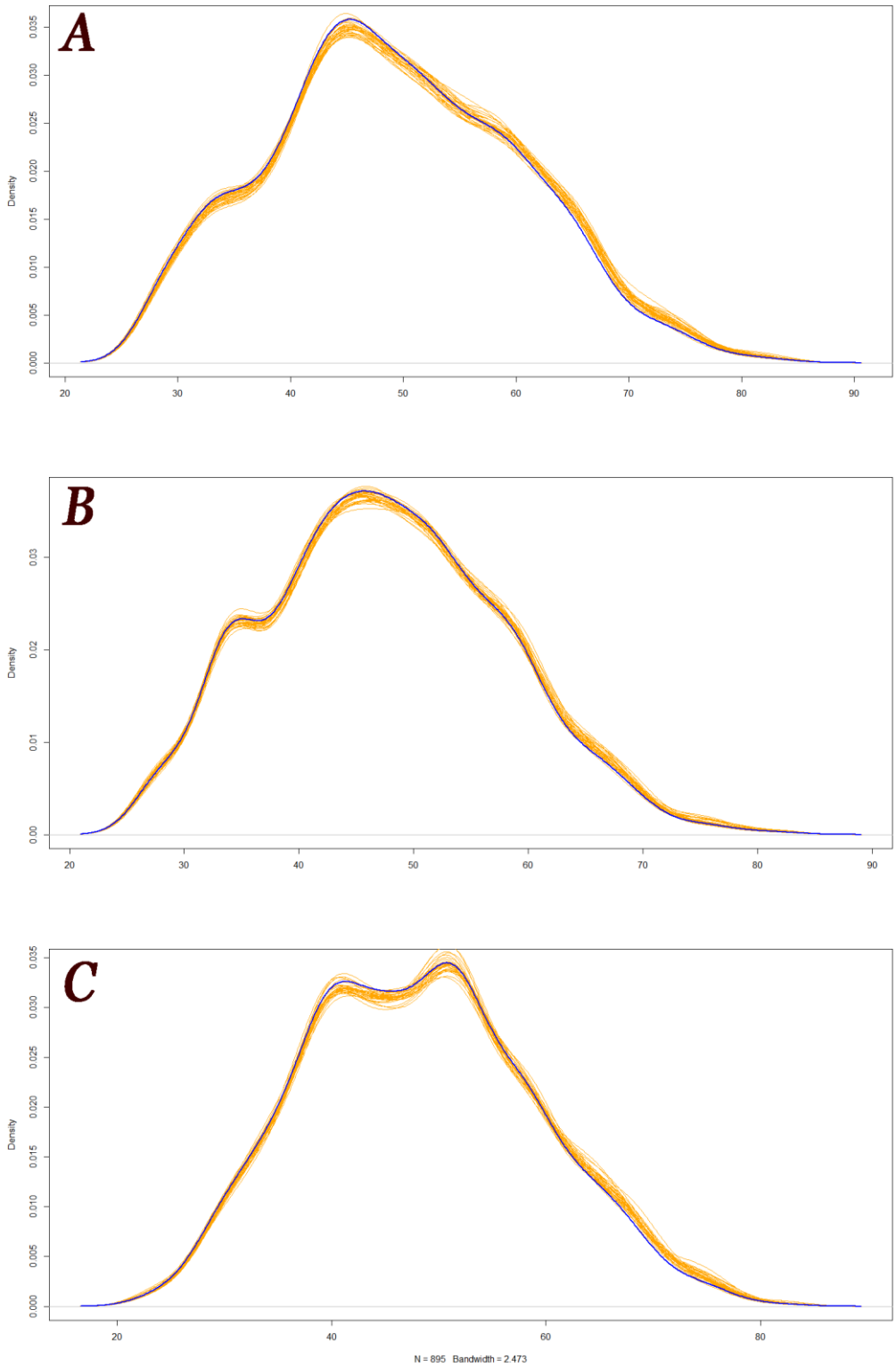
SUPPLEMENTAL FIGURE 1 MACRONUTRIENT DOSES DURING THE FIRST WEEK IN PICU ADMINISTERED TO THE TESTED PATIENT POPULATION

Daily amount of total energy in kcal/kg/day, and the daily amounts of total substrates in g/kg/day are shown for the first 7 days in the pediatric intensive care unit (PICU). Bars represent the mean and the whiskers represent the standard error of the mean (SEM). The red bars represent the early-PN group and the green bars represent the late-PN group.

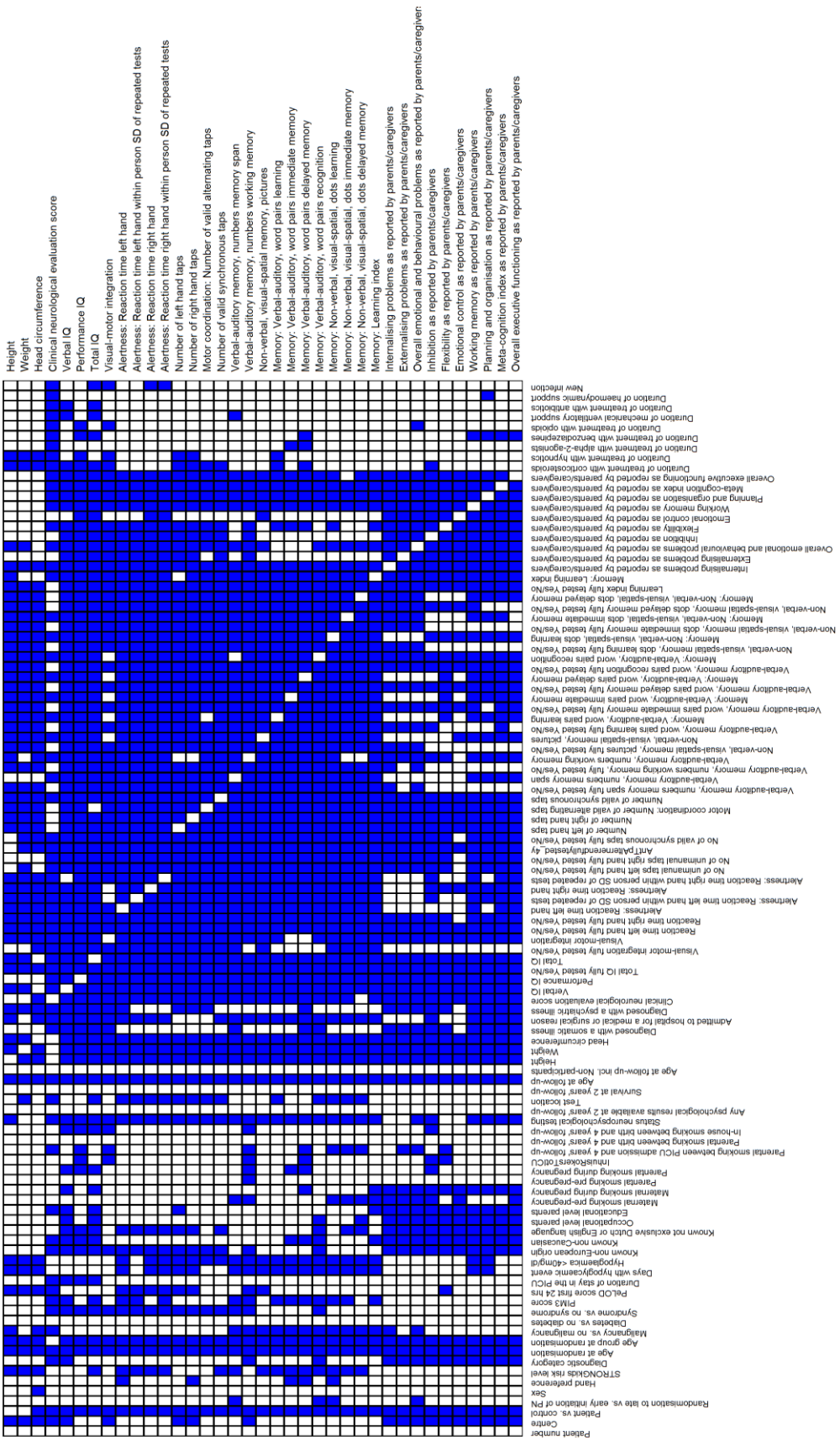


SUPPLEMENTAL FIGURE 2 IMPUTATION CONVERGENCE FOR SELECTED NEUROCOGNITIVE TEST RESULTS

Mean and standard deviation of imputed values in each of 31 datasets over 100 iterations for **A)** Emotional and behavioral problems as reported by parents/caregivers — T-score: Internalizing problems **B)** Externalizing problems **C)** Total problems.

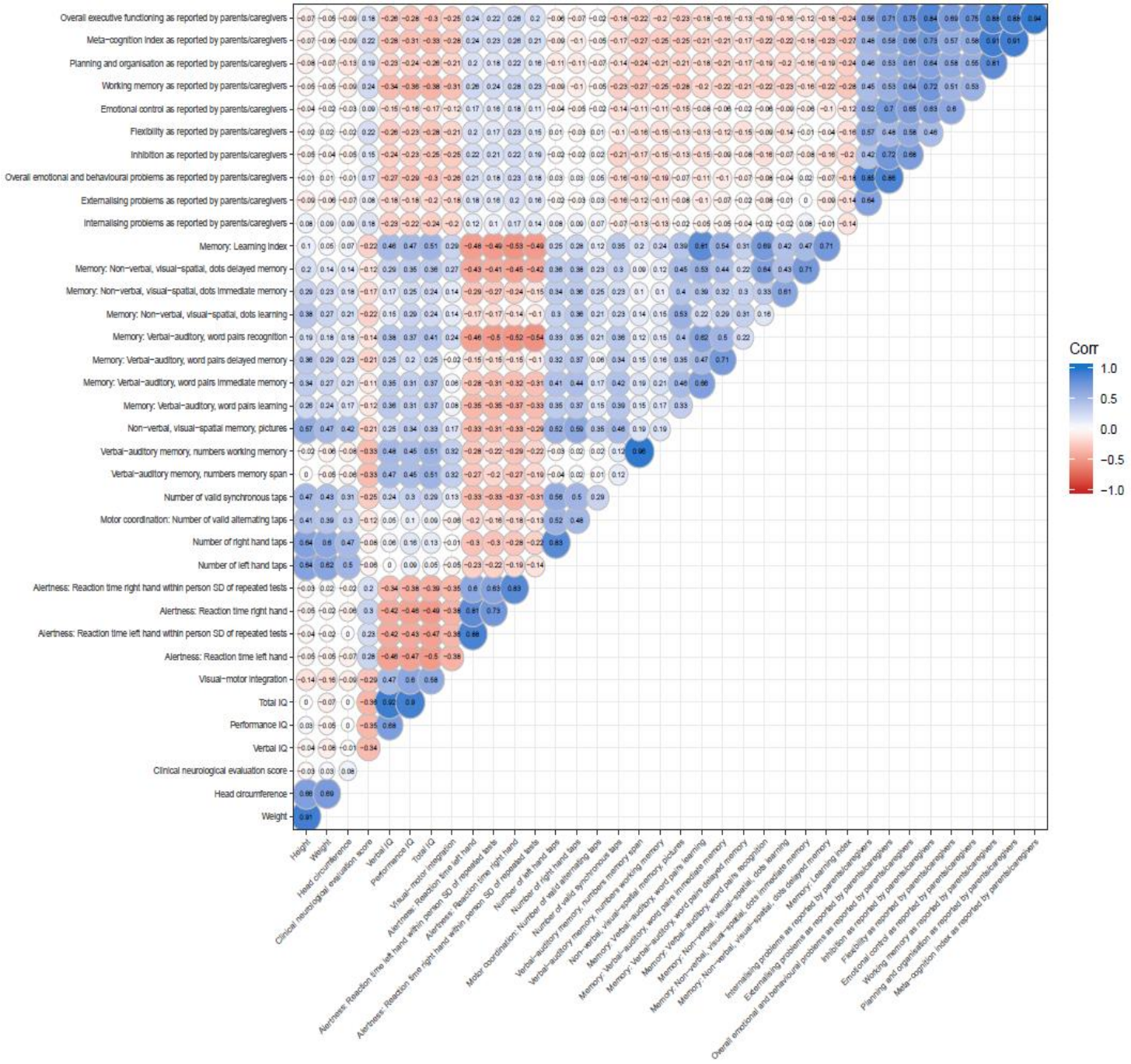


SUPPLEMENTAL FIGURE 3 DENSITY ESTIMATES OF THE OBSERVED AND IMPUTED VALUES FOR SELECTED NEUROCOGNITIVE TEST RESULTS
Density estimated for observed values (in blue) and for each imputed dataset (in orange) for **A)** Emotional and behavioral problems as reported by parents/caregivers — T-score: Internalizing problems **B)** Externalizing problems **C)** Total problems.



SUPPLEMENTAL FIGURE 4 MULTIPLE IMPUTATION PREDICTOR VARIABLES

Missing values for the variables in each row are imputed based on models that use as predictors only the column variables highlighted in blue. The predictor variables are selected as described in **Supplemental Method 4**.



SUPPLEMENTAL FIGURE 5 CORRELATION MATRIX OF PHYSICAL, NEUROCOGNITIVE AND PSYCHOSOCIAL OUTCOMES

The correlation matrix shows the correlation between all physical, neurocognitive and emotional/behavioural outcomes. Blue shades represent a positive correlation, red shades represent inverse correlations. Darker coloured shading represents a stronger correlation. For the statistical methodology of this matrix, see **Supplemental Method 6**.

Supplemental Tables

SUPPLEMENTAL TABLE 1.1 DEMOGRAPHICS AND OTHER PATIENT CHARACTERISTICS UPON PICU ADMISSION, ACUTE OUTCOMES AND POST-RANDOMISATION TREATMENTS IN THE PICU OF PARTICIPATING PATIENTS WHO WERE TOO DISABLED FOR NEUROCOGNITIVE TESTING AND THOSE WHO UNDERWENT NEUROCOGNITIVE TESTING

	Participating patients too disabled for neurocognitive testing N=84	Neurocognitively tested patients N=684	P
Demographics			
Age at 4-years' follow-up - yr	9·0 (5·6)	7·3 (4·3)	0·008
Sex			0·87
Male	49 (58·3%)	393 (57·5%)	
Female	35 (41·7%)	291 (42·5%)	
Known non-Caucasian race[a]	11 (13·1%)	53 (7·8%)	0·09
Known non-European origin[a]	20 (23·8%)	129 (18·9%)	0·27
Known not exclusive Dutch or English language	20 (23·8%)	158 (23·1%)	0·88
Socioeconomic status			
Educational level parents[b]			0·001
Educational level 1	9 (10·7%)	30 (4·4%)	
Educational level 1·5	4 (4·8%)	51 (7·5%)	
Educational level 2	21 (25·0%)	157 (23·0%)	
Educational level 2·5	11 (13·1%)	116 (17·0%)	
Educational level 3	10 (11·9%)	183 (26·8%)	
Educational level unknown	29 (34·5%)	147 (21·5%)	
Occupational level parents[c]			<0·0001
Occupational level 1	3 (3·6%)	7 (1·0%)	
Occupational level 1·5	6 (7·1%)	63 (9·2%)	
Occupational level 2	19 (22·6%)	108 (15·8%)	
Occupational level 2·5	5 (6·0%)	69 (10·1%)	
Occupational level 3	5 (6·0%)	118 (17·3%)	
Occupational level 3·5	0 (0·0%)	53 (7·8%)	
Occupational level 4	10 (11·9%)	102 (14·9%)	
Occupational level unknown	36 (42·9%)	164 (24·0%)	
Patient characteristics upon PICU admission			
Randomization			0·11
Early PN	48 (57·1%)	328 (48·0%)	
Late PN	36 (42·9%)	356 (52·1%)	
Infant (age<1y) at randomization	36 (42·9%)	331 (48·4%)	0·33
STRONGkids risk level[d]			0·15
Medium	71 (84·5%)	613 (89·6%)	
High	13 (15·5%)	71 (10·4%)	
PeLOD score, first 24h in PICU [e]	22·8 (12·4)	20·0 (11·6)	0·03
PIM3 score[f]	-3·0 (1·5)	-3·5 (1·4)	0·001
PIM3 probability of death - % [g]	9·1 (13·6)	6·6 (11·7)	0·001
Diagnostic category			<0·0001
Surgical			
Abdominal	1 (1·2%)	68 (9·9%)	
Burns	0 (0·0%)	3 (0·4%)	
Cardiac	28 (33·3%)	291 (42·5%)	
Neurosurgery-Traumatic brain injury	10 (11·9%)	58 (8·5%)	
Thoracic	1 (1·2%)	38 (5·6%)	

Transplantation	1 (1·2%)	11 (1·6%)	
Orthopaedic surgery-Trauma	12 (14·3%)	19 (2·8%)	
Other	1 (1·2%)	25 (3·7%)	
Medical			
Cardiac	0 (0·0%)	23 (3·4%)	
Gastrointestinal-Hepatic	2 (2·4%)	2 (0·3%)	
Oncologic-Haematologic	0 (0·0%)	6 (0·9%)	
Neurologic	9 (10·7%)	42 (6·1%)	
Renal	0 (0·0%)	0 (0%)	
Respiratory	11 (13·1%)	70 (10·2%)	
Other	8 (9·5%)	28 (4·1%)	
Malignancy	3 (3·6%)	38 (5·6%)	0·44
Diabetes	0 (0·0%)	0 (0·0%)	>0·99
Syndrome[h]	48 (57·1%)	63 (9·2%)	<0·0001
Known parental smoking between birth and PICU admission	17 (20·2%)	151 (22·1%)	0·70
Acute effects of randomization and post-randomization treatments in PICU			
Duration of stay in the PICU – days	7·5 (14·6)	7·8 (16·0)	0·57
Patients who acquired a new infection in PICU	10 (11·9)	96 (14·0%)	0·59
Duration of mechanical ventilatory support – days	5·2 (10·8)	5·0 (11·7)	0·72
Number of days with hypoglycemia <40mg/dl – days	0·2 (0·8)	0·1 (0·5)	0·97
Duration of antibiotic treatment – days	4·9 (9·6)	5·4 (14·2)	0·81
Duration of hemodynamic support – days	1·9 (3·6)	2·7 (7·7)	0·71
Duration of treatment with opioids – days	3·2 (4·5)	5·0 (9·3)	0·01
Duration of treatment with benzodiazepines – days	4·2 (10·7)	4·4 (10·2)	0·35
Duration of treatment with hypnotics – days	1·0 (1·9)	1·5 (6·0)	0·79
Duration of treatment with alpha-2-agonists – days	0·9 (6·6)	1·1 (6·8)	0·22
Duration of treatment with corticosteroids - days	1·0 (1·9)	1·2 (3·9)	0·03

Data are n (%) or mean (SD).

[a] Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.

[b] The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Method 4**).

[c] The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Method 4**).

[d] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.[33]

[e] Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.[34]

[f] Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.[35]

[g] Pediatric Index of Mortality 3 (PIM3) probability of death, ranging from 0% to 100%, with higher percentages indicating a higher probability of death in PICU.[35]

[h] A pre-randomization syndrome or illness *a priori* defined as affecting or possibly affecting neurocognitive development (**Supplemental Method 3**).

Abbreviations: BMI, body mass index; NA, not applicable (values only known when the patients were seen at follow-up, or not applicable for healthy control children); PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition; SEM, standard error of the mean.

SUPPLEMENTAL TABLE 1.2 PHYSICAL DEVELOPMENT AND PARENT-REPORTED OUTCOMES AT 4 YEARS' FOLLOW-UP OF PARTICIPATING PATIENTS WHO WERE TOO DISABLED FOR NEUROCOGNITIVE TESTING AND THOSE WHO UNDERWENT NEUROCOGNITIVE TESTING

	Participating patients too disabled for neurocognitive testing N=84		Neurocognitively tested patients N=684		P
	Number (%) of available data per outcome	Outcome result	Number (%) of available data per outcome	Outcome result	
Height - cm	77 (91·7%)	118·1 (24·2)	655 (95·8%)	120·9 (23·1)	0·04
Weight - kg	84 (100·0%)	25·6 (14·0)	647 (94·6%)	27·0 (17·1)	0·45
BMI - kg/m2	77 (91·7%)	17·4 (3·7)	646 (94·4%)	16·9 (3·4)	0·13
Head circumference - cm	83 (98·8%)	50·1 (3·7)	649 (94·9%)	51·9 (2·7)	<0·0001
Diagnosed with a somatic illness	42 (50·0%)	30 (71·4%)	523 (76·5%)	280 (53·5%)	0·02
Diagnosed with a psychiatric illness	76 (90·5%)	11 (14·5%)	609 (89·0%)	53 (8·7%)	0·10
Admitted to hospital for a medical or surgical reason	81 (96·4%)	73 (90·1%)	657 (96·1%)	435 (66·2%)	<0·0001
Clinical neurological evaluation score (range, 0-8)[a]	57 (67·9%)	4·3 (1·8)	616 (90·1%)	0·6 (1·3)	<0·0001
Executive functioning as reported by parents/caregivers - T-score[a]					
Inhibition	36 (42·9%)	61·8 (14·8)	556 (81·3%)	49·6 (11·8)	<0·0001
Flexibility	36 (42·9%)	59·0 (14·8)	557 (81·4%)	49·1 (10·6)	<0·0001
Emotional control	36 (42·9%)	56·1 (15·1)	557 (81·4%)	48·9 (10·5)	0·004
Working memory	35 (41·7%)	67·5 (12·4)	557 (81·4%)	51·5 (11·8)	<0·0001
Planning and organization	35 (41·7%)	62·0 (17·4)	557 (81·4%)	50·2 (11·2)	<0·0001
Meta-cognition index	35 (41·7%)	64·9 (15·7)	556 (81·3%)	50·3 (11·7)	<0·0001
Total score	35 (41·7%)	64·1 (16·8)	555 (81·1%)	49·7 (11·8)	<0·0001
Emotional and behavioral problems as reported by parents/caregivers - T-score[a]					
Internalizing problems	44 (52·4%)	55·7 (10·4)	565 (82·6%)	50·4 (11·2)	0·006
Externalizing problems	44 (52·4%)	53·4 (13·0)	565 (82·6%)	48·5 (10·3)	0·02
Total problems	44 (52·4%)	56·6 (12·2)	565 (82·6%)	49·7 (11·0)	0·0007

[a] Higher scores reflect worse performance.

Abbreviations: BMI, body mass index; IQ, intelligence quotient; PICU, pediatric intensive care unit; PN, parenteral nutrition; SD, standard deviation

SUPPLEMENTAL TABLE 2.1 MULTIVARIABLE LINEAR REGRESSION ANALYSES DETERMINING SIGNIFICANT AND INDEPENDENT ASSOCIATIONS BETWEEN RISK FACTORS AND INTERNALISING PROBLEMS AS REPORTED BY THE PARENTS/CAREGIVERS AT 4 YEARS' FOLLOW-UP WITHIN THE PATIENT POPULATION THAT UNDERWENT NEUROCOGNITIVE TESTING

	Model adjusted for risk factors		Model further adjusted for acute effects of late-PN vs early-PN		Model further adjusted for post-randomization treatments	
Variable	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**
Randomization to late vs· early initiation of PN	***-1·880 (-3·690 to -0·071)***	***0·042***	***-1·702 (-3·541 to 0·173)***	***0·070***	***-1·625 (-3·470 to 0·219)***	***0·084***
Centre						
Leuven vs Edmonton	0·019 (-8·653 to 8·691)	0·997	-0·024 (-8·717 to 8·668)	0·996	0·082 (-8·725 to 8·888)	0·985
Rotterdam vs Edmonton	0·627 (-7·840 to 9·094)	0·884	0·424 (-8·050 to 8·898)	0·922	0·292(-8·316 to 8·899)	0·947
Male vs female sex	1·165 (-0·681 to 3·011)	0·216	1·062 (-0·789 to 2·913)	0·260	1·187(-0·674 to 3·049)	0·211
Right vs left hand preference	-0·130 (-2·867 to 2·608)	0·926	-0·230 (-2·981 to 2·522)	0·870	-0·063 (-2·831 to 2·706)	0·964
Medium vs high STRONGkids risk level[a]	-3·074 (-6·178 to 0·031)	0·052	-2·727 (-5·867 to 0·413)	0·089	-2·667 (-5·893 to 0·559)	0·105
Diagnostic category (as compared with cardiac surgery)						
Surgical						
Abdominal	0·627 (-3·109 to 4·363)	0·742	0·631 (-3·103 to 4·365)	0·740	0·530 (-3·258 to 4·317)	0·783
Burns	-2·005 (-14·952 to 10·942)	0·761	-2·825 (-15·865 to 10·215)	0·671	-2·919 (-16·09 to 10·255)	0·664
Neurosurgery - traumatic brain injury	1·887 (-1·990 to 5·764)	0·339	1·848 (-2·028 to 5·725)	0·349	1·689 (-2·200 to 5·577)	0·394
Thoracic	-1·197 (-5·380 to 2·986)	0·574	-1·233 (-5·422 to 2·955)	0·563	-0·961 (-5·192 to 3·270)	0·655
Transplantation	-0·617 (-8·396 to 7·162)	0·876	-1·493 (-9·446 to 6·461)	0·712	-1·241 (-10·413 to 7·930)	0·790
Orthopedic surgery-trauma	-0·573 (-6·724 to 5·577)	0·855	-0·821 (-6·987 to 5·346)	0·794	-0·917 (-7·153 to 5·318)	0·772
Other	1·849 (-3·448 to 7·147)	0·761	1·418 (-3·947 to 6·783)	0·604	0·920 (-4·527 to 6·368)	0·740
Medical						
Cardiac	-0·611 (-6·202 to 4·980)	0·830	-1·314 (-6·929 to 4·300)	0·646	-1·436 (-7·200 to 4·328)	0·625
Gastrointestinal-hepatic	-4·742 (-25·519 to 16·035)	0·652	-4·641 (-25·442 to 16·160)	0·659	-4·818 (-25·658 to 16·022)	0·648
Hematologic-oncologic	-2·598 (-12·953 to 7·758)	0·622	-2·529 (-12·915 to 7·857)	0·633	-2·829 (-13·968 to 8·309)	0·618
Neurologic	-2·208 (-6·747 to 2·331)	0·339	-2·106 (-6·655 to 2·444)	0·363	-2·322 (-7·005 to 2·361)	0·330
Respiratory	-1·383 (-4·996 to 2·230)	0·452	-1·414 (-5·059 to 2·231)	0·446	-1·294 (-5·025 to 2·437)	0·496
Other	-0·106 (-4·796 to 4·585)	0·965	-0·255 (-4·953 to 4·443)	0·915	0·177 (-4·795 to 5·148)	0·944
Infant (age<1y) vs child at randomization	-4·643 (-6·591 to -2·694)	<0·0001	-4·610 (-6·602 to -2·618)	<0·0001	-4·874 (-6·911 to -2·836)	<0·0001
Malignancy vs no malignancy	2·107 (-2·272 to 6·486)	0·345	2·061 (-2·328 to 6·450)	0·357	2·049 (-2·421 to 6·519)	0·368
Syndrome vs no syndrome[b]	1·699 (-1·521 to 4·919)	0·300	1·520 (-1·732 to 4·772)	0·359	1·906 (-1·400 to 5·211)	0·258
PIM3 score (per point added)[c]	0·526 (-0·364 to 1·416)	0·246	0·401 (-0·529 to 1·330)	0·397	0·326 (-0·607 to 1·260)	0·492

PeLOD score first 24 hrs (per point added)[d]	0·000 (-0·117 to 0·117)	0·995	-0·007 (-0·125 to 0·110)	0·906	-0·014 (-0·133 to 0·105)	0·822
Known non-European origin vs other[e]	0·166 (-3·549 to 3·881)	0·930	0·073 (-3·652 to 3·798)	0·969	0·024 (-3·707 to 3·755)	0·990
Known non-Caucasian vs other[e]	-2·306 (-7·355 to 2·743)	0·368	-2·178 (-7·227 to 2·872)	0·396	-2·025 (-7·097 to 3·047)	0·431
Known not exclusive Dutch or English language vs other	1·709 (-1·020 to 4·438)	0·219	1·922 (-0·830 to 4·673)	0·171	1·794 (-0·968 to 4·555)	0·202
Socioeconomic status						
Educational level parents (as compared with level 1)[f]						
Educational level 1·5	-2·778 (-8·831 to 3·274)	0·367	-2·491 (-8·585 to 3·603)	0·422	-2·240 (-8·415 to 3·935)	0·476
Educational level 2	-1·811 (-7·261 to 3·639)	0·514	-1·374 (-6·880 to 4·133)	0·624	-1·536 (-7·121 to 4·049)	0·589
Educational level 2·5	-4·396 (-10·040 to 1·248)	0·126	-4·025 (-9·707 to 1·657)	0·164	-3·934 (-9·700 to 1·803)	0·178
Educational level 3	-4·973 (-10·619 to 0·672)	0·084	-4·540 (-10·228 to 1·149)	0·117	-4·629 (-10·387 to 1·128)	0·115
Educational level unknown	-2·630 (-8·386 to 3·125)	0·369	-2·324 (-8·109 to 3·462)	0·429	-2·354 (-8·182 to 3·474)	0·427
Occupational level parents (as compared with level 1)[g]						
Occupational level 1·5	1·679 (-7·871 to 11·229)	0·730	1·529 (-8·054 to 11·113)	0·754	1·092 (-8·523 to 10·706)	0·824
Occupational level 2	0·238 (-9·230 to 9·706)	0·961	0·149 (-9·361 to 9·659)	0·975	-0·395 (-9·928 to 9·138)	0·935
Occupational level 2·5	-2·341 (-12·174 to 7·492)	0·640	-2·473 (-12·346 to 7·400)	0·623	-2·704 (-12·618 to 7·209)	0·592
Occupational level 3	-0·226 (-9·745 to 7·492)	0·963	-0·300 (-9·840 to 9·239)	0·951	-0·660 (-10·210 to 8·890)	0·892
Occupational level 3·5	1·781 (-8·264 to 11·826)	0·728	1·651 (-8·423 to 11·725)	0·747	1·442 (-8·654 to 11·538)	0·779
Occupational level 4	-1·396 (-11·172 to 8·379)	0·779	-1·547 (-11·359 to 8·265)	0·757	-2·074 (-11·907 to 7·759)	0·679
Occupational level unknown	1·793 (-7·686 to 11·273)	0·710	1·682 (-7·850 to 11·214)	0·729	1·478 (-8·060 to 11·017)	0·761
Parental smoking between birth and PICU admission vs no smoking	0·827 (-1·321 to 2·974)	0·450	0·863 (-1·290 to 3·016)	0·431	0·950 (-1·203 to 3·103)	0·386
New infection vs no new infection			1·333 (-1·910 to 4·576)	0·420	0·436 (0·802 to -2·972)	3·844
Duration of stay in the PICU (per day added)			0·083 (-0·055 to 0·220)	0·237	0·164 (0·279 to -0·134)	0·461
Days with hypoglycemic event (per day added)			-0·049 (-1·813 to 1·714)	0·956	-0·369 (0·708 to -2·298)	1·561
Duration of mechanical ventilatory support (per day added)			-0·094 (-0·276 to 0·088)	0·310	-0·095 (0·340 to -0·291)	0·101
Duration of treatment with antibiotics (per day added)					-0·127 (0·375 to -0·407)	0·154
Duration of hemodynamic support (per day added)					-0·013 (0·898 to -0·215)	0·188
Duration of treatment with corticosteroids (per day added)					0·020 (0·905 to -0·316)	0·357
Duration of treatment with opioids (per day added)					-0·001 (0·996 to -0·284)	0·282
Duration of treatment with benzodiazepines (per day added)					0·173 (0·227 to -0·108)	0·454
Duration of treatment with hypnotics (per day added)					-0·012 (0·931 to -0·286)	0·262
Duration of treatment with alpha-2-agonists (per day added)					-0·183 (0·153 to -0·434)	0·068

For internalizing problems as reported by parents, higher scores reflect more problems.

[a] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.[33]

*[b] A pre-randomization syndrome or illness a priori defined as affecting or possibly affecting neurocognitive development (**Supplemental Method 3**)*

[c] Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.[35]

[d] Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.[34]

[e] Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.[36]

*[f] The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Method 4**).*

*[g] The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Method 4**).[18]*

Abbreviations: PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition.

SUPPLEMENTAL TABLE 2.2 MULTIVARIABLE LINEAR REGRESSION ANALYSES DETERMINING SIGNIFICANT AND INDEPENDENT ASSOCIATIONS BETWEEN RISK FACTORS AND EXTERNALISING PROBLEMS AS REPORTED BY THE PARENTS/CAREGIVERS AT 4 YEARS' FOLLOW-UP WITHIN THE PATIENT POPULATION THAT UNDERWENT NEUROCOGNITIVE TESTING

	Model adjusted for risk factors		Model further adjusted for acute effects of late-PN vs early-PN		Model further adjusted for post-randomization treatments	
Variable	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**
Randomization to late vs· early initiation of PN	***-1·731 (-3·433 to -0·028)***	***0·046***	***-1·645 (-3·379 to 0·090)***	***0·063***	***-1·51 (-3·242 to 0·219)***	***0·086***
Centre						
Leuven vs Edmonton	-3·194 (-11·275 to 4·886)	0·438	-3·337 (-11·463 to 4·790)	0·420	-2·708 (-10·865 to 5·449)	0·51
Rotterdam vs Edmonton	-4·080 (-11·970 to 3·811)	0·310	-4·365 (-12·279 to 3·549)	0·279	-4·168 (-12·145 to 3·809)	0·30
Male vs female sex	1·987 (0·306 to 3·667)	0·021	1·922 (0·235 to 3·609)	0·026	2·058 (0·362 to 3·754)	0·017
Right vs left hand preference	0·299 (-2·443 to 3·041)	0·830	0·284 (-2·452 to 3·020)	0·838	0·526 (-2·236 to 3·287)	0·70
Medium vs high STRONGkids risk level[a]	-1·276 (-4·212 to 1·660)	0·394	-1·211 (-4·189 to 1·766)	0·424	-1·380 (-4·461 to 1·700)	0·37
Diagnostic category (as compared with cardiac surgery)						
Surgical						
Abdominal	-1·137 (-4·542 to 2·268)	0·512	-1·150 (-4·562 to 2·263)	0·508	-1·192 (-4·630 to 4·711)	0·496
Burns	2·862 (-4·331 to 5·632)	0·643	2·764 (9·451 to 14·978)	0·657	1·044 (-11·235 to 13·322)	0·867
Neurosurgery - traumatic brain injury	-0·836 (-9·256 to 14·980)	0·653	-0·807 (-4·459 to 2·846)	0·665	-1·174 (-4·826 to 2·477)	0·527
Thoracic	-0·543 (-4·486 to 2·813)	0·786	-0·545 (-4·479 to 3·390)	0·786	-0·359 (-4·292 to 3·575)	0·857
Transplantation	-4·324 (-4·465 to 3·379)	0·241	-4·343 (-11·742 to 3·056)	0·249	-6·763 (-15·230 to 1·770)	0·119
Orthopedic surgery-trauma	-2·478 (-11·568 to 2·921)	0·385	-2·524 (-8·144 to 3·096)	0·378	-2·685 (-8·333 to -2·964)	0·350
Other	0·650 (-4·331 to 5·632)	0·798	0·453 (-4·575to 5·480)	0·860	-0·359 (-5·428 to 4·711)	0·889
Medical						
Cardiac	0·085 (-5·026 to 5·195)	0·974	-0·210 (-5·378 to 4·957)	0·936	-0·280 (-5·636 to 5·075)	0·918
Gastrointestinal-hepatic	-6·568 (-24·963 to 11·828)	0·482	-6·447 (-24·862 to 11·968)	0·490	-6·618 (-24·971 to 11·734)	0·477
Hematologic-oncologic	-5·302 (-14·848 to 4·245)	0·276	-5·263 (-14·876 to 4·350)	0·283	-7·950 (-18·288 to 2·388)	0·131
Neurologic	-3·142 (-7·219 to 0·934)	0·130	-3·122 (-7·217 to 0·973)	0·135	-3·883 (-8·063 to 0·297)	0·068
Respiratory	-1·308 (-4·669 to 2·052)	0·445	-1·575 (-4·970 to 1·820)	0·362	-1·416 (-4·878 to 2·045)	0·421
Other	-2·207 (-6·608 to 2·195)	0·325	-2·468 (-6·881 to 1·946)	0·273	-2·788 (-7·433 to 1·858)	0·238
Infant (age<1y) vs child at randomization	0·085 (-3·814 to -0·112)	0·038	-1·929 (-3·820 to -0·039)	0·046	-1·930 (-3·864 to 0·003)	0·050
Malignancy vs no malignancy	-1·963 (-1·993 to 6·212)	0·313	2·077 (-2·034 to 6·188)	0·321	1·233 (-2·948 to 5·414)	0·562
Syndrome vs no syndrome[b]	1·045 (-0·206 to 1·471)	0·139	0·861 (-2·032 to 3·754)	0·559	1·754 (-1·173 to 4·682)	0·239
PIM3 score (per point added)[c]	0·632 (-0·118 to 0·098)	0·851	0·567 (-0·303 to 1·438)	0·201	0·497 (-0·373 to 1·367)	0·262
PeLOD score first 24 hrs (per point added)[d]	-0·010 (-5·192 to 1·861)	0·353	-0·010 (-0·119 to 0·099)	0·854	-0·028 (-0·234 to 0·114)	0·498

Known non-European origin vs other[e]	-1·665 (-4·125 to 5·649)	0·758	-1·591 (5·129 to 1·946)	0·377	-3·444 (-8·677 to 1·789)	0·196
Known non-Caucasian vs other[e]	0·762 (-0·483 to 4·868)	0·108	0·824 (-4·064 to 5·713)	0·739	0·165 (-6·148 to 6·479)	0·958
Known not exclusive Dutch or English language vs other	2·192 (-3·814 to -0·112)	0·038	2·243 (-0·441 to 4·926)	0·101	-2·959 (-6·951 to 1·033)	0·146
Socioeconomic status						
Educational level parents (as compared with level 1)[f]						
Educational level 1·5	-0·738 (-6·223 to 4·747)	0·792	-0·787 (-6·313 to 4·739)	0·780	0·169 (-5·412 to 5·751)	0·952
Educational level 2	0·941 (-3·973 to 5·855)	0·707	1·121 (-3·845 to 6·087)	0·657	1·720 (-3·280 to 6·719)	0·499
Educational level 2·5	-2·784 (-7·912 to 2·345)	0·286	-2·608 (-7·774 to 2·557)	0·321	-2·013 (-7·196 to 3·171)	0·445
Educational level 3	-4·599 (-9·875 to 0·678)	0·087	-4·448 (-9·759 to 0·862)	0·100	-3·841 (-9·179 to 1·497)	0·157
Educational level unknown	-1·506 (-6·824 to 3·813)	0·578	-1·378 (-6·726 to 3·970)	0·612	-0·718 (-6·076 to 4·640)	0·792
Occupational level parents (as compared with level 1)[g]						
Occupational level 1·5	-2·079 (-10·773 to 6·615)	0·639	-2·325 (-11·046 to 6·397)	0·601	-3·048 (-11·746 to 5·650)	0·492
Occupational level 2	0·088 (-8·613 to 8·789)	0·984	-0·177 (-8·922 to 8·568)	0·968	-0·847 (-9·576 to 7·883)	0·848
Occupational level 2·5	-2·387 (-11·413 to 6·639)	0·604	-2·712 (-11·774 to 6·351)	0·557	-3·471 (-12·537 to 5·595)	0·452
Occupational level 3	0·527 (-8·275 to 9·329)	0·906	0·344 (-8·483 to 9·171)	0·939	-0·141 (-8·941 to 8·660)	0·974
Occupational level 3·5	-0·920 (-10·204 to 8·365)	0·846	-1·190 (-10·505 to 8·125)	0·802	-1·568 (-10·882 to 7·746)	0·740
Occupational level 4	-1·592 (-10·639 to 7·454)	0·730	-1·904 (-10·989 to 7·181)	0·681	-2·735 (-11·812 to 6·341)	0·554
Occupational level unknown	-0·963 (-9·764 to 7·838)	0·830	-1·260 (-10·107 to 7·587)	0·780	-1·575 (-10·400 to 7·251)	0·726
Parental smoking between birth and PICU admission vs· no smoking	1·797(-0·309 to 3·903)	0·094	1·781 (-0·332 to 3·893)	0·098	1·895 (-0·203 to 3·993)	0·076
New infection vs no new infection			-0·648 (-3·674 to 2·378)	0·674	-2·044 (-5·248 to 1·160)	0·210
Duration of stay in the PICU (per day added)			0·046 (-0·077 to 0·170)	0·461	0·086 (-0·187 to 0·359)	0·536
Days with hypoglycemic event (per day added)			-0·231 (-1·904 to 1·441)	0·786	-0·449 (-2·265 to 1·367)	0·627
Duration of mechanical ventilatory support (per day added)			-0·008 (-0·174 to 0·158)	0·924	-0·057 (-0·237 to 0·122)	0·527
Duration of treatment with antibiotics (per day added)					-0·096 (-0·355 to 0·163)	0·466
Duration of hemodynamic support (per day added)					0·017 (-0·168 to 0·202)	0·859
Duration of treatment with corticosteroids (per day added)					0·244 (-0·077 to 0·566)	0·135
Duration of treatment with opioids (per day added)					-0·015 (-0·266 to 0·236)	0·906
Duration of treatment with benzodiazepines (per day added)					0·176 (-0·073 to 0·425)	0·646
Duration of treatment with hypnotics (per day added)					0·214 (-0·038 to 0·466)	0·095
Duration of treatment with alpha-2-agonists (per day added)					-0·273 (-0·504 to -0·043)	0·020

For internalizing problems as reported by parents, higher scores reflect more problems.

[a] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.[33]

*[b] A pre-randomization syndrome or illness a priori defined as affecting or possibly affecting neurocognitive development (**Supplemental Method 3**)*

[c] Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.[35]

[d] Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.[34]

[e] Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.[36]

*[f] The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Method 4**).*

*[g] The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Method 4**).[18]*

Abbreviations: PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition.

SUPPLEMENTAL TABLE 2.3 MULTIVARIABLE LINEAR REGRESSION ANALYSES DETERMINING SIGNIFICANT AND INDEPENDENT ASSOCIATIONS BETWEEN RISK FACTORS AND TOTAL EMOTIONAL AND BEHAVOURIAL PROBLEMS AS REPORTED BY THE PARENTS/CAREGIVERS AT 4 YEARS' FOLLOW-UP WITHIN THE PATIENT POPULATION THAT UNDERWENT NEUROCOGNITIVE TESTING

	Model adjusted for risk factors		Model further adjusted for acute effects of late-PN vs early-PN		Model further adjusted for post-randomization treatment effects	
Variable	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**	**β estimate (95% CI)**	**P-value**
Randomization to late vs early initiation of PN	***-2·442 (-3·433 to -0·028)***	***0·046***	***-2·242 (***-4·043 to -0·4043)	***0·015***	***-2·163 (-3·960 to -0·365)***	***0·019***
Centre						
Leuven vs Edmonton	-2·834 (-11·275 to 4·886)	0·438	-2·687 (-11·115 to 5·742)	0·531	-2·172 (-10·667 6·324)	0·616
Rotterdam vs Edmonton	-2·360 (-11·970 to 3·811)	0·310	-2·476 (-10·732 to 5·742)	0·556	-2·506 (-10·848 to 5·837)	0·555
Male vs female sex	1·483 (0·306 to 3·667)	0·021	1·411 (-0·360 to 3·183)	0·118	1·558 (-0·224 to 3·339)	0·086
Right vs left hand preference	0·019 (-2·443 to 3·041)	0·830	-0·067 (-2·865 to 2·731)	0·962	0·190 (-2·625 to 3·004)	0·894
Medium vs high STRONGkids risk level[a]	-2·630 (-4·212 to 1·660)	0·394	-2·369 (-5·452 to 0·713)	0·132	-2·445 (-5·610 to 0·721)	0·130
Diagnostic category (as compared with cardiac surgery)						
Surgical						
Abdominal	-0·652 (-4·542 to 2·268)	0·512	-0·679 (-4·249 to 2·891)	0·709	-0·703 (-4·301 to 2·895)	0·701
Burns	1·540 (-9·256 to 14·980)	0·643	0·682 (-12·001 to 13·365)	0·916	-0·229 (-12·993 to 12·536)	0·972
Neurosurgery - traumatic brain injury	0·503 (-4·486 to 2·813)	0·653	0·433 (-3·290 to 4·157)	0·819	0·086 (-3·633 to 3·805)	0·964
Thoracic	-1·323 (-4·465 to 3·379)	0·786	-1·354 (-5·435 to 2·727)	0·515	-1·091 (-5·197 to 3·015)	0·602
Transplantation	-2·856 (-11·568 to 2·921)	0·241	-3·506 (-11·188 to 4·176)	0·370	-4·678 (-13·497 to 4·141)	0·298
Orthopedic surgery-trauma	-0·542 (-8·086 to 3·129)	0·385	-0·778 (-6·692 to 5·137)	0·796	-0·892 (-6·840 to 5·056)	0·768
Other	0·705 (-4·331 to 5·632)	0·798	0·379 (-4·881 to 5·640)	0·887	-0·426 (-5·742 to 4·891)	0·875
Medical						
Cardiac	-1·239 (-5·026 to 5·195)	0·974	-1·771 (-7·124 to 3·582)	0·516	-1·964 (-7·501 to 3·573)	0·486
Gastrointestinal-hepatic	-5·844 (-24·963 to 11·828)	0·482	-5·716 (-24·994 to 13·563)	0·559	-5·919 (-25·143 to 13·305)	0·544
Hematologic-oncologic	-5·495 (-14·848 to 4·245)	0·276	-5·668 (-15·844 to 4·507)	0·274	-7·908 (-18·814 to 2·998)	0·155
Neurologic	-4·072 (-7·219 to 0·934)	0·130	-3·963 (-8·229 to 0·303)	0·069	-4·661 (-9·032 to -0·289)	0·037
Respiratory	-2·226 (-4·669 to 2·052)	0·445	-2·311 (-5·835 to 1·214)	0·198	-2·282 (-5·887 to 1·323)	0·214
Other	-2·103 (-6·608 to 2·195)	0·325	-2·285 (-6·863 to 2·293)	0·327	-2·352 (-7·170 to 2·466)	0·338
Infant (age<1y) vs child at randomization	-4·487 (-3·814 to -0·112)	0·038	-4·406 (-6·347 to -2·464)	<0·0001	-4·536 (-6·517 to -2·553)	<0·0001
Malignancy vs no malignancy	3·260 (-1·993 to 6·212)	0·313	3·273 (-0·978 to 7·524)	0·131	2·827 (-1·492 to 7·145)	0·199
Syndrome vs no syndrome[b]	2·110 (-1·819 to 3·909)	0·474	1·892 (-1·198 to 4·983)	0·229	2·610 (-0·519 to 5·738)	0·102
PIM3 score (per point added)[c]	0·582 (-0·206 to 1·471)	0·139	0·450 (-0·451 to 1·351)	0·327	0·375 (-0·527 to 1·276)	0·414
PeLOD score first 24 hrs (per point added)[d]	-0·007 (-0·118 to 0·098)	0·851	-0·014 (-0·128 to 0·100)	0·807	-0·029 (-0·144 to 0·086)	0·615

Known non-European origin vs other[e]	-0·459 (-5·192 to 1·861)	0·353	-0·513 (-4·143 to 3·118)	0·781	-0·575 (-4·201 to 3·050)	0·755
Known non-Caucasian vs other[e]	-0·943 (-4·125 to 5·649)	0·758	-0·850 (-5·866 to 4·166)	0·738	-0·582 (-5·609 to 4·445)	0·819
Known not exclusive Dutch or English language vs other	1·987 (-0·483 to 4·868)	0·108	2·172 (-0·532 to 4·876)	0·115	2·133 (-0·572 to 4·837)	0·122
Socioeconomic status						
Educational level parents (as compared with level 1)[f]						
Educational level 1·5	-1·687 (-6·223 to 4·747)	0·792	-1·543 (-7·337 to 4·252)	0·601	-0·819 (-6·677 to 5·040)	0·784
Educational level 2	-0·445 (-3·973 to 5·855)	0·707	-0·095 (-5·296 to 5·106)	0·971	0·126 (-5·125 to 5·377)	0·962
Educational level 2·5	-4·285 (-7·912 to 2·345)	0·286	-3·979 (-9·365 to 1·407)	0·147	-3·577 (-9·000 to 1·846)	0·195
Educational level 3	-5·469 (-9·875 to 0·678)	0·087	-5·138 (-10·638 to 0·362)	0·067	-4·864 (-10·405 to 0·677)	0·085
Educational level unknown	-2·716 (-6·824 to 3·813)	0·578	-2·467 (-8·024 to 3·091)	0·383	-2·127 (-7·708 to 3·453)	0·453
Occupational level parents (as compared with level 1)[g]						
Occupational level 1·5	1·882 (-10·773 to 6·615)	0·639	1·757 (-7·374 to 10·888)	0·706	1·105 (-8·007 to 10·217)	0·812
Occupational level 2	1·570 (-8·613 to 8·789)	0·984	1·513 (-7·619 to 10·644)	0·745	0·781 (-8·335 to 9·897)	0·866
Occupational level 2·5	-0·537 (-11·413 to 6·639)	0·604	-0·636 (-10·101 to 8·829)	0·895	-1·220 (-10·689 to 8·248)	0·800
Occupational level 3	1·799 (-8·275 to 9·329)	0·906	1·736 (-7·453 to 10·925)	0·711	1·242 (-7·921 to 10·405)	0·790
Occupational level 3·5	1·154 (-10·204 to 8·365)	0·846	1·011 (-8·701 to 10·723)	0·838	0·678 (-9·025 to 10·381)	0·891
Occupational level 4	0·701 (-10·639 to 7·454)	0·730	0·546 (-8·937 to 10·028)	0·910	-0·254 (-9·721 to 9·214)	0·958
Occupational level unknown	1·571 (-9·764 to 7·838)	0·830	1·481 (-7·699 to 10·661)	0·751	1·142 (-8·012 to 10·296)	0·806
Parental smoking between birth and PICU admission vs· no smoking	1·450 (-0·309 to 3·904)	0·094	1·451 (-0·677 to 3·579)	0·181	1·571 (-0·547 to 3·689)	0·146
New infection vs no new infection			1·215 (-1·941 to 4·370)	0·450	-0·185 (-3·501 to 3·131)	0·913
Duration of stay in the PICU (per day added)			0·057 (-0·071 to 0·186)	0·380	0·105 (-0·180 to 0·389)	0·471
Days with hypoglycemic event (per day added)			-0·343 (-2·051 to 1·366)	0·694	-0·517 (-2·380 to 1·346)	0·586
Duration of mechanical ventilatory support (per day added)			-0·046 (-0·218 to 0·127)	0·601	-0·080 (-0·266 to 0·107)	0·401
Duration of treatment with antibiotics (per day added)					-0·115 (-0·386 to 0·155)	0·402
Duration of hemodynamic support (per day added)					-0·017 (-0·209 to 0·174)	0·858
Duration of treatment with corticosteroids (per day added)					0·166 (-0·161 to 0·493)	0·320
Duration of treatment with opioids (per day added)					-0·054 (-0·317 to 0·210)	0·690
Duration of treatment with benzodiazepines (per day added)					0·277 (0·011 to 0·543)	0·041
Duration of treatment with hypnotics (per day added)					0·134 (-0·129 to 0·398)	0·316
Duration of treatment with alpha-2-agonists (per day added)					-0·281 (-0·523 to -0·039)	0·023

For total emotional and behavioral problems as reported by parents, higher scores reflect more problems.

[a] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.[33]

[b] *A pre-randomization syndrome or illness a priori defined as affecting or possibly affecting neurocognitive development (**Supplemental Method 3**)*
[c] *Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.[35]*
[d] *Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.[34]*
[e] *Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.[36]*
[f] *The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Method 4**).*
[g] *The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Method 4**).[18]*
Abbreviations: PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition.

SUPPLEMENTAL TABLE 3 COMPARISON OF PATIENTS RANDOMISED TO LATE-PN DURING PICU STAY WITH HEALTHY CONTROL CHILDREN FOR THE TESTS SIGNIFICANTLY AFFECTED BY THE RANDOMISED INTERVENTION

Neurocognitive testing	P-value
Internalizing problems as reported by parents/caregivers	0·103
Externalizing problems as reported by parents/caregivers	0·313
Total behavioral and emotional problems as reported by parents/caregivers	0·085

SUPPLEMENTAL TABLE 4 DEMOGRAPHICS, POST-RANDOMISATION TREATMENTS IN THE PICU, AND ACUTE OUTCOMES OF PATIENTS FOR WHOM DNA METHYLATION STATUS WAS ANALYSED ON THE LAST DAY OF PICU STAY

	Tested post-PICU population	
	Early-PN N=192	**Late-PN N=211**
Demographics		
Age at 4-years' follow-up - yr	7·6 (4·2)	7·5 (4·1)
Sex		
Male	111 (57·8%)	125 (59·2%)
Female	81 (42·2%)	86 (40·8%)
Known non-Caucasian race[a]	13 (6·8%)	7 (3·3%)
Known non-European origin[a]	41 (21·4%)	30 (14·2%)
Known not exclusive Dutch or English language	49 (25·5%)	49 (23·2%)
Socioeconomic status		
Educational level parents[b]		
Educational level 1	6 (3·1%)	11 (5·2%)
Educational level 1·5	17 (8·9%)	12 (5·7%)
Educational level 2	43 (22·4%)	53 (25·1%)
Educational level 2·5	27 (14·1%)	34 (16·1%)
Educational level 3	55 (28·7%)	60 (28·4%)
Educational level unknown	44 (22·9%)	41 (19·4%)
Occupational level parents[c]		
Occupational level 1	0 (0·0%)	5 (2·4%)
Occupational level 1·5	18 (9·4%)	27 (12·8%)
Occupational level 2	33 (17·2%)	35 (16·6%)
Occupational level 2·5	19 (9·9%)	16 (7·6%)
Occupational level 3	34 (17·7%)	45 (21·3%)
Occupational level 3·5	14 (7·3%)	14 (6·6%)
Occupational level 4	31 (16·2%)	37 (17·5%)
Occupational level unknown	43 (22·4%)	32 (15·2%)
Patient characteristics upon PICU admission		
Infant (age<1y) at randomization	71 (37·0%)	91 (43·1%)
STRONGkids risk level[d]		
Medium	184 (95·8%)	196 (92·9%)
High	8 (4·2%)	15 (7·1%)
PeLOD score, first 24h in PICU [e]	23·7 (10·3)	24·1 (10·4)
PIM3 score[f]	-3·6 (1·3)	-3·7 (1·1)
PIM3 probability of death - % [g]	6·1 (10·9)	4·5 (8·0)
Diagnostic category		
Surgical		
Abdominal	7 (3·7%)	6 (2·8%)
Burns	1 (0·5%)	0 (0·0%)
Cardiac	109 (56·8%)	123 (58·3%)

Neurosurgery-Traumatic brain injury	17 (8·9%)	19 (9·0%)
Thoracic	9 (4·7%)	11 (5·2%)
Transplantation	0 (0·0%)	4 (1·9%)
Orthopedic surgery-Trauma	10 (5·2%)	5 (2·4%)
Other	5 (2·6%)	8 (3·8%)
Medical		
Cardiac	5 (3·7%)	5 (2·4%)
Gastrointestinal-Hepatic	1 (0·5%)	1 (0·5%)
Oncologic-Hematologic	0 (0·0%)	2 (1·0%)
Neurologic	13 (6·8%)	10 (4·7%)
Respiratory	8 (4·2%)	8 (3·8%)
Other	7 (3·7%)	9 (4·3%)
Malignancy	14 (7·3%)	12 (5·7%)
Diabetes	0 (0·0%)	0 (0·0%)
Syndrome[h]	15 (7·8%)	23 (10·9%)
Known parental smoking between birth and PICU admission	31 (16·1%)	42 (19·9%)
Duration of stay in the PICU – days	5·6 (11·7)	3·6 (3·5)
Patients who acquired a new infection in PICU	23 (12·0%)	9 (4·3%)
Duration of mechanical ventilatory support – days	3·1 (5·7)	2·2 (2·6)
Number of days with hypoglycemia <40 mg/dl – days	0·1 (0·6)	0·2 (0·5)
Post-randomization treatments effects		
Duration of antibiotic treatment – days	3·7 (8·9)	2·1 (3·0)
Duration of hemodynamic support – days	2·4 (8·4)	1·6 (2·8)
Duration of treatment with opioids – days	4·6 (9·8)	2·9 (3·1)
Duration of treatment with benzodiazepines – days	3·1 (7·9)	1·6 (2·4)
Duration of treatment with hypnotics – days	1·1 (5·9)	0·5 (1·0)
Duration of treatment with alpha-2-agonists – days	0·3 (1·6)	0·1 (1·1)
Duration of treatment with corticosteroids - days	0·8 (1·9)	0·5 (1·8)

Data are n (%) or mean (SD).

[a] Participants were classified according to race and geographical origin by the investigators. These classifications were performed to capture ethnical and regional differences in the frequency of consanguinity, which may adversely affect cognitive performance.[33]

*[b] The education level is the average of the paternal and maternal educational level, and calculated based upon the 3-point scale subdivisions as made by the Algemene Directie Statistiek (Belgium; statbel.fgov.be/nl/) and the Centraal Bureau voor de Statistiek (The Netherlands; statline.cbs.nl): Low (=1), middle (=2) and high (=3) educational level (**Supplemental Method 4**).*

*[c] The occupation level is the average of the paternal and maternal occupation level, which is calculated based upon the International Isco System 4-point scale for professions (**Supplemental Method 4**).*

[d] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1 to 3 indicating medium risk, and a score of 4 to 5 indicating high risk.[33]

[e] Pediatric Logistic Organ Dysfunction (PeLOD) scores range from 0 to 71, with higher scores indicating more severe illness.[34]

[f] Pediatric Index of Mortality 3 (PIM3) scores, with higher scores indicating a higher risk of mortality.[35]

[g] Pediatric Index of Mortality 3 (PIM3) probability of death, ranging from 0% to 100%, with higher percentages indicating a higher probability of death in PICU.

*[h] A pre-randomization syndrome or illness a priori defined as affecting or possibly affecting neurocognitive development (**Supplemental Method 3**).*

Abbreviations: BMI, body mass index; PeLOD, pediatric logistic organ dysfunction score; PICU, pediatric intensive care unit; PIM3, pediatric index of mortality 3 score; PN, parenteral nutrition; SD, standard deviation.

SUPPLEMENTAL TABLE 5 ADDED EXPLANATORY POWER PROVIDED BY THE CPG-SITES DIFFERENTIALLY METHYLATED BY EARLY-PN IN PREDICTING THE NEUROCOGNITIVE OUTCOMES ADVERSELY AFFECTED BY EARLY-PN

	Non-linear regression models			
	Without randomized intervention		With randomized intervention	
Outcome assessed at 4-years follow-up	**R^2** [a]	**Fold-increase in R^2** [b]	**R^2** [c]	**Fold-increase in R^2** [d]
Emotional/Behavioral: Internalizing problems as reported by parents/caregivers	0·618	2·026	0·619	1·851
Emotional/Behavioral: Externalizing problems as reported by parents/caregivers	0·603	1·813	0·603	1·710
Emotional/Behavioral: Total problems as reported by parents/caregivers	0·613	1·868	0·614	1·771

[a] Optimism-corrected R^2 of multivariable non-linear random forest regression models for outcomes assessed at 4-years follow-up including 37 differentially methylated CpG-sites and baseline risk factors.

[b] Optimism-corrected R^2 fold-increase with respect to multivariable non-linear regression models including baseline risk factors only.

[c] Optimism-corrected R^2 of multivariable non-linear random forest regression models for outcomes assessed at 4-years follow-up including 37 differentially methylated CpG-sites, baseline risk factors and the randomized intervention.

[d] Optimism-corrected R^2 fold-increase with respect to multivariable non-linear random forest regression models only including baseline risk factors and the randomized intervention.

*All models were evaluated in 100 bootstrapped replicates. Baseline risk factors included age, center, race,[36] gender, geographic origin,[36] language, history of malignancy, diabetes, a predefined "syndrome" (**Supplemental Method 3**), diagnosis and severity of illness (PIM3 and PeLOD),[34,35] and risk of malnutrition (STRONGkids)].[33] R^2 for non-linear models computed according to Rubin's rules.[37]*

Abbreviations: PN, parenteral nutrition.

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CHAPTER 6 - INTERNATIONAL SURVEY OF DE-IMPLEMENTATION OF INITIATING PARENTERAL NUTRITION EARLY IN PEDIATRIC INTENSIVE CARE UNITS

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1. ABSTRACT

Background: Initiating parenteral nutrition (PN) within 24 hours in critically ill children is inferior to withholding PN during the first week, as was found in the PEPaNIC study. The aims of this study were to investigate de-implementation of early initiation of PN at PICUs worldwide, and to identify factors influencing de-implementation.

Methods: A cross-sectional online survey was conducted (May – October 2017), consisting of 41 questions addressing current PN practices, the degree of de-implementation, and factors affecting de-implementation.

Results: We analyzed 81 responses from 39 countries. Of these 81 respondents, 53 (65%) were aware of the findings of the PEPaNIC study, and 43 (53%) have read the article. In these 43 PICUs, PN was completely withheld during the first week in 10 PICUs, of which 5 already withheld PN (12%), and 5 de-implemented early initiation of PN (12%). Partial de-implementation was reported by 17 (40%) and no de-implementation by 16 (37%). Higher de-implementation rates were observed when the interpreted level of evidence and grade of recommendation of PEPaNIC was high. Predominant reasons for retaining early initiation of PN were concerns on withholding amino acids, the safety in undernourished children and neonates, and the long-term consequences. Furthermore, the respondents were waiting for updated guidelines.

Conclusions: One year after the publication of the PEPaNIC trial, only two-thirds of the respondents was aware of the study results. Within this group, early initiation of PN was de-implemented completely in 12% of the PICUs, while 40% asserted partial de-implementation. Increasing the awareness, addressing the intervention-specific questions and more frequently revising international guidelines might help to accelerate de-implementation of ineffective, unproven or harmful healthcare.

2. BACKGROUND

Optimal nutrition is considered essential to improve outcome in the pediatric intensive care unit (PICU) but large well-designed randomized, controlled trials (RCTs) with clinically relevant outcome measures are lacking.(1, 2) The limited evidence leads to a wide variation in nutritional practices between individual intensivists, PICUs and countries. This variation includes timing of and thresholds for the initiation of parenteral nutrition (PN), as measured by a worldwide survey with a point-prevalence.(3) According to this survey completed in 2014, in 20% of the PICUs, PN was initiated within 24 hours after admission, and in 55% of the PICUs within 48 hours.(3) The international guidelines at that time were based on small studies with surrogate outcome measures, observations, and expert opinion, and could not provide clear recommendations on the timing of initiating PN in critically ill children.(4, 5) In 2016, the results of the large, international, multicenter, RCT 'PEPaNIC' (Pediatric Early versus Late PN in Critically Illness) were published.(6) This RCT showed that administering PN within 24 hours after PICU admission (Early-PN; the standard therapy) was clinically inferior to withholding PN during the first week of PICU admission (Late-PN).(6) Withholding PN during the first week prevented new infections, shortened intensive care dependency, the duration of mechanical ventilation and hospital stay. Based on the impact of these findings, and the scarcity of evidence for the early use of PN in PICUs, one could expect that currently, initiation of supplemental PN is delayed until after the first week of critical illness in the majority of PICUs.

De-implementation or de-adoption is described as 'reducing or stopping low-value, ineffective, harmful or unproven care'.(7-9) However, rational and quantitative evidence are only part of the driving forces for decision making and only 49% of the interventions is supported or contradicted by the available evidence.(7, 10) Little is known about the factors that influence the extend and pace of de-implementation.(8, 11) Moreover, currently, only 10% of the de-implementation research has focused on pediatric healthcare.(9)

In this study, we explored the degree of early de-implementation of initiating PN in the first week in PICUs and barriers for de-implementation with a survey among physicians and dieticians across PICUs worldwide.

3. METHODS

This electronic (LimeSurvey GmbH version 2.06lts) cross-sectional survey was conducted between May and October 2017. It consisted of 41 questions and was provided in English, French and Spanish. The full questionnaire used for this survey can be found as supplement to this article (**Supplemental Method 1**). In brief, the survey was developed to collect information in different echelons. The first part collected general information of the respondents and responding PICUs, the second part focused on the current practice of PN in the responding PICU, and the third part investigated the awareness of the results of the PEPaNIC trial. Subsequently, the respondents who had read the findings of this study prior to our survey were requested to participate in the final part of the survey in which they were asked to grade the quality of evidence of the PEPaNIC trial according to the Scottish Intercollegiate Guidelines Network (SIGN) system that was provided in the survey.(12) Finally, they were asked whether and how the PEPaNIC results has changed the current practice of initiating PN in their PICU, and which factors have influenced the degree of de-implementation in their PICU.

The survey was piloted by independent clinicians in two different centers (Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands and the University Hospital of Leuven, Belgium) to test the clarity, relevance and clinical sensibility of the questionnaire, and the questionnaire was adapted accordingly. Data from this pilot were not included in the final analyses and survey results. The survey was electronically distributed among members of the World Federation of Pediatric Intensive and Critical Care Societies (WFPICCS) by newsletter and Twitter, and to specific members of the European Society of Pediatric and Neonatal Intensive Care (ESPNIC). Reminders were sent three times with six-week intervals. If more than one questionnaire was present for a PICU, the answers were weighed by the inverse of the number of completed questionnaires per center in order to process conflicting statements within one PICU, without disrupting the weight of the answers per PICU.

Main outcome was the degree of de-implementation (fidelity), with complete de-implementation defined as withholding PN until day 8 of PICU admission. Partial de-implementation was defined as postponed initiation of PN (but still initiated prior to day 8 in PICU) and/or decreased amount of PN as compared with nutritional practices before the results from PEPaNIC, or only administering PN during the first week in specific patient groups. Secondary outcomes were supporting factors and barriers for de-implementation.

Statistical analysis was performed using IBM SPSS statistics version 24. All answers were categorical, and were expressed as numbers and proportions.

4. RESULTS

Response

Since the survey was distributed via Twitter, ESPNIC and WFPICCS, with unknown number of PICUs in their databases, the exact number of invited PICUs is unknown. A total of 88 completed questionnaires were received, of which one was removed because the respondent worked in a Neonatal Intensive Care Unit.

TABLE 1 CHARACTERISTICS OF THE RESPONDING PEDIATRIC INTENSIVE CARE UNITS

Characteristic	No. of PICUs (n=81)
Continent	
Europe	39 (48%)
South America	14 (17%)
Asia	12 (15%)
North America	12 (15%)
Africa	2 (3%)
Oceania	2 (3%)
Hospital type	
University children's hospital	37 (46%)
University hospital	24 (30%)
General hospital	18 (21%)
Other	2 (3%)
Type of PICU	
Multidisciplinary/mixed	75 (93%)
Medical	4 (5%)
Cardiac	1 (1%)
Surgical	1 (1%)
Combination of PICU	
Not combined	66 (82%)
With neonatal ICU	10 (12%)
With adult ICU	4 (5%)
With adult and neonatal ICU	1 (1%)
Size of PICU	
1-10 beds	33 (41%)
11-20 beds	28 (35%)
21-30 beds	16 (50%)
>30 beds	4 (6%)
Paediatric admissions (patients/year)	
1-250	7 (9%)
251-500	29 (36%)
501-750	18 (22%)
751-1000	7 (9%)
1001-1250	7 (9%)
>1250	13 (16%)
Mechanically ventilated patients	
<25 %	9 (11%)
25-50 %	31 (38%)
50-75 %	25 (31%)
>75 %	16 (20%)

PICU = pediatric intensive care unit; ICU = intensive care unit

From the remaining 87 questionnaires, the answers of nine respondents from three centers needed to be pooled per center by weighing the answers according to the number of completed questionnaires per center. The 3 pooled responses per center were kept for analyses, and the individual responses were removed (**Figure 1**). Finally, responses from 81 PICUs in 39 countries on 6 continents were analyzed (**Figure 2**). Of the respondents, 74% were (pediatric) intensivists, 12% were dieticians or nutritionists, 6% were pediatricians, 5% were nurses or nurse practitioners, and 3% were anesthesiologists.

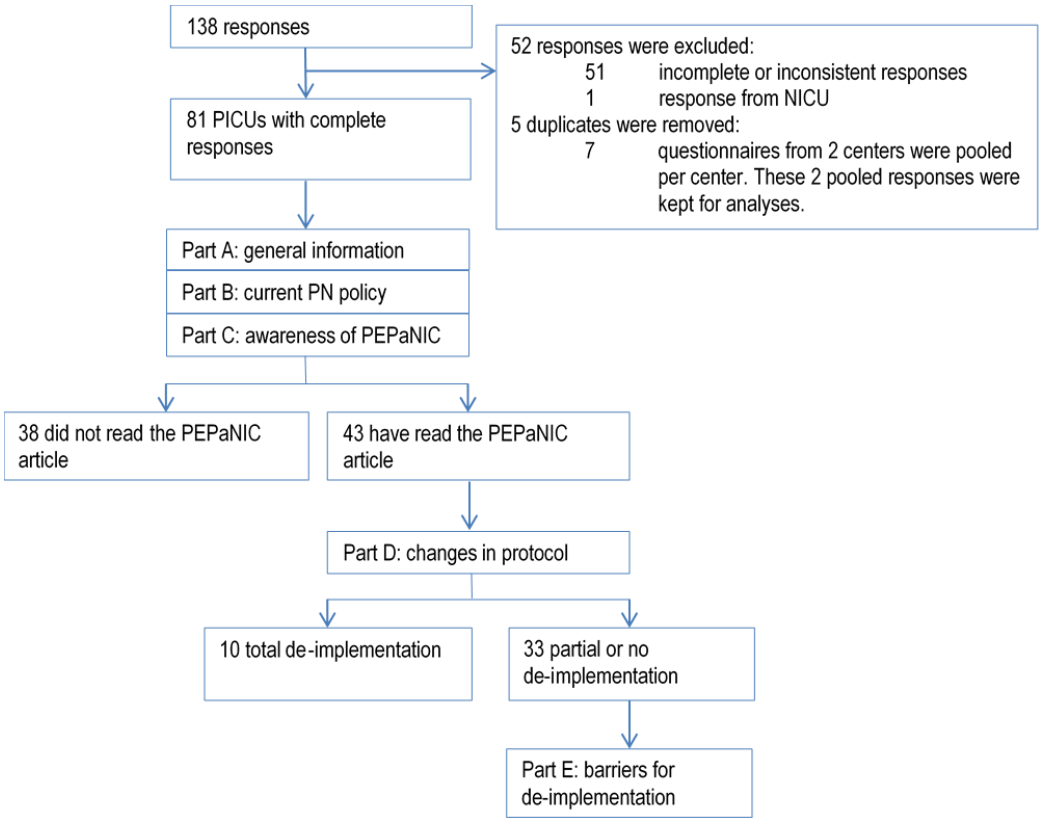

FIGURE 1 FLOWCHART OF THE RESPONSES AND BUILD-UP OF THE SURVEY
NICU = neonatal intensive care unit; PN = parenteral nutrition

Of the responding PICUs, 39 (48%) were located in Europe, 14 (17%) in South America, 12 (15%) in North America, and 12 (15%) in Asia (**Table 1**). The majority of the PICUs had 251-750 pediatric admissions per year (**Table 1**). All PICU demographics are displayed in **Table 1**.

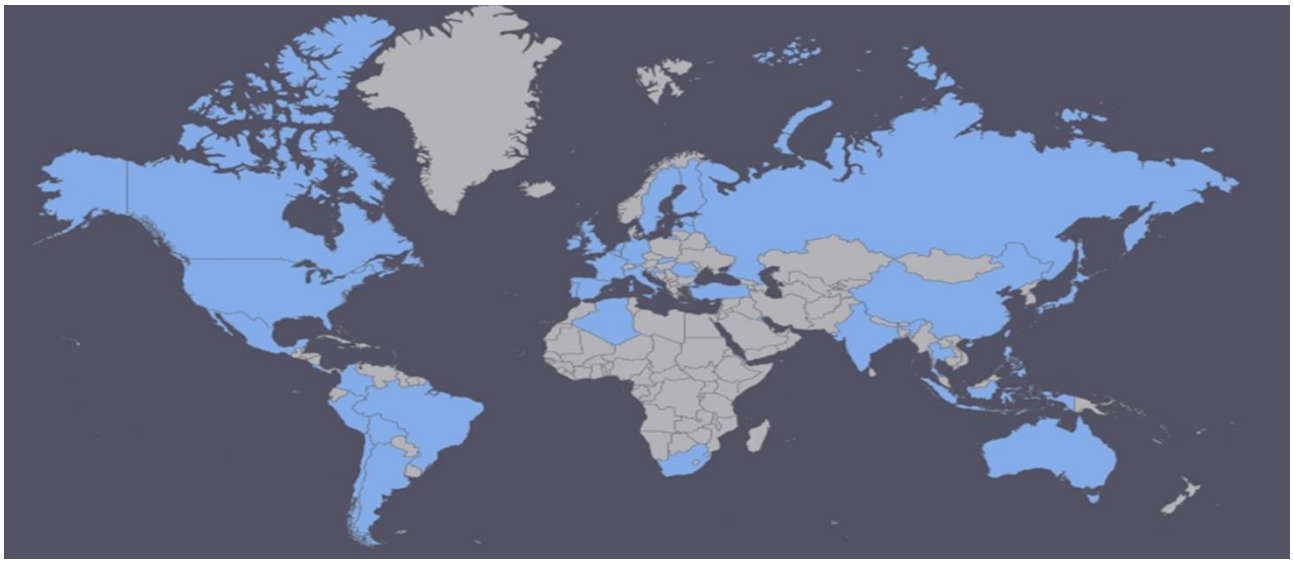

FIGURE 2 PARTICIPATING PICUS: 81 RESPONSES FROM 39 COUNTRIES (IN BLUE), COVERING SIX CONTINENTS
Created with: https://www.amcharts.com/visited_countries/

Current PN practices in PICUs

In 50 of the 81 PICUs (62%), a nutritional protocol regarding PN was present. Most of the protocols were based on international guidelines (27 of 50, 54%), 8 of 50 (16%) on national guidelines, and 15 of 50 (30%) on the opinion of the staff. Respondents from 10 of the 81 PICUs (12%) would always start PN if enteral nutrition (EN) is insufficient, and 4 (5%) would never start PN. In 43 of the 81 PICUs (53%), supplemental PN would be started if enteral nutrition covered less than 80% of the target goals, at 20 (25%) of the PICUs if EN covered less than 50%, and 4 (5%) of the PICUs handled another threshold. PN administration via peripheral intravenous access was possible in 58 of the 81 PICUs (72%).

Regarding the timing of PN initiation, amino acids would be started within 48 hours when a child was (expected to be) intolerable to EN in 37 of the 81 PICUs (46%). Initiation of amino acids was postponed beyond the first week in 4 of the 81 PICUs (5%; **Figure 3**). Lipids would be started within 48 hours in 34 of the 81 PICUs (42%; **Figure 3**). Lipids would be initiated beyond the first week in 4 of the 81 PICUs (5%; **Figure 3**). Targeted glucose intake during the first 12-24 hours varied between 1-4 mg/kg/min and 8-10 mg/kg/min. In most cases, 4-6 mg/kg/min was targeted in children who weighed less than 10 kilograms (38 of 81 PICUs, 47%), 1-4 mg/kg/min in children who weighed 10-30 kilograms (50 of 81 PICUs, 62%) and also in children weighing more than 30 kilograms (62 of 81 PICUs, 77%). Of the 81 respondents, 73 (90%) would administer vitamins and trace elements routinely.

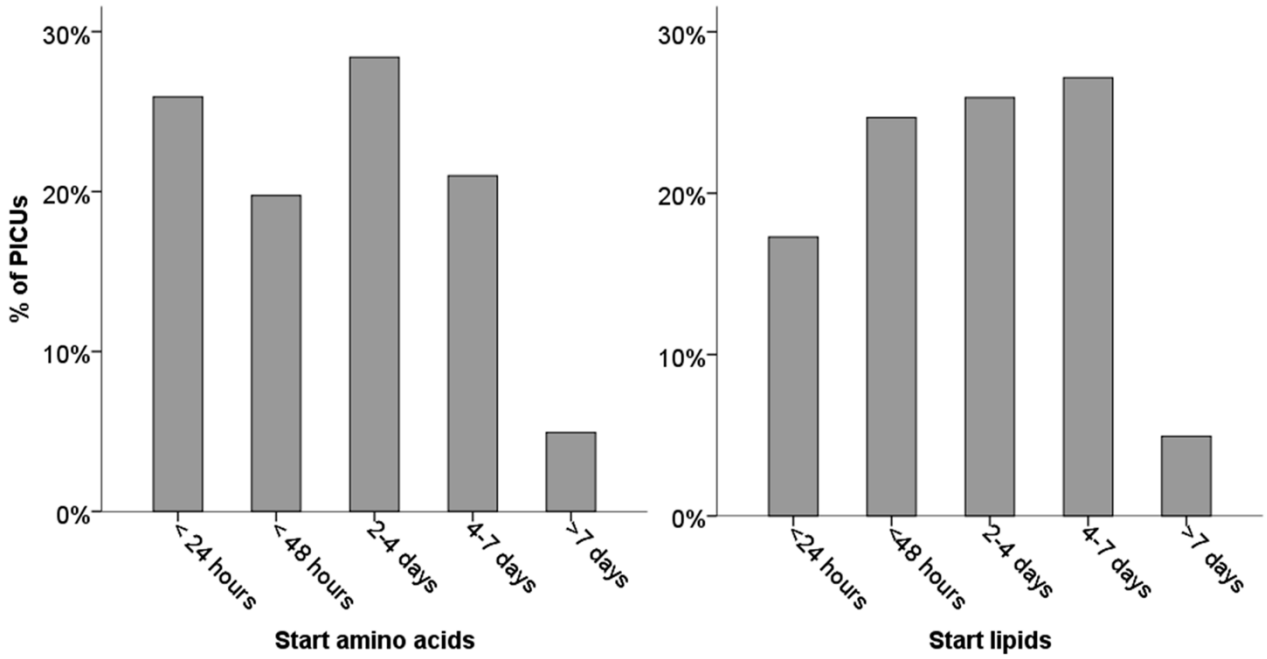

FIGURE 3 TIME TO INITIATE PARENTERAL NUTRITION WHEN ENTERAL NUTRITION IS (EXPECTED TO BE) INSUFFICIENT
PICU = pediatric intensive care unit

De-implementation of initiating PN early during critical illness

Fifty-three of the 81 respondents (65%) answered to be familiar with the results from the PEPaNIC trial, and 43 (53%) reported to have read the original article. Those who have read the article were larger PICUs and all multidisciplinary/mixed, and reported higher proportions of mechanically ventilated patients (**Supplemental Table 1**). The majority of those who have read the article would start PN if EN was <50%, whereas the majority of those who have not read the article would start PN if EN was <80% of target (**Supplemental Table 1**). Furthermore, those who have read the article would start amino acids more often within 48 hours than those who did not read the article (**Supplemental Table 1**).

Of the 43 respondents who have read the article, 9 (21%) interpreted the level of evidence of the PEPaNIC trial as level 1, 25 (58%) as level 2, and 9 (21%) as level 3. Furthermore, 8 (19%) of these 43 respondents interpreted the grade of recommendation as A (shall be recommended), 17 (39%) as B (should be recommended), and 18 (42%) as 0 (can/may be recommended). These 43 respondents all completed the final part of the survey questions on de-implementation of early PN initiation in their PICU (**Figure 1**). Complete de-implementation of early PN initiation, due to the results of PEPaNIC, was reported by 12% (5 of 43) and another 5 (12%) declared to already withhold PN during the first week prior to PEPaNIC (**Figure 4**). Partial de-implementation was asserted by 17 (40%) of the respondents (**Figure 4**). Of these 17 respondents, 16 reported to give PN during the first week only in specific patient groups (11 to neonates, 11 to

undernourished children, and 4 to other, unspecified patients), and 3 respondents declared to have postponed the timing of initiation and/or decreasing the amount of amino acids or lipids. Sixteen (37%) of the 43 PICUs reported no de-implementation, and continued to administer PN early during PICU admission. Ten of these PICUs would start PN within 48 hours after admission, of which 6 within 24 hours.

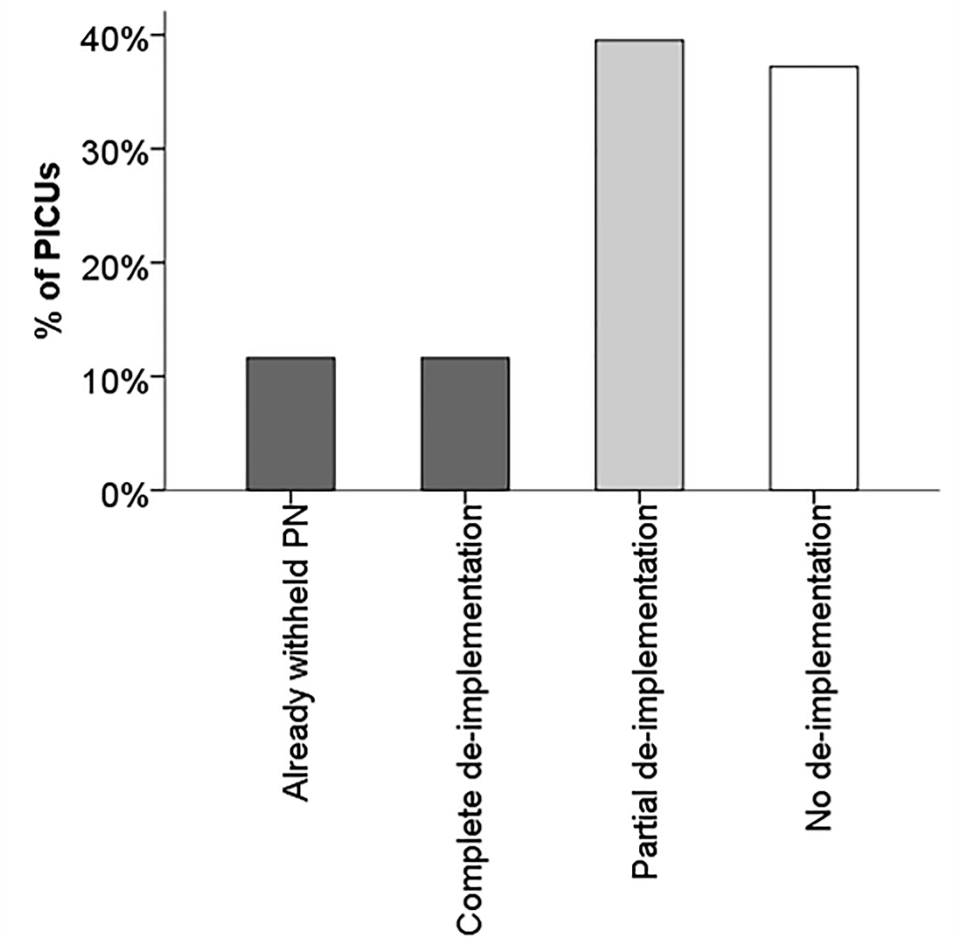

FIGURE 4 DE-IMPLEMENTATION OF EARLY PARENTERAL NUTRITION DURING THE FIRST WEEK OF PAEDIATRIC CRITICAL ILLNESS
PICU = pediatric intensive care unit; PN = parenteral nutrition

Associations between PICU/respondent characteristics and de-implementation

The degree of de-implementation within the characteristics of the PICUs/respondents is described in **Table 2**. Higher proportions of complete de-implementation were observed in PICUs from which the respondent rated the level of evidence and grade of recommendation high as compared with those PICUs who rated them lower (**Supplemental Table 2**).

Barriers for de-implementation

As familiarity of study results are a condition of studying de-implementation, we started off with making this distinction. Of the respondents, only 65% was familiar with the PEPaNIC study and only 43 (53%) had actually read the article. Of these 43, 33 respondents reported no or partial de-implementation and were asked for reasons not to adopt withholding PN during the first week (**Figure 1**). The most distinct arguments were those that addressed the safety of postponing PN. The perception that withholding PN would be harmful to children who were undernourished on admission (barrier for 17 respondents, 52%) and neonates (barrier for 11 respondents, 33%) were important barriers. Another major concern was the conviction that parenteral amino acids should be provided during the acute phase of critical illness (mentioned by 15 respondents, 46%). Further arguments represented the need for additional confirmation of the results from the PEPaNIC trial: waiting for replicating studies (11 respondents; 33%), waiting for updated international guidelines (11 respondents; 33%), and waiting for long term outcome results (8 respondents; 24%) (**Table 2**). Interestingly, 9 (27%) respondents reported that the results from the PEPaNIC trial were discussed within their staff but this had not led to de-implementation of early PN initiation because of lack of consensus (**Table 2**).

TABLE 2 BARRIERS FOR DE-IMPLEMENTATION (>1 ANSWER PER PICU POSSIBLE) IN THE 33 PICUS THAT HAVE PARTIALLY OR NOT DE-IMPLEMENTED EARLY ADMINISTRATION OF PN

Barriers	**No of PICUs (n=33)**
Safety issues	
Not convinced of the safety and/or efficacy in undernourished children	17
Convinced that critically ill children need amino acids in the acute phase of illness	15
Not convinced of the safety and/or efficacy in neonates	11
Convinced that critically ill children need lipids in the acute phase of illness	6
Not convinced of the safety in general	4
Convinced that critically ill children need more glucose in the acute phase of illness	2
Confirmation of results	
Waiting for updated international guidelines[a]	11
Waiting for replicating studies	11
Waiting for long term results	8
Don't consider these results to be cost-effective	1
Structural reasons	
Non-consensus within staff	9
Other[b]	5
Lack of nutritional protocol	2
Because of logistic reasons (i.e. arrangements with pharmacy)	1
Total number of reasons	**103**

PICU = pediatric intensive care unit; PN = parenteral nutrition

[a]Respondents from Europe: n=7, North America: n=2, South America: n=2 and Africa: n=1

[b]Provided answers: the PEPaNIC results are not generalizable to our PICU: n=3; PN is administrated rarely in our center: n=1; we are currently changing our PN strategies: n=1.

5. DISCUSSION

This survey showed that nutritional practices continue to vary greatly among PICUs as was previously reported.(3) Despite the dearth of evidence in the field of nutritional support in the PICU, in the current survey only about two-thirds of the respondents asserted to be familiar with the results from the PEPaNIC trial and approximately half had read the article. Among these respondents, PN was completely withheld during the first week in almost a quarter of the PICUs, and most PICUs had partially de-implemented early PN initiation, which meant predominantly that early PN would only be given to specific patient groups. Reported barriers for de-implementation were predominantly based on the conviction that PN during the first week of critical illness is necessary in neonates and undernourished children, and especially amino acids were viewed to be essential.

Although this de-implementation rate might be considered low, it is to be expected given the relative short time between publication of PEPaNIC and the survey (approximately 1 year). It has been shown that it takes more than a decade from publication to implementation into practice.(13, 14) An important first step in this process is to create awareness of new insights.(15) Interestingly, our survey pointed out that even if the existing evidence in the field is scarce and new results from a large, international study are published in a high-impact, open access journal, only two-thirds of the PICUs was aware of these results.

Besides awareness of new results, (de-)implementation depends on inhibiting and supporting factors. Previous studies have identified the following influences: believe in the benefits for the targeted population, financial implications, organizational structure, caregiver's motivation to change current practice, feasibility, quality of the evidence, credibility of the working group, relevance and generalizability of the research.(16-19) Indeed, most of these factors were mentioned in our survey as arguments not to change current practice. We will discuss those barriers/facilitators that could guide us to enhance early de-implementation.

In our survey, 76% still administered PN during the first week to all critically ill children or specific patient groups, because they believed in the benefit of early initiation of PN. Despite the fact that early-PN appeared to be even more harmful in neonates than in older children, and more harmful in children at the highest risk of malnutrition, as was already reported in the PEPaNIC article,(6) neonates and undernourished children were predominant barriers. After the survey, additional detailed subgroup analyses of neonates and undernourished children were published, which showed that withholding PN was clinically beneficial in these patients as well.(20, 21) Concerns on withholding PN in critically ill children might be explained by several assumptions. Since undernourishment on admission has been associated with worse clinical outcomes, it is

assumed that providing (parenteral) nutrition can improve clinical outcome by promoting anabolism. In small RCTs, higher provision of energy and protein/amino acids resulted in a positive protein balance.(22, 23) Subsequently, it was assumed that this would also lead to improved clinical outcome. These assumptions regarding PN might have reduced the faith in the controversial results from the PEPaNIC study, which is also reflected in a number of respondents who requested for repeat studies. Currently, we could identify one single center RCT on ClinicalTrials.gov, which is designed to randomize 80 critically ill children to receive supplemental PN within 12 or 96 hours after admission.(24) However, for clinicians working in combined adult/pediatric ICUs, PEPaNIC could have been considered as a repeat study. Withholding PN for a week in critically ill adults has been included in the 'choosing wisely campaign', a list made by specialty societies of possible unnecessary healthcare recommendations.(25) This might explain why PN was completely withheld in critically ill children during the first week in all of the combined adult/pediatric ICUs. Additionally, since evidence for withholding PN during the first week in critically ill adults has already been published first in 2011,(26) the time between evidence from research and de-implementation in practice might play a role. Furthermore, a significant proportion of the respondents mentioned the request for updated guidelines. When the survey was distributed, the most recent international guidelines were developed in 2005 and 2009. In the meantime, these guidelines have been updated by the leading expert nutrition societies,(27, 28) which means that the time between previous and current versions of the guidelines was 8 to 13 years. The fact that updated guidelines were awaited by a significant proportion of respondents stresses the importance of up-to-date guidelines. Hence, more frequent updates of the international guidelines might enhance (de-)implementation.

Despite the factors that hamper de-implementation, we have observed a shift in the timing of initiation of PN in critically ill children. In 2013, a worldwide survey was conducted, addressing nutritional practices in the PICU.(3) In this survey, the majority (55%) of the PICUs reported to start PN within 48 hours, and 20% within 24 hours. Furthermore, PN was completely withheld in only 3.5% of the PICUs before the PEPaNIC results were published.(3) Comparing these results to the results of our study, there seems to be a shift towards initiation of PN between day 2 to 7 and an increase in complete de-implementation of early PN, although this cannot be concluded confidently as the responding PICUs were not exactly the same.

Limiting the delay in de-implementation is of particular importance in case of harm by an intervention – which was the case in early-PN – or cost-ineffectiveness. Based on our results and existing literature, de-implementation might be accelerated by increasing awareness, gaining trust on the efficacy and safety of stopping the intervention, and facilitating up-to-date international guidelines. An important aspect to take into account is that the personal willingness and readiness to change a practice differs widely, which is illustrated by the 'theory of the diffusion of innovation' by Rogers et al.(15) According to this theory, the

PICUs who had de-implemented early PN in our survey could be the 'Early Adopters', who generally have the highest degree of opinion leadership.(15) Hence, the next step to increase awareness and gain support, demands the Innovators and Early Adopters to distribute the knowledge within their networks. Furthermore, the concerns on the efficacy and safety of stopping the intervention (in our case withholding PN) should be addressed if possible. Since the launch of this survey, several secondary analyses have investigated the main concerns, such as the harm associated with administration of amino acids,(29) the efficacy and safety of withholding PN in undernourished children(21) and neonates,(20) the long-term effects on physical and neuropsychological functions,(30) and the cost-effectiveness of withholding PN.(31) All these new findings were supportive for de-implementation of early-PN. Additionally, underlying mechanisms are currently explored.(32) Finally, since many clinical practices depend on the international opinion, de-implementation might be accelerated if the international guidelines would be revised more frequently in order to cover the most up-to-date evidence.

The strength of our study is the widespread responses from 39 countries. However, some limitations should also be addressed. First, responses from 81 PICUs are a small fraction of all PICUs worldwide, which might decrease generalisability. Possibly, only physicians interested in nutrition might have responded to our survey, which poses a risk of selection bias. Second, some answers from the respondents could potentially have been socially desirable, as this survey has been conducted by the PEPaNIC study group. Furthermore, some respondents gave inconsistent answers. We have analyzed all answers as provided by the respondent to avoid incorrect interpretation. And third, with this survey, we have measured theoretical de-implementation based upon the answers of the respondents, without measuring real PN practices. A previous survey addressing nutritional practices in PICUs, in which the questionnaire was followed by a point-prevalence, illustrated that the respondents often overestimated their practices.(3)

6. CONCLUSIONS

One year after the publication of the PEPaNIC trial, only two-thirds of the respondents was aware of the study results. Within this group, complete de-implementation of starting PN in the first week of critical illness was done in 12% of the PICUs worldwide, and partial de-implementation was done in 40% of the PICUs. Another 12% of PICUs already withheld PN during the first week. Important barriers for not de-implementing early PN were concerns on the efficacy and safety of withholding PN, and waiting for updated international guidelines. Increasing the awareness, addressing the intervention-specific questions and more frequently revising the international guidelines might help to accelerate de-implementation of ineffective, unproven or harmful healthcare.

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SUPPLEMENTARY MATERIAL

Supplemental methods

Supplemental Method 1: Questionnaire

Methods 1: Questionnaire

Part A: General information

1. What is your country of work?

2. What is the name of your institution?

3. What type of hospital do you work in?
   - general
   - university
   - university-children's
   - other, specify

4. What is your profession?
   - (pediatric) intensivist
   - anesthesiologist
   - pediatrician
   - surgeon
   - dietician/nutritionist
   - Nurse/nurse practitioner
   - other, specify

5. How many years of experience do you have working in a PICU?
   - 1-5
   - 6-10
   - 11-20
   - >20

6. What type of a PICU do you work in?
   - multidisciplinary/mixed
   - surgical
   - cardiac
   - medical
   - other, specify

7. Is the PICU combined with an adult ICU or a neonatal ICU?
   - Not combined
   - neonatal
   - adult
   - Both neonatal and adult

8. What is the number of pediatric ICU beds in your unit (until age 18 years)?
   - 1-10

□ 11-20
□ 21-30
□ >30

9. What is the average number of pediatric admissions per year in your unit (until age 18 years)?

□ 1-250
□251-500
□501-750
□751-1000
□1001-1250
□>1250

10. What is the average proportion of mechanically (invasive) ventilated pediatric patients per year in your unit (until age 18 years)?

□ <25%
□ 25-50%
□ >50-75%
□ >75%

Part B: Parenteral nutrition in your PICU

Please fill in your <u>current practice</u> regarding PN for critically ill pediatric patients.

1. Is there a nutritional protocol regarding PN used in your PICU?
   Yes / No
   If Yes → after C, answer D1
   If No → after C, answer D2

2. What is the basis of your nutritional protocol?
   □ International guideline → go to B3
   □ National guideline → go to B4
   □ Opinion of the staff → go to B4

3. Which guideline?
   □ European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)
   □ American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.)
   □ Adult international guideline

4. How much glucose is administered during the first 12-24 hours of admission:
   - <10 kg □ 1-4 mg/kg/min (1.4-5.8 g/kg/day) □ 4-6 mg/kg/min (5.8-8.6 g/kg/day) □ 6-8 mg/kg/min (8.6-11.5 g/kg/day) □ 8-10 mg/kg/min (11.5-14.4 g/kg/day)
   - 10-30 kg □ 1-4 mg/kg/min (1.4-5.8 g/kg/day) □ 4-6 mg/kg/min (5.8-8.6 g/kg/day) □ 6-8 mg/kg/min (8.6-11.5 g/kg/day) □ 8-10 mg/kg/min (11.5-14.4 g/kg/day)
   - >30 kg □ 1-4 mg/kg/min (1.4-5.8 g/kg/day) □ 4-6 mg/kg/min (5.8-8.6 g/kg/day) □ 6-8 mg/kg/min (8.6-11.5 g/kg/day) □ 8-10 mg/kg/min (11.5-14.4 g/kg/day)

5. At what point would you start amino acids in a child (expected to be) intolerable to enteral feeds?
   - □ < 24 hours
   - □ < 48 hours
   - □ 2-4 days
   - □ 4-7 days
   - □ >7 days

6. At what point would you start lipids in a child (expected to be) intolerable to enteral feeds?
   - □ < 24 hours
   - □ < 48 hours
   - □ 2-4 days
   - □ 4-7 days
   - □ >7 days

7. When enteral nutrition is provided but is insufficient to meet target goals, would PN be added?
   - □ No
   - □ Yes, always
   - □ Yes, if enteral nutrition covers < 80% of target calories
   - □ Yes, if enteral nutrition covers < 50% of target calories
   - □ other

8. If PN is given in combination with enteral nutrition: at what moment (percentage of nutritional target achieved by enteral nutrition) is PN stopped?
   - □ If enteral nutrition covers 100% of target calories
   - □ If enteral nutrition covers > 80% of target calories
   - □ If enteral nutrition covers > 50% of target calories
   - □ Other

9. How is PN provided in your institution?
   - □ Pharmacy-customized, age/weight specific
   - □ Commercial mixed bags
   - □ Other

10. Is it possible to administer PN without a central venous line? Yes / No

11. Do you routinely administer vitamins and trace elements? Yes / No

Part C: Awareness of the Early versus Late PN in Critically Ill Children (PEPaNIC) study

The results of the international, multicenter, randomized, controlled trial Pediatric Early versus Late Parenteral Nutrition in Critical Illness (PEPaNIC) have been published in the New England Journal of Medicine in March 2016. The article and supplemental material have been attached to the invitation email.

1. Are you familiar with the results of this study? Yes / No

2. Did you read this article before you filled out this survey? Yes / No

3. Did you read the supplemental material before you filled out this survey? Yes / No

4. How would you rate the level of evidence?

- □ 1 (high to excellent)
- □ 2 (moderate to high)
- □ 3 (low)
- □ 4 (expert opinion)

5. How would you rate the grade of recommendation?

- □ A (shall be recommended)
- □ B (should be recommended)
- □ 0 (can/may be recommended)
- □ GPP (Good Practice Points)

Part D1: Change in Nutritional Practice

In the PEPaNIC study, in children who were allocated to the Late PN group, during the first week of critical illness, PN was withheld completely (meaning: low amounts of glucose (<2 mg/kg/min), no amino acids and no lipids were administered). Late PN resulted in lower percentage of new infections and shorter duration of PICU stay compared to Early PN.

1. Has the local nutritional protocol of your PICU, concerning the initiation or amount of PN, been changed due to the results of this study?

- □ **No** change. We already withheld PN during the first week → go to D9, after D: go to E4
- □ **No** change. We still administer PN during the first week in all children → go to D9
- □ **Yes**, we have changed our practice. We now withhold PN during the first week → go D6 & D8, after D: go to E4
- □ **Yes**, we have changed our protocol <u>partially</u> or only in specific patients. We withhold or decreased component(s) of PN → go to D2

2. Please specify: during the first week of critical illness in children… (multiple choice)

- □ We still administer PN in the first week <u>only</u> in specific patient groups (i.e. neonates, malnourished children, specific diseases) → go to D3
- □ We withhold or decrease only some of the macronutrients (glucose, amino acids, lipids) → go to D5
- □ Other, namely …. → go to D5

3. Which patient group(s) continued receiving PN during the first week? (multiple choice)

- □ neonates (<1 month)
- □ malnourished children
- □ other, namely …

4. At which day was/is PN started in this patient group?

Neonates:

- □ < 24 hours
- □ < 48 hours
- □ 2-4 days
- □ 4-7 days
- □ >7 days

Malnourished children:

- □ < 24 hours
- □ < 48 hours
- □ 2-4 days
- □ 4-7 days
- □ >7 days

Other:

□ < 24 hours
□ < 48 hours
□ 2-4 days
□ 4-7 days
□ >7 days

→ go to D9

5. Did you change the administration of parenteral amino acids during the first week due to the results of this study? (multiple choice)

□ No → go to D7
□ Yes, we have changed the timing of initiation of amino acids → go to D6
□ Yes, we have changed the amount of amino acids → go to D7

6. At which day were amino acids started <u>before</u> the change in protocol:

□ < 24 hours
□ < 48 hours
□ 2-4 days
□ 4-7 days
□ >7 days

7. Did you change the administration of parenteral lipids during the first week due to the results of this study? (multiple choice)

□ No → go to D9
□ Yes, we have changed the timing of initiation of lipids → go to D8
□ Yes, we have changed the amount of lipids → go to D9

8. At which day were parenteral lipids started <u>before</u> the change in protocol:

□ < 24 hours
□ < 48 hours
□ 2-4 days
□ 4-7 days
□ >7 days

9. Did you lower the amount of glucose administered intravenously during the first week due to the results of this study? Yes / No

10. Keeping the results of the study in mind, when a child deteriorates clinically <u>after the first week</u> (i.e. sepsis), would you then discontinue PN? Yes / No.

Part D2: Change in Nutritional Practice

In the PEPaNIC study, in children who were allocated to the Late PN group, during the first week of critical illness, PN was withheld completely (meaning: low amounts of glucose (<2 mg/kg/min), no amino acids and no lipids were administered). Late PN resulted in lower percentage of new infections and shorter duration of PICU stay compared to Early PN.

1. Has the local nutritional practice of your PICU, concerning the initiation or amount of PN, been changed due to the results of this study?

□ **No** change. We already withheld PN during the first week → go to D9, after D: go to E4

□ **No** change. We still administer PN during the first week in all children → go to D9

□ **Yes**, we have changed our practice. We now withhold PN during the first week → go D6 & D8, after D: go to E4

□ **Yes**, we have changed our practice <u>partially</u> or only in specific patients. We withhold or decreased component(s) of PN → go to D2

2. Please specify: during the first week of critical illness in children… (multiple choice)

□ We still administer PN in the first week <u>only</u> in specific patient groups (i.e. neonates, malnourished children, specific diseases) → go to D3

□ We withhold or decrease only some of the macronutrients (glucose, amino acids, lipids) → go to D5

□ Other, namely …. → go to D5

3. Which patient group(s) continued receiving PN during the first week? (multiple choice)

□ neonates (<1 month)

□ malnourished children

□ other, namely …

4. At which day was/is PN started in this patient group?

Neonates:

□ < 24 hours

□ < 48 hours

□ 2-4 days

□ 4-7 days

□ >7 days

Malnourished children:

□ < 24 hours

□ < 48 hours

□ 2-4 days

□ 4-7 days

□ >7 days

Other:

□ < 24 hours

□ < 48 hours

□ 2-4 days

□ 4-7 days

□ >7 days

→ go to D9

5. Did you change the administration of parenteral amino acids during the first week due to the results of this study? (multiple choice)

□ No → go to D7

□ Yes, we have changed the timing of initiation of amino acids → go to D6

□ Yes, we have changed the amount of amino acids → go to D7

6. At which day were amino acids started <u>before</u> the change in practice:

□ < 24 hours

□ < 48 hours

□ 2-4 days

□ 4-7 days

□ >7 days

7. Did you change the administration of parenteral lipids during the first week due to the results of this study? (multiple choice)

- □ No → go to D9
- □ Yes, we have changed the timing of initiation of lipids → go to D8
- □ Yes, we have changed the amount of lipids → go to D9

8. At which day were parenteral lipids started before the change in practice:

- □ < 24 hours
- □ < 48 hours
- □ 2-4 days
- □ 4-7 days
- □ >7 days

9. Did you lower the amount of glucose administered intravenously during the first week due to the results of this study? Yes / No

10. Keeping the results of the study in mind, when a child deteriorates clinically after the first week (i.e. sepsis), would you then discontinue PN? Yes / No.

Part E: Reasons for not implementing Late PN

1. What is/are reason(s) for not withholding PN in your PICU during the first week of critical illness in children? (multiple choice)

- □ waiting for replicating studies
- □ not convinced of the safety
- □ not convinced of the safety and/or efficacy in neonates
- □ not convinced of the safety and/or efficacy in malnourished children
- □ convinced that critically ill children need amino acids in the acute phase of illness
- □ convinced that critically ill children need more glucose in the acute phase of illness
- □ convinced that critically ill children need lipids in the acute phase of illness
- □ waiting for long term results.
- □ don't consider these results to be cost-effective
- □ waiting for updated international guidelines
- □ lack of nutritional protocol
- □ non-consensus within staff
- □ Because of logistic reasons (i.e. arrangements with pharmacy) (→ go to E2)
- □ Other, namely …..

All answers (except logistic reasons)→go to E4

2. When enteral nutrition is insufficient to meet nutritional targets, do you intend to withhold PN during the first week of critical illness in the future?

- □ No, I intend to start PN as soon as possible
- □ Yes, I intend to withhold PN for less than 7 days in the future (→ go to E3)
- □ Yes, I intend to withhold PN during the first week in the future (→ go to E4)

3. At which day do you intend to start PN in the future?

- □ < 24 hours
- □ < 48 hours
- □ 2-4 days

- 4-7 days
- >7 days

4. Do you have any comments on this survey? (not mandatory)

5. If you would like to be informed about the results of this survey, please fill in your name and email-address. (not mandatory)

Supplemental Tables

SUPPLEMENTAL TABLE 1 CHARACTERISTICS AND NUTRITIONAL PRACTICES OF PICUS OF WHICH THE RESPONDENT HAD READ THE ARTICLE VERSUS THOSE WHO HAVE NOT READ THE ARTICLE

Characteristic / nutritional practice	Have read the article (n=43)	Did not read the article (n=38)
Continent		
Europe	22 (51.2%)	17 (44.7%)
South America	8 (18.6%)	6 (15.8%)
Asia	2 (4.7%)	10 (26.3%)
North America	8 (18.6%)	4 (10.5%)
Africa	2 (4.7%)	0 (0%)
Oceania	1 (2.3%)	1 (2.6%)
Hospital type		
University children's hospital	26 (60.5%)	11 (28.9%)
University hospital	10 (23.3%)	14 (36.8%)
General hospital	6 (14.0%)	12 (31.6%)
Other	1 (2.3%)	1 (2.6%)
Type of PICU		
Multidisciplinary/mixed	43 (100%)	32 (84.2%)
Medical	0 (0%)	4 (10.5%)
Cardiac	0 (0%)	1 (2.6%)
Surgical	0 (0%)	1 (2.6%)
Combination of PICU		
Not combined	35 (81.4%)	31 (81.6%)
With neonatal ICU	5 (11.6%)	5 (13.2%)
With adult ICU	3 (7.0%)	1 (2.6%)
With adult and neonatal ICU	0 (0%)	1 (2.6%)
Size of PICU		
1-10 beds	12 (27.9%)	21 (55.3%)
11-20 beds	16 (37.2%)	12 (31.6%)
21-30 beds	12 (27.9%)	4 (10.5%)
>30 beds	3 (7.0%)	1 (2.6%)
Paediatric admissions (patients/year)		
1-250	3 (7.0%)	4 (10.5%)
251-500	13 (30.2%)	16 (42.1%)
501-750	9 (20.9%)	9 (23.7%)
751-1000	4 (9.3%)	3 (7.9%)
1001-1250	5 (11.6%)	2 (5.3%)
>1250	9 (20.9%)	4 (10.5%)
Mechanically ventilated paediatric patients		
<25%	6 (14.0%)	3 (7.9%)
25-50%	13 (30.2%)	18 (47.4%)
50-75%	14 (32.6%)	11 (28.9%)
>75%	10 (23.3%)	6 (15.8%)
Add PN if EN is insufficient		
No	2 (4.7%)	2 (5.3%)
Yes, if EN <50%	13 (30.2%)	7 (18.4%)
Yes, if EN <80%	21 (4.7%)	22 (57.9%)
Yes, always	5 (11.6%)	5 (13.2%)
Other	2 (4.7%)	2 (5.3%)
Start amino acids		
<24 hours	12 (27.9%)	9 (23.7)
<48 hours	5 (11.6%)	11 (28.9%)
2-4 days	12 (27.9%)	9 (23.7%)
4-7 days	10 (23.3%)	7 (18.4%)
>7 days	4 (9.3%)	0 (0.0%)
Start lipids		
<24 hours	9 (20.9%)	5 (13.2%)
<48 hours	9 (20.9%)	11 (28.9%)
2-4 days	11 (25.6%)	10 (26.3%)
4-7 days	11 (25.6%)	11 (28.9%)
>7 days	3 (7.0%)	1 (2.6%)

PICU = pediatric intensive care unit; ICU = intensive care unit; PN = parenteral nutrition

SUPPLEMENTAL TABLE 2 DISTRIBUTION OF THE DEGREE OF DE-IMPLEMENTATION WITHIN THE CHARACTERISTICS OF THE 43 PICUS/RESPONDENTS WHO HAVE ANSWERED PART D OF THE QUESTIONNAIRE

Characteristic	No de-implementation	Partial de-implementation	Complete de-implementation	Already withheld PN
Continent				
Europe	2 (9%)	8 (36%)	8 (36%)	2 (9%)
South America	2 (25%)	4 (50%)	0 (0%)	2 (25%)
Asia	2 (100%)	0 0%)	0 (0%)	0 (0%)
North America	3 (39%)	3 (38%)	1 (13%)	1 (13%)
Africa	1 (50%)	1 (50%)	0 (0%)	0 (0%)
Oceania	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Combination of PICU				
Not combined	14 (40%)	15 (43%)	3 (9%)	3 (9%)
With neonatal ICU	2 (40%)	2 (40%)	0 (0%)	1 (20%)
With adult ICU	0 (0%)	0 (0%)	2 (67%)	1 (33%)
With adult and neonatal ICU	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Size of PICU				
1-10 beds	5 (42%)	4 (33%)	1 (8%)	2 (7%)
11-20 beds	5 (31%)	6 (38%)	3 (19%)	2 (13%)
21-30 beds	6 (50%)	5 (42%)	1 (8%)	0 (0%)
>30 beds	0 (0%)	2 (67%)	0 (0%)	1 (33%)
Experience of respondent (years)				
1-5	0 (0%)	3 (50%)	2 (33%)	1 (17%)
6-10	5 (71%)	2 (29%)	0 (0%)	0 (0%)
11-20	6 (46%)	3 (23%)	1 (8%)	3 (23%)
>20	5 (29%)	9 (53%)	2 (12%)	1 (6%)
Nutritional protocol present				
Yes	12 (41%)	10 (35%)	4 (14%)	3 (10%)
No	2 (29%)	7 (50%)	1 (7%)	2 (14%)
Rated level of evidence				
4	0 (0%)	0 (0%)	0 (0%)	0 (0%)
3	7 (78%)	2 (22%)	0 (0%)	0 (0%)
2	7 (28%)	11 (44%)	2 (8%)	5 (20%)
1	2 (22%)	4 (44%)	3 (33%)	0 (0%)
Rated grade of recommendation				
Good Practice Points	0 (0%)	0 (0%)	0 (0%)	0 (0%)
0	10 (56%)	6 (33%)	0 (0%)	2 (11%)
B	3 (18%)	9 (53%)	2 (12%)	3 (18%)
A	3 (38%)	2 (25%)	3 (38%)	0 (0%)

PICU = pediatric intensive care unit; ICU = intensive care unit; PN = parenteral nutrition

CHAPTER 7 - GENERAL DISCUSSION AND PERSPECTIVES

GENERAL DISCUSSION

Years after being admitted to the PICU and independent of the underlying condition or illness, critically ill children suffer from a severe legacy, characterized by impaired growth, neurocognitive development and physical functioning.[1-4] Such a long-term legacy jeopardizes educational opportunities and reduces general well-being and quality of life of these children.[5,6] As compared with healthy peers, this long-term footprint of critical illnesses could also prevent these patients from "fitting in" and finding a place in society while growing up. Given that over the last decades, mortality of critical illness in children has decreased and short-term intensive care outcomes have improved,[7] research in pediatric critical illness should focus more on longer-term morbidity endpoints in order to further improve outcome.[8] Recent research has shown that not only short-term but also long-term outcomes of pediatric critical illness are in fact modifiable by changing certain aspects of the intensive care management in the PICU.[9-13]

Neurocognitive development and growth of children require a normal function of several neuro-endocrine axes, among which the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis play a key role.[14-17] In **Chapter 3** and **Chapter 4** of this PhD thesis, we have documented the changes in the HPT-axis and the HPA axis that occur during the acute phase of critical illness in children, in patients who had been included in the PEPaNIC RCT. We could confirm the presence of the non-thyroidal illness syndrome (NTI) upon PICU admission, characterized by low serum concentrations of TSH, T4 and T3, and elevated rT3 serum concentrations, which is in line with earlier observations in the adult critically ill population. A striking difference with adult patients was the finding in critically ill children that plasma total and free cortisol concentrations were only briefly elevated and to a much lesser extent than in adults,[18,19] despite similarly suppressed circulating plasma levels of binding-proteins and equally downregulated cortisol breakdown. Unexpectedly, we observed that plasma ACTH levels were not elevated upon PICU admission and decreased quickly further over time. Given that circulating plasma free cortisol levels were no longer elevated, the low plasma ACTH did not appear to be attributable to free cortisol-induced feedback inhibition. Other factors, such as exposure to phthalates that can leach from the indwelling medical devices or the use of certain drugs in the PICU could theoretically contribute to such ACTH suppression in the absence of elevated free cortisol.[18,20,21] Alternatively, one could speculate that the set point for feedback inhibition could be lowered in the context of critical illness. Such a re-set of setpoint for feedback inhibition has previously been suggested as a potential explanation for the low circulating TSH serum levels in patients with NTI.[22-24] Interestingly, since we documented that plasma ACTH concentrations were further suppressed by (high dose)

corticosteroid treatment, it seems that ACTH secretion in patients not treated with corticosteroids is not fully suppressed and that cortisol-induced feedback inhibition at the pituitary and/or hypothalamic level is still functional.

Next in Chapter 3 and 4, we have also assessed the predictive value of the changes in both the HPT and HPA axis for intensive care outcomes. Upon PICU admission, the severity of the NTI, with low T4, low T3 and high rT3, was independently associated with worse intensive care outcomes. This is not a novel finding, though,[25-27] and whether it reflects causality or rather adaptation to severe illnesses remains a topic of debate.[28-31] Regarding the HPA axis, our findings were slightly different, as worse short-term outcomes were found to be predicted, independently, by lower ACTH plasma levels and by higher cortisol plasma levels. As also the treatment with (high dose) corticosteroids was found to be independently associated with poor intensive care outcome, this raised the possibility that, in critically ill children, it may not be appropriate to acutely increase the systemic cortisol availability. Of course, such a hypothesis can only be tested by RCTs. However, our new data do suggest that such future RCTs should incorporate adequately planned interim safety analyses. Currently, multicenter RCTs on hydrocortisone in pediatric severe sepsis (NCT00732277) and septic shock (NCT03401398) are ongoing but do not mention the risks that we have identified.

Finally, in Chapter 3 and 4, we assessed the role of the feeding strategy in bringing about these hormonal changes in pediatric critical illness and how this relates to the intensive care outcomes of critically ill children. This is an important question, given that fasting can substantially alter the hormonal levels of the HPT and HPA axis in healthy subjects.[32,33] In the context of the PEPaNIC RCT, we could investigate the impact of randomization to either initiating supplemental PN within the first 24 hours of PICU admission (early-PN), which comes down to early full feeding, in comparison with omission of PN until beyond the first week in PICU (late-PN) on the HPT and HPA axis changes that occur over time during pediatric critical illness. Given that late-PN comes down to virtual fasting in most PICU patients, this study allowed to compare the impact of such virtual fasting with early full feeding in critically ill children. For the HPT axis, virtual fasting in the first days in PICU was found to aggravate NTI over time, as compared to early full feeding. Statistically, the further peripheral inactivation of thyroid hormone by late-PN, reflected by a further lowering of the T3/rT3 ratio, contributed to the short-term clinical benefits of late-PN versus early PN, whereas the further accentuation of the central component of NTI, reflected by a further lowering of T4, appeared to counteract the outcome benefit of late-PN. In other words, despite the fact that late-PN further lowered T4 over time, the further decrease of the T3/rT3 ratio evoked by late-PN appeared to be dominant, as overall, the use of late-PN was beneficial for intensive care outcomes, also in this sub-analysis of the large PEPaNIC RCT. This interesting finding suggested that the central component of the NTI could be a maladaptive response of the body to critical illness, whereas the peripheral inactivation of thyroid hormone could be beneficial. If this would be

correct, then treatment with T3 or T4 during critical illness might not be appropriate as it could contribute to worse outcome. However, the data do suggest that attempts to restore the central component of the NTI, by administration of TRH, while allowing peripheral changes to take place as directed by the needs of the disease, could be worth investigating in future RCTs. Unlike what we observed for the HPT axis, late-PN versus early-PN did not affect the changes that occur within the HPA axis during critical illness in children. This finding was in line with the previously published results obtained in the critically ill adult population.[19] Not seeing an effect of the feeding strategy on the HPA axis, which is present in healthy subjects, could be explained by the fact that critically ill patients no longer have a diurnal pattern in the activity of the HPA-axis and that ACTH is suppressed via other more potent signals. Also, late-PN patients were not fully fasted as they received some enteral feeding and small amounts of IV glucose which could have prevented the expected fasting-induced changes within the HPA-axis.

The outcomes studied in **Chapter 3** and **Chapter 4** are all short-term, intensive care outcomes. However, it remains unknown whether long-term outcome of critically ill children could be affected by the illness-induced neuro-endocrine alterations. As a first step in trying to answer this question, it was necessary to accurately document the longer-term outcomes of the PEPaNIC patients. Although a follow-up analysis was already performed 2 years after inclusion in the PEPaNIC RCT,[34] the research team had *a priori* planned to repeat such study 4 years later,[35] as at that time many more children would have reached a testable age. As reported in **Chapter 5** of this PhD thesis, we have performed this 4-year follow-up of the PEPaNIC RCT, evaluating growth, physical function and broad neurocognitive development. This 4-year follow-up study of the PEPaNIC RCT included 684 patients who were tested neurocognitively. Taking into account important information on how patients were doing 4 years after being included in the RCT, which involves knowing how many patients had died and how many patients were too disabled to be tested at this time point, this came down to a loss to follow-up, with no information available for these patients, of 33.8%. As compared with other long-term follow-up studies,[36,37] this represents quite a limited loss to follow-up. Such excellent recruitment rate could be attributed to an excellent collaboration and communication among participating centers, as well as the high motivation and efforts of the local multidisciplinary follow-up teams on a daily basis. The professionalism of our multicenter team was key in this success, by organizing regular meetings to monitor inclusion rates and solving problems that were identified, all driven by a high standard of scientific integrity. The results of the 4-year follow-up study revealed that children who were admitted to the PICU, still showed an important legacy of the critical illness, characterized by impaired growth and impaired neurocognitive development as compared with matched healthy peers. In addition, critically ill children who benefited in the short-term from late-PN also showed less parent- or caregiver reported emotional and behavioral problems 4 years later, as compared with children who had received supplemental PN early during PICU admission. Moreover, the data showed that for this developmental domain, the late-PN patients were no longer different from healthy

matched control children, which means that the abnormalities were fully explained by the use of early-PN. In the previously reported 2-year follow-up study, the most striking differences between early-PN and late-PN patients were present for executive functioning, which was improved by late-PN. Interestingly, from previously performed pediatric psychosocial research, it is known that normal executive functioning is essential to develop normal behavior.[38,39] With the PEPaNIC children getting older at the 4-year follow-up, it is thus not surprising that the emotional and behavioral problems were more prominently present. The next question is which part of the broad long-term legacy of critical illness is attributable to the neuro-endocrine changes which are reported in **Chapter 3** and **Chapter 4**. This remains to be investigated.

Epigenetic changes, more specifically altered DNA methylation, may be a biological basis for long-term sequelae evoked by environmentally deleterious exposures and by illness. Our group has shown that *de novo* alterations in DNA methylation in 159 CpG-sites occurred during critical illness in children.[40] Interestingly, this study also revealed that, adjusted for risk factors, early-PN was the cause of the altered DNA methylation in 37 of these 159 CpG-sites. The altered DNA methylation of these CpG-sites occurred mainly in non-protein-coding DNA, intergenic DNA regions, and gene bodies of protein-coding genes, in particular those involved in growth and brain development.[40] In our study at 4-year follow-up, as reported in **Chapter 5**, we could show that the behavioral problems attributed to the use of early-PN were found to be at least partially mediated by the adversely altered DNA methylation by early-PN. Many of these CpG-sites that played a mediating role for the harmful effect of early-PN on behavior at 4 year follow-up were the same as the ones that explained the early-PN induced impaired executive functioning at 2-year follow-up.[40] This important finding provided further support for the clinical data that already suggested that normal executive functioning is essential to later develop normal behavior. At first sight, it may be surprising and perhaps counterintuitive that a short exposure to critical illness and the associated nutritional management in the PICU could evoke such important long-term sequelae. However, we have now shown that epigenetic changes provide a possible biological basis for this phenomenon. The identification of a biological basis of a clinical problem also opens perspectives for prevention and treatment. Ideally, the epigenetic changes that are observed during critical illness should be prevented. This was found to be the case for a part of the DNA methylation changes, which were prevented by omitting the use of early-PN. However, the early-PN mediated alteration in DNA methylation only accounted for 37 of the 159 identified CpG-sites that are altered by critical illness.[40] Hence, other contributing factors must play a role and perhaps some of these are also modifiable. However, if the modification in methylation of the remaining CpG-sites is only due to the critical illness itself or to unmodifiable factors, therapeutic intervention should be studied. Interestingly, recent advanced genome editing technologies such as CRISPR-Cas might represent a theoretical option to be considered in the future.[41,42] Another unanswered question related to the biological basis of the long-term legacy of critical illness is whether the observed DNA methylation changes are present in all cell types. If these DNA

methylation changes would also be present in reproductive cells, and if they would be permanent, it would be possible that the offspring of these children could also be affected. This potential transgenerational effect of critical illness and its management is worth investigating. A longer-term follow-up study with documentation of the DNA methylation status of the future offspring of the currently studied patients and comparing these with the data from their parents would allow to begin to address this important question.

The data presented in this PhD project, as well as the data that were previously generated by our group, are both clinically robust and hypothesis generating when it comes down to preventing harm by avoiding supplemental PN early during PICU stay. However, the process of de-implementation of the use of early-PN in critically ill children worldwide does not seem easy. In **Chapter 6**, we report the results of our worldwide survey, which we distributed among the members of the World Federation of Pediatric Intensive and Critical Care Societies (WFPICCS) and the European Society of Paediatric and Neonatal Intensive Care (ESPNIC), one year after publication of the PEPaNIC RCT in a high impact journal (NEJM) in 2016. The results of this survey showed that only one third of the 81 responders were aware of the PEPaNIC study results. The responders were active in 43 PICUs worldwide, mostly located in Europe (48%), but also in South America (17%), North America (15%) and Asia (15%). Only 10 of the 43 PICUs changed their protocol to total de-implementation of early-PN because of the PEPaNIC RCT 2016 results. The 33 remaining PICUs performed partial or no de-implementation at all. Barriers for de-implementation were mostly safety concerns about the possibility that withholding PN would be harmful for undernourished children and neonates. However, the results of the more recently published secondary sub-analyses of the PEPaNIC RCT of the critically ill term neonates[43] and the undernourished children upon PICU admission[44] showed that withholding PN for one 1 week was clinically superior to early-PN also in these subgroups. Apart from a higher risk of hypoglycemia in neonates up to 4 weeks of age in the late-PN group, there was no difference in safety outcomes between both randomization groups. Another barrier for de-implementation of early-PN was the conviction that parenteral amino acids should be provided during the acute phase of critical illness. After the survey was closed, however, our group analyzed which macronutrient best explained the harm caused by early-PN.[45] Interestingly, the early administration of amino acids, even in low doses, was independently associated with worse intensive care outcomes. In contrast, higher doses of glucose administered early in the course of illness seemed somewhat protective. Finally, responders who only partially or did not de-implement the use of early-PN at all, were waiting for long-term results, replicating studies and updated international guidelines. In 2018, recent guidelines on pediatric parenteral nutrition of the ESPGHAN/ESPEN/ESPR/CSPEN working group were published, recommending the withholding of parenteral nutrition, including amino acids, for 1 week in critically ill children.[46] A recently published expert statement of the European Society of Pediatric and

Neonatal Intensive Care (ESPNIC) on nutritional support for critically ill children, recommends considering withholding PN during the first week in PICU in neonates and children, independent of their nutritional state.[47] Unfortunately, no replicating studies with a randomized controlled study design have been performed since the publication of the PEPaNIC RCT. Also, no ongoing recruiting trials on clinicaltrials.gov were found. To address the concern of the long-term effects of withholding PN early during PICU admission, the robustness of the later on published results of the 2 year follow-up[34] and the here reported 4 year follow-up study could provide the awaited for reassurance. Also, via the identification of a biological basis of the long-term harm caused by the use of early-PN, as shown after 2 years[40] and 4 years and described in this thesis manuscript, which again was mostly explained by the use of amino acids rather than lipids or glucose, de-implementation could be further supported.

As already alluded to in the parts above, the work presented in this PhD thesis has shed light on future research questions and opens perspectives for innovation. First, other potentially modifiable factors that play a role in the long-term legacy of critical illness in children should be investigated. Indeed, currently 3 modifiable factors have been identified: the use of early-PN, hyperglycemia, and phthalate exposure, as these have shown to negatively affect the long-term outcome of critically ill children. Hence, other iatrogenic factors such as drugs could also play a role. Indeed, certain types of drugs used in the PICU have already been identified as associated with neurocognitive outcomes 2 and 4 years after critical illness. The use of benzodiazepines was identified as independently associated with worse outcomes and the use of α2-agonists was found to independently relate to better outcomes. Further exploring these interesting associations via randomized controlled trials appears necessary. Second, it is possible to interfere with the hormonal changes that occur during critical illness in children, and the impact on short-term and long-term outcomes of such interventions should be investigated via RCTs. These could imply the study of TRH to correct the central component of the NTI, and the study of CRH and/or ACTH to increase the suppressed ACTH levels. Third, the altered DNA methylation status as a molecular basis of the harm induced by early-PN on long-term neurocognitive development provides inspiration for a broad range of new research questions. The link between the altered DNA methylation status of the CpG-sites and gene expression could be further explored. In addition, although possibly inducing less stable changes in gene expression, also other forms of epigenetic alterations are worth investigating as potential mediators, such as histone modification and micro RNAs. Finally, transgenerational effects of DNA methylation changes induced by critical illness and its management should be investigated in the offspring of patients who were critically ill as a child.

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SUMMARY

Summary

Children are admitted to the pediatric intensive care unit (PICU) when they acutely need vital organ support to avoid imminent death. Although most of the children need intensive care for only a few days and most of them seemingly recover well from the acute insult, many of them are confronted with long-term consequences after critical illness as observed years after hospital discharge. This legacy, which remains largely unexplained, broadly affects neurocognitive development, but also growth and quality of life of the children, independently of pre-existing illnesses or conditions.

Previous research has shown that the long-term adverse outcome after critical illness is modifiable by changing certain conditions during the acute PICU management. Our group previously performed the large multicenter PEPaNIC randomized controlled trial (RCT), which included 1440 critically ill children, and demonstrated that withholding supplemental parenteral nutrition during the first week in PICU (late-PN), and thus accepting a macronutrient deficit, was clinically superior to initiating supplemental PN within the first 24 hours of admission (early-PN). Late-PN accelerated recovery and decreased the risk of acquiring a new infection during PICU admission. Interestingly, also in the longer-term 2 years after inclusion, late-PN was shown to improve and even normalize several neurocognitive outcomes, in particular the executive function of inhibitory control, as compared with early-PN. Whether impairments in physical and neurocognitive domains observed at 2-years follow-up persist or disappear, or whether other problems may emerge in relation to the randomized intervention remained unclear. The acute phase of critical illness is also hallmarked by several neuroendocrine changes, possibly influenced by the macronutrient deficit caused by late-PN, which could affect development and growth of the children in the long term. This PhD thesis first documented the changes in the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-adrenal (HPA) axis in the acute phase of pediatric critical illness, and assessed the impact of accepting a macronutrient deficit with late-PN hereon. It also investigated the long-term effects of pediatric critical illness and an early macronutrient deficit on neurocognitive development, growth and physical functioning 4 years after PICU admission. The final objective of this thesis was to assess the de-implementation of early-PN in PICUs worldwide.

In the first part of this PhD project, we confirmed the presence of non-thyroidal illness (NTI) upon PICU admission, with low serum concentrations of TSH, T4 and T3, and elevated rT3 serum concentrations. The severity of NTI upon admission was independently associated with worse intensive care outcomes. NTI is also known to occur with fasting. Consistent with the mimicking of a fasting response, the macronutrient deficit in patients from the late-PN group resulted in a worsening of NTI over the first few days in PICU as compared with early-PN patients. Interestingly, in a statistical mediation analysis, the peripheral inactivation of thyroid

hormone, which was further accentuated by late-PN, contributed to the outcome benefit of late-PN and thus appeared to be a beneficial response to critical illness. In contrast, the central component of NTI attributable to suppressed TSH and evidenced by the decrease in T4, counteracted the late-PN outcome benefit and appeared to be a maladaptive response. Whether treating the central component of NTI with TRH infusion in the PICU improves outcome of critically ill children requires further investigation in adequately powered RCTs.

In the second part, the changes in the HPA axis during the acute phase of pediatric critical illness were documented. In contrast with critically ill adults, cortisol levels in critically ill children were only briefly elevated upon PICU admission and became normal thereafter, despite low binding-proteins and persistently suppressed cortisol metabolism. As ACTH was normal upon admission and decreased quickly thereafter, cortisol availability was not driven by increased ACTH. On day 3 of admission, high cortisol and low ACTH levels independently predicted poor outcome. Also, treatment with corticosteroids in the PICU further suppressed ACTH and was independently associated with poor outcomes. These findings suggest that exogenously increasing cortisol availability during the acute phase of critical illness in children might be inappropriate, and that future studies on corticosteroid treatment should plan safety analyses, as harm may be possible.

In the third part of this PhD project, we presented the results of the 4-year follow-up study of the PEPaNIC RCT, which investigated the effect of late-PN versus early-PN on anthropometrics, health status, parent- or caregiver-reported executive functions and emotional/behavioral problems, and clinical tests for intelligence, visual-motor integration, alertness, motor coordination and memory. Four years after inclusion, these children scored worse for almost every developmental domain as compared with matched healthy children. However, part of the impairment, more specifically the parent- or caregiver-reported internalizing, externalizing and total emotional/behavioral problems, could be prevented in patients from the late-PN group who did not receive supplemental PN in the first week of PICU admission. The emotional/behavioral problems thus attributable to the use of early-PN were found to be at least partially mediated by adversely altered DNA-methylation by early-PN during PICU stay. Hence, the avoidance of early-PN induced alterations in DNA-methylation status during PICU stay could be a biological mediator of the protection against emotional/behavioral problems 4 years later as observed with late-PN. Taken together, these findings provide strong further support in favor of the de-implementation of administering PN during the first week in PICU.

In the last part, the de-implementation of early PN was assessed in PICUs worldwide, by analyzing the results of a survey that was distributed one year after publication of the PEPaNIC RCT. The survey was completed by respondents of 81 different PICUs in 39 countries. At the time of the survey, two-thirds of the respondents was aware of the PEPaNIC study results, of whom 12% already did not initiate supplemental PN in the first week in their PICU. Another 12% started de-implementing early-PN after reading the PEPaNIC RCT paper, and 40% partially de-implemented early-PN by postponing the initiation of PN (though still initiated prior to day 8 in PICU) and/or providing decreased amounts of PN. Barriers for not de-implementing early-PN were concerns about the efficacy and (long-term) safety of late-PN, and waiting for updated international guidelines. In the meantime, recently published guidelines, the work presented in this PhD thesis as well as other research performed by our group, could already provide reassuring answers to the concerns practitioners had who did not de-implement early-PN. In the future, frequent careful monitoring of the nutritional practices in PICUs worldwide should be continued, in order to find strategies to accelerate de-implementation of ineffective, unproven or harmful healthcare.

In conclusion, in this PhD thesis, we showed that the peripheral inactivation of thyroid hormone during pediatric critical illness, which is further accentuated by accepting a macronutrient deficit in the first week in the PICU, might be a beneficial response, whereas the central component of NTI might be maladaptive. This implies that future research should focus on treatment of the central component to improve outcome. Next, we showed that systemic cortisol availability is elevated only transiently in critically ill children and is not driven by elevated ACTH. As low ACTH and high cortisol, as well as corticosteroid treatment, predicted poor outcome, exogenously increasing cortisol availability during the acute phase might be inappropriate. We further showed that children experience a severe legacy in multiple domains of their physical and neurocognitive development 4 years after critical illness. Whether the disturbances in the HPT or HPA axis may contribute remains to be investigated. However, part of this legacy, more specifically the emotional and behavioral problems, can be prevented by withholding of supplemental parenteral nutrition in the first week in the PICU. Prevention of altered DNA methylation was found to be a potential biological mediator hereof. These findings can provide reassuring answers to some of the concerns raised by the respondents of the survey who did not de-implement early-PN in their PICU.

SAMENVATTING

Samenvatting

Kritiek zieke kinderen die opgenomen worden op de pediatrische intensieve zorgen afdeling hebben acute nood aan ondersteuning van de vitale organen om een nakend overlijden te vermijden. Hoewel de meeste kinderen op deze afdeling slechts gedurende een paar dagen nood hebben aan intensieve zorgen, en de meesten ook snel lijken te herstellen van de acute aandoening, ondervinden velen van hen belangrijke lange termijn gevolgen, zelfs jaren na hun opname. Deze “erfenis” van lange termijn gevolgen blijft grotendeels onverklaard, en heeft een weerslag op de verschillende domeinen van neurocognitieve ontwikkeling, groei en levenskwaliteit, en dit onafhankelijk van onderliggende aandoeningen of ziekten.

Eerder onderzoek toonde aan dat deze gevolgen op lange termijn tot op zekere hoogte beïnvloedbaar zijn door het wijzigen van enkele specifieke factoren tijdens het verblijf op de afdeling intensieve zorgen. Onze onderzoeksgroep voerde eerder de grote multicentrische PEPaNIC gerandomiseerde en gecontroleerde studie uit. Deze studie includeerde 1440 kritiek zieke kinderen om het effect te bestuderen van het vroeg starten van aanvullende parenterale voeding (binnen 24 uur na opname, (vroege-PN) bij ontoereikende enterale voeding ten opzichte van het niet toedienen van dergelijke aanvullende parenterale voeding gedurende de eerste 7 dagen van de opname op de pediatrische intensieve zorgen afdeling (late-PN). Het aanvaarden van een tekort aan macronutriënten door het toepassen van late-PN bleek klinisch beter te zijn dan de vroege-PN strategie, gezien kinderen in de late-PN groep sneller herstelden en een lager risico hadden op het ontwikkelen van een nieuwe infectie tijdens de opname. De 2-jaar opvolgingsstudie van de PEPaNIC studie toonde bovendien aan dat late-PN ook verschillende neurocognitieve uitkomsten verbeterde en zelfs normaliseerde in deze kinderen, en in het bijzonder de executieve functie van inhibitorische controle. Het was echter onduidelijk of de verstoorde ontwikkeling in fysieke en neurocognitieve domeinen die vastgesteld werd 2 jaar na inclusie zouden persisteren of verdwijnen in een latere fase. Het was ook mogelijk dat andere problemen gerelateerd aan de gerandomiseerde interventie pas later aan de oppervlakte zouden komen na 4 jaar. De acute fase van kritieke ziekte is ook gekenmerkt door verschillende neuro-endocriene veranderingen, mogelijks beïnvloed door het tekort aan macronutriënten veroorzaakt door late-PN, die mogelijks ook ontwikkeling en groei kunnen beïnvloeden op lange termijn. Dit doctoraatsproject documenteerde eerst de veranderingen in de hypothalamus-hypofyse-schildklieras en in de hypothalamus-hypofyse-bijnieras, en onderzocht de impact van het tekort aan macronutriënten onder late-PN hierop. Het onderzocht ook de effecten op lange termijn van pediatrische kritieke ziekte en het vroege tekort aan macronutriënten op neurocognitieve ontwikkeling, groei en fysiek functioneren 4 jaar na opname op de pediatrische intensieve zorgen afdeling. Het laatste objectief van deze doctoraatsthesis was het onderzoeken van de de-implementatie van vroege-PN op pediatrische intensieve zorgen afdelingen wereldwijd.

In het eerste deel van deze doctoraatsthesis bevestigden we eerst het optreden van het “non-thyroidal illness syndroom” (NTI) bij opname, met lage serumconcentraties van TSH, T4 en T3, en verhoogde serumconcentraties van rT3. De ernst van NTI bij opname was onafhankelijk geassocieerd met slechtere uitkomst op de intensieve zorgen afdeling. Het is bekend dat NTI ook voorkomt bij vasten. Consistent met het nabootsen van een vastenrespons, resulteerde het tekort aan macronutriënten in de patiënten in de late-PN groep in een verergering van NTI over de eerste dagen tijdens opname op de pediatrische intensieve zorgen afdeling in vergelijking tot de respons bij patiënten in de vroege-PN groep. Bovendien toonde een statistische mediatieanalyse aan dat de perifere inactivatie van schildklierhormoon, die verder werd geaccentueerd door late-PN over de eerste dagen na opname, bijdroeg tot het gunstige effect van late-PN op korte termijn, en dus een gunstig antwoord lijkt te zijn van het lichaam op kritieke ziekte. Daarentegen bleek de centrale component van NTI, toe te schrijven aan het onderdrukte TSH en weerspiegeld in de lage T4 concentraties, het gunstige effect van late-PN op de korte termijn tegen te werken. Hieruit valt af te leiden dat de centrale component van NTI mogelijks een schadelijke respons betekent. Verder onderzoek onder de vorm van gerandomiseerde studies met voldoende bewijskracht moet uitwijzen of het behandelen van deze centrale component met TRH-infusie de uitkomst van kritiek zieke kinderen kan verbeteren.

In het tweede deel werden de veranderingen in de hypothalamus-hypofyse-bijnier as tijdens de acute fase van kritieke ziekte in kinderen gedocumenteerd. In tegenstelling tot kritiek zieke volwassenen, waren de cortisolwaarden in kinderen slechts kort gestegen bij admissie op de pediatrische intensieve zorgen afdeling, en normaliseerden ze nadien snel ondanks lage bindingseiwitten en een persisterende onderdrukking van het cortisolmetabolisme. De cortisolbeschikbaarheid was niet gedreven door een gestegen ACTH, gezien ACTH-waarden normaal waren bij opname en nadien snel daalden. Op dag 3 van opname voorspelden zowel hoge cortisolwaarden en lage ACTH-waarden onafhankelijk slechte uitkomsten op korte termijn. De behandeling met corticosteroïden onderdrukte verder de ACTH-waarden en was ook onafhankelijk geassocieerd met slechte klinische uitkomsten. Deze bevindingen suggereren dat het exogeen verhogen van cortisolbeschikbaarheid tijdens de acute fase van kritieke ziekte in kinderen niet gepast lijkt, en dat toekomstige studies over behandeling met corticosteroïden veiligheidsanalyses moeten inplannen, gezien dergelijke behandeling mogelijks schade induceert.

In het derde deel van deze doctoraatsthesis werden de resultaten van de 4-jaar opvolgingsstudie van de PEPaNIC studie voorgesteld, waarin het effect werd bestudeerd van late-PN ten opzichte van vroege-PN op antropometrie, gezondheidsstatus, executieve functies en emotionele en gedragsproblemen zoals gerapporteerd door ouders of zorgverleners, en op klinische testen voor intelligentie, visuele-motorische integratie, alertheid, motorische coördinatie en geheugen. Vier jaar na inclusie scoorden deze kinderen slechter op bijna elk ontwikkelingsdomein, in vergelijking met hun gezonde leeftijdsgenoten. Een deel van

deze problematiek, en meer specifiek de internaliserende, externaliserende en totale emotionele/gedragsproblemen gerapporteerd door ouders of zorgverleners, kon echter voorkomen worden in patiënten in de late-PN groep die geen aanvullende parenterale voeding kregen tijdens de eerste week van hun opname op de pediatrische intensieve zorgen afdeling. De emotionele/gedragsproblemen die dus te wijten zijn aan het gebruik van vroege-PN waren ook minstens gedeeltelijk gemedieerd door ongunstige wijzigingen in het DNA methylatieprofiel veroorzaakt door vroege-PN tijdens opname. Bijgevolg, kan het vermijden van wijzigingen in DNA methyalatiestatus geïnduceerd door vroege-PN tijdens het verblijf op de intensieve zorgen afdeling dus een biologische mediator zijn van de bescherming die de late-PN strategie biedt tegen emotionele/gedragsproblemen 4 jaar later. Kortom, deze bevindingen vormen een belangrijke verdere ondersteuning om de de-implementatie van het toedienen van parenterale voeding tijdens de eerste week op de pediatrische intensieve zorgen afdeling door te voeren.

In een laatste deel werd de de-implementatie van vroege-PN in pediatrische intensieve zorgen afdelingen wereldwijd onderzocht, door het analyseren van de resultaten van een enquête die een jaar na publicatie van de PEPaNIC studie werd verspreid. De enquête werd ingevuld door zorgverleners werkzaam in 81 verschillende pediatrische intensieve zorgen afdelingen in 39 verschillende landen. Op het moment van de enquête was twee-derde van de zorgverleners op de hoogte van de resultaten van de PEPaNIC studie, waarvan 12% voordien al geen parenterale voeding meer toediende aan kritiek zieke kinderen op hun afdeling. Een andere 12% hiervan startte met de de-implementatie van vroege-PN na het lezen van het PEPaNIC artikel, en 40% de-implementeerde gedeeltelijk door het uitstellen van het opstarten van parenterale voeding (maar nog steeds met opstart vóór dag 8) en/of door het toedienen van kleinere hoeveelheden van parenterale voeding. Wanneer vroege-PN helemaal niet werd gede-implementeerd, gaven de zorgverleners aan dat de reden hiervoor was dat ze bezorgd waren over de doeltreffendheid en de (lange termijn) veiligheid van late-PN, of dat er gewacht werd op de aanpassingen van officiële internationale richtlijnen. In de tussentijd kunnen zowel de recent gepubliceerde richtlijnen, het werk uit deze doctoraatsthesis en het andere onderzoek verricht door onze onderzoeksgroep reeds geruststellende antwoorden bieden op de zorgen van deze zorgverleners die nog geen de-implementatie van vroege-PN toepasten. In de toekomst blijft het frequent en nauwkeurig opvolgen van de nutritionele gebruiken op de pediatrische intensieve zorgen afdelingen nodig, zodat strategieën om snellere de-implementatie van niet-effectieve, niet-bewezen en zelfs schadelijke zorg kunnen ontwikkeld worden.

Uit het werk beschreven in deze doctoraatsthesis kunnen we verscheidene belangrijke conclusies trekken met belangrijke klinische implicaties. We toonden aan dat de perifere inactivatie van schildklierhormoon tijdens kritieke ziekte in kinderen, die verder geaccentueerd wordt door het aanvaarden van een tekort aan macronutriënten in de eerste week op de pediatrische intensieve zorgen afdeling, mogelijks een nuttige

respons is, terwijl de centrale component van NTI maladaptief lijkt te zijn. Dit impliceert dat verder onderzoek zou moeten focussen op de behandeling van de centrale component om klinische uitkomsten te verbeteren. Daarnaast toonden we aan dat systemische cortisolbeschikbaarheid slechts kortdurend gestegen is in kritiek zieke kinderen, en niet kan gedreven zijn door gestegen ACTH. Gezien lage ACTH- en hoge cortisolwaarden, alsook de behandeling met corticosteroïden, allen slechte uitkomst op korte termijn voorspellen, lijkt het exogeen verhogen van cortisolbeschikbaarheid tijdens de acute fase van kritieke ziekte bij kinderen niet gepast. Verder toonden we aan dat de kinderen ernstige gevolgen dragen in meerdere domeinen van fysieke en neurocognitieve ontwikkeling 4 jaar na hun kritieke ziekte. Een deel van deze gevolgen kan vermeden worden door het weerhouden van parenterale voeding in de eerste week tijdens opname op de pediatrische intensieve zorgen afdeling. De preventie van wijzigingen in DNA methylatiestatus kan een biologische mediator zijn hiervan. Deze bevindingen voorzien geruststellende antwoorden op een aantal vragen van zorgverleners die volgens een wereldwijde enquête nog geen de-implementatie van vroege-PN toepasten op hun afdeling.

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Scientific acknowledgments, personal contribution and conflict of interest

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PERSONAL CONTRIBUTION

Chapter 1, 2 and 7
An Jacobs wrote these chapters.

Chapter 3 and 4
An Jacobs contributed to the writing of the statistical analysis plan, the laboratory and statistical analyses, and the writing of the paper.

Chapter 5
An Jacobs contributed to the gathering of the data, the statistical analyses and the writing of the paper.

Chapter 6
An Jacobs contributed to the design and translation of the survey and the writing of the paper.

CONFLICT OF INTEREST

None of the funding sources mentioned above were involved in the study design, patient recruitment, data collection and analysis, or in the interpretation of the results. None of the authors report any conflict of interest.

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Curriculum Vitae
An Jacobs

1. EMPLOYMENT

- PhD Student, Laboratory of Intensive Care Medicine (2016-present), KU Leuven, Leuven, Belgium.
- Medical Doctor, Trainee in Pediatrics (2013-present, training will be completed in July 2020), University Hospitals Leuven, Leuven, Belgium

2. EDUCATION AND PROFESSIONAL EXPERIENCE

2016 – present	Doctoral training, Doctoral School of Biomedical Sciences, KU Leuven, Belgium.
2013 – present	Master after Master in Specialised Medicine, Pediatrics
2016	Specialist training in Pediatrics, Stellenbosch University Hospital, Cape Town, South Africa.
2015	Specialist training in Neonatology, St-Augustinus Hospital, Wilrijk, Belgium
2014 – 2015	Specialist training in Pediatrics, St-Luc UCL University Hospital, Woluwe, Belgium.
2013 – 2014	Specialist training in Pediatrics, St-Vincentius Hospital, Antwerp, Belgium.
2009 – 2013	Master's degree in medicine, Magna Cum Laude, Catholic University of Leuven, Belgium.
2006 – 2009	Bachelor's degree in medicine, Catholic University of Leuven, Belgium.
2000 – 2006	Secondary School degree in Science-Mathematics, College of Montfort, Rotselaar, Belgium.

3. CERTIFICATES AND AWARDS

2018	**Outstanding Abstract Award** with travel grant for "Prognostic value of the nonthyroidal illness syndrome in the pediatric intensive care unit and impact hereon of nutritional management in relation to clinical outcomes" at the Endocrine Society's Annual Meeting, Chicago, US
2018	**Presidential Poster Competition Winner** in the Pediatric Endocrinology category at the Endocrine Society's Annual Meeting, Chicago, US
2017	**Young Investigator Award**, 27th Meeting of the Belgian Endocrine Society, Terhulpen
2017	Certificate in **Writing for a general audience**, Instituut voor Levende Talen, KU Leuven, Leuven
2017	Certificate in **Advanced Paediatric Life Support**, Advanced Life Support Group, Malle
2017	Certificate Course in **Abdominal ultrasonography**, Vlaamse Vereniging voor Echografie, Leuven
2016	Certificate **Presentation and seminar skills for biomedical researchers**, Instituut voor Levende Talen, KU Leuven, Leuven
2016	Certificate in training in **Applied Good Clinical Practice** for investigators & Site Personnel, European Forum for Good Clinical Practice and the UZ Leuven Clinical Trial Center, Leuven
2015	Certificate in **European Paediatric Life Support**, European Resuscitation Council, Leuven
2014	Certificate in **Neonatal Life Support**, European Resuscitation Council, Aartselaar

4. PRESENTATIONS AT CONFERENCES AND SYMPOSIA

1. **An Jacobs**, Ilse Vanhorebeek, Inge Derese, Sarah Vander Perre, Pieter Wouters, Sascha Verbruggen, Koen Joosten, Greet Van den Berghe. Changes in the hypothalamic-pituitary-adrenal axis during pediatric critical illness. Oral presentation at the 101st Annual Meeting of the Endocrine Society – ENDO, New Orleans, US, March 2019.

2. **An Jacobs**, Ilse Vanhorebeek, Inge Derese, Sarah Vander Perre, Esther van Puffelen, Sören Verstraete, Lies Pauwels, Sascha Verbruggen, Pieter J Wouters, Lies langouche, Gonzalo Garcia Guerra, Koen Joosten, Greet Van den Berghe. Prognostic value of the nonthyroidal illness syndrome in the pediatric intensive care unit and impact hereon of nutritional management in relation to clinical outcomes. Oral and poster presentation at the 100th Annual Meeting of the Endocrine Society – ENDO, Chicago, US, March 2018.

3. **An Jacobs**, Ilse Vanhorebeek, Inge Derese, Sarah Vander Perre, Esther van Puffelen, Sören Verstraete, Lies Pauwels, Sascha Verbruggen, Pieter J Wouters, Lies langouche, Gonzalo Garcia Guerra, Koen Jossten, Greet Van den Berghe. Prognostic value of the nonthyroidal illness syndrome in the pediatric intensive care unit and impact hereon of nutritional management in relation to clinical outcomes. Poster presentation at the 27th meeting of the Belgian Endocrine Society, Terhulpen, October 2017.

4. **An Jacobs**, Frederic De Meulder, François Eyskens. Floppy infant syndrome associated with vitamin B12 deficiency in the mother. Poster presentation at the 42th annual meeting of the Belgische Vereniging voor Kindergeneeskunde, Brugge, March 2014

5. Publications

- **An Jacobs**, Karolijn Dufler, Renate Eveleens, José Hordijk, Hanna Van Cleemput, Ines Verlinden, Pieter Wouters, Liese Mebis, Gonzalo Garcia Guerra, Koen Joosten, Sascha Verbruggen, Fabian Güiza, Ilse Vanhorebeek, Greet Van den Berghe. Long-term developmental impact of withholding parenteral nutrition in pediatric-ICU: a 4-year follow-up of the PEPaNIC randomized controlled trial. Accepted for publication in The Lancet Child & Adolescent Health, in press.
- **An Jacobs**, Inge Derese, Sarah Vander Perre, Pieter J Wouters, Sascha Verbruggen, Jaak Billen, Pieter Vermeersch, Gonzalo Garcia Guerra, Koen Jossten, Ilse Vanhorebeek, Greet Van den Berghe. Dynamics and prognostic value of the hypothalamus-pituitary-adrenal axis responses to pediatric critical illness and association with corticosteroid treatment: a prospective observational study. Intensive Care Medicine 2020;46:70-81 **(IF 18.9)**
- Lies Langouche, **An Jacobs**, Greet Van den Berghe. Nonthyroidal illness syndrome across the ages. Journal of the Endocrine Society 2019;3:2313-2325. **(no IF yet)**
- **An Jacobs**, Ilse Vanhorebeek, Greet Van den Berghe. Nonthyroidal illness in critically ill children. Current Opinion Endocrinology, Diabetes and Obesity 2019;26:241-249.**(IF 3.2)**
- **An Jacobs,** Ines Verlinden, Ilse Vanhorebeek, Greet Van den Berghe. Early Supplemental Parenteral Nutrition in Critically Ill Children: An Update. Journal of Clinical Medicine 2019;8,830. **(IF 5.6)**
- **An Jacobs**, Inge Derese, Sarah Vander Perre, Esther van Puffelen, Sören Verstraete, Lies Pauwels, Sascha Verbruggen, Pieter Wouters, Lies Langouche, Gonzalo Garcia Guerra, Koen Joosten, Ilse Vanhorebeek, Greet Van den Berghe . The non-thyroidal illness syndrome in critically ill children: prognostic value and impact of nutritional management. Thyroid 2019:29:480-492. **(IF 7.5)**
- **An Jacobs**, Marine Flechet, Ilse Vanhorebeek, Sören Verstraete, Catherine Ingels, Michael P Casaer, Gerardo Soto-Campos, Sascha C Verbruggen, Koen F Joosten, Fabian Güiza, Greet Van den Berghe. Performance of pediatric mortality prediction scores for pediatric intensive care unit mortality and 90-day mortality. Pediatric Critical Care Medicine 2019;20(2):113-119. **(IF 3.0)**
- Govindan Malarvannan, Matthias Onghena, Sören Verstraete, Esther van Puffelen, **An Jacobs**, Ilse Vanhorebeek, Sascha Verbruggen, Koen F Joosten, Greet Van den Berghe, Philippe Jorens. Phthalate and alternative plasticizers in indwelling medical devices in pediatric intensive care units. Journal of Hazardous Materials 2019;363:64-72. **(IF 7.6)**
- Esther van Puffelen, **An Jacobs**, Charlotte J.M. Verdoorn, Koen F.M. Joosten, Greet van den Berghe, Erwin Ista, Sascha C.A.T. Verbruggen. Worldwide survey of de-implementation of initiating parenteral nutrition early in paediatric intensive care units. BMC Health Services Research 2019;19:279. **(IF 1.9)**
- Sören Verstraete, Sascha C Verbruggen, José A Hordijk, Ilse Vanhorebeek, Karolijn Dulfer, Fabian Güiza, Esther van Puffelen, **An Jacobs**, Sandra Leys, Astrid Durt, Hanna Van Cleemput, Renate D Eveleens, Gonzalo Garcia Guerra, Pieter J Wouters, Koen F Joosten, Greet Van den Berghe. Long-term developmental effects of withholding parenteral nutrition for 1 week in the paediatric intensive care unit: a 2-year follow-up of the PEPaNIC international, randomised, controlled trial. Lancet of Respiratory Medicine 2019;7(2):141-153. **(IF 22.9)**
- **An Jacobs**, François Vermeulen, Kris de Boeck, Kristina Casteels, Marijke Proesmans. Clinical outcome of CF patients with CF related diabetes: do we need to change our policy? Open Journal of Pathology 2016;6:32-40. **(IF 0.6)**
- **An Jacobs**, Gert Van Assche, August Van Olmen. Cytomegalovirus-colitis bij exacerbatie van inflammatoire darmziekte: pathogene rol of merker van Ernst? Tijdschrift voor Geneeskunde 2013;69:816-824. **(no IF yet)**
- **An Jacobs**, Frederic De Meulder, François Eyskens. Floppy infant syndrome associated with vitamin B12 deficiency in the mother. Tijdschrift van de Belgische kinderarts 2014;16:406 (abstract). **(no IF yet)**

6. Extracurricular activities

- Running and hiking, skiing, kickboxing and cycling.
- Board member of Jong VVK (Vlaamse Vereniging voor Kindergeneeskunde), which aims to improve the education for trainees in Pediatrics in Flanders
- Former member of KLJ (Katholieke Landelijke Jeugd) on regional and national level, where I organised training courses and education sessions for adolescents volunteering in youth work.