ACUTE STRESS RESPONSE

IN CRITICALLY ILL CHILDREN

ENDOCRINE ASPECTS

CIP-gegevens Koninklijke Bibliotheek, Den Haag

ISBN 90-56-770-462

Printed by PrintPartners Ipskamp, Enschede
Design cover and drawings: Ramses P.W.G. de Pauw, Antwerpen
Lay-out: Berry P.L.M. den Brinker en Marieke den Brinker, Amsterdam

This research presented in this thesis was financially supported by Pharmacia Sweden, now Pfizer.

The printing of this thesis was financially supported by Pfizer Nederland.

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ACUTE STRESS REACTIE
IN KINDEREN MET LEVENSBEDREIGENDE ZIEKTE
ENDOCRIENE ASPECTEN

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 8 februari 2006 om 11.45 uur

door

Marieke den Brinker
geboren te Amsterdam

Promotiecommissie

Promotor:	Prof.dr. A.C.S. Hokken-Koelega
Overige leden:	Prof.dr. F.H. de Jong Prof.dr. D. Tibboel Prof.ir. T.J. Visser
Copromotor:	Dr.K.F.M. Joosten

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INTRODUCTION

General introduction

The first person to introduce the term “stress (response)” was Hans Selye in 1955 and he described it as “the non-specific response of the body to any demand” (1). As all living beings strive for homeostasis, which is the ability to maintain the consistency of their internal milieu despite changes in their surroundings (2), the stress response may also be defined as: the body’s non-specific response to maintain homeostasis of the internal environment. One ultimate example of stress is critical illness (3).

The effects of critical illness are manifold and not the least among them are the profound changes seen in endocrine and metabolic systems. Initially, hormones primarily under the control of hypothalamic-pituitary axis and the autonomic nervous system enact the endocrine response to critical illness. Importantly, these endocrine responses are under control of factors from the immune system, such as cytokines.

It has been proposed that critical illness falls, at least in adults, into two endocrine and metabolically distinct phases: acute and chronic (Figure 1) (4). The endocrine changes during the acute phase of critical illness consist primarily of activation of the so-called “catabolic pathways” (cortisol) and peripheral inactivation of “anabolic pathways” (low IGF-I and testosterone levels), whereas pituitary activity is essentially maintained or amplified. In critically ill adults, these changes have consistently been viewed as adaptive or beneficial, as they may redirect and reduce energy consumption, postpone anabolism and, at the same time, activate the immune response while protecting the host against deleterious biological effects of the latter. In contrast to the changes seen in the acute phase of critical illness, prolonged critical illness is characterized, at least in adults, by reduced pulsatile secretion of anterior pituitary hormones, which correlate positively with reduced activity of target tissues, with cortisol secretion as the notable exception as levels remain elevated through a peripheral drive (4). Unlike the situation during the acute phase of critical illness, hormonal changes seen during the prolonged phase of adult critical illness have been assumed not to be adaptive.

The course of critical illness in children differs from adults. Critical illness in children may develop quickly and if children survive the impact of acute critical illness, it is rapidly followed by recovery. Unlike adults, chronic or protracted critical illness is fairly uncommon in children. Although in adults the endocrine and metabolic changes during the acute phase of critical illness have been viewed as adaptive it is still unclear to which extent some of these defense mechanisms may hypo- or hyper-respond and as a consequence be harmful. Understanding of the endocrine and metabolic changes of pediatric critical illness, especially those in the acute phase, is important and may improve outcome, as it allows the rational use of pharmaceutical interventions. It may also reveal endocrine and metabolic changes to be used as prognostic markers. This prompted us to study and describe the acute

stress response of critically ill children, due to meningococcal sepsis or septic shock or after open-heart operation, within the first days of admission on the pediatric intensive care (PICU). This thesis focuses on three hormonal axes: the hypothalamic-pituitary-adrenal (HPA) axis, the hypothalamic-pituitary-thyroid (HPT) axis and the growth hormone-insulin-like growth factor-I (GH-IGF-I) axis.

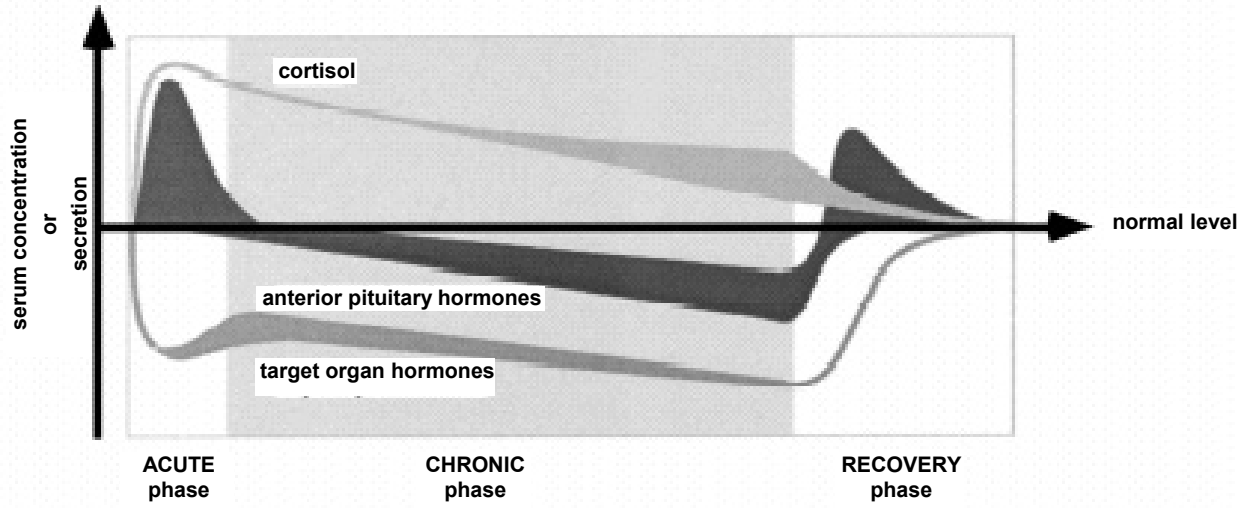

Figure 1. Simplified concept of the pituitary-dependent changes during the course of critical illness in adults (adapted form Van den Berghe {Van den Berghe, 1998 #455}). In the acute phase of illness (first hours to a few days after onset), the secretory activity of the anterior pituitary is essentially maintained or amplified, whereas anabolic target organ hormones are inactivated. Cortisol levels are elevated in concert with ACTH. In the chronic phase of protracted critical illness (intensive care dependent for weeks), the secretory activity of the anterior pituitary appears uniformly suppressed in relation to reduced circulating levels of target organ hormones. Impaired anterior pituitary hormone secretion allows the respective target organ hormones to decrease proportionately over time, with cortisol being a notable exception, the circulating levels of which remain elevated through a peripheral drive, a mechanism that ultimately may also fail. The onset of recovery is characterized by restored sensitivity of the anterior pituitary to reduced feedback control.

The hypothalamic-pituitary-adrenal axis

The adrenal cortex produces steroid hormones, which can be divided into mineralocorticoids, glucocorticoids and adrenal androgens (5). *Mineralocorticoids*, principally aldosterone, regulate renal retention of sodium, which influences intravascular volume and blood pressure. *Glucocorticoids*, principally cortisol, also known as the “stress hormone”, have a wide variety of bodily functions, such as maintaining vascular tone, endothelial and vascular integrity, potentiating the vasoconstrictor actions of catecholamines, muting the inflammatory cascade and promoting substrate mobilization (6). *Adrenal androgens* play a role in the mid-childhood growth spurt. These steroid hormones are produced in the three zones of the adrenal cortex, which appear histologically and functionally different. In anatomical order from outside to inside these zones are named: *zona glomerulosa*,

zona fasciculata and *zona reticularis*, producing mineralocorticoids, glucocorticoids and adrenal androgens, respectively. Figure 2 presents these steroidogenic pathways starting from the common substrate cholesterol. After birth the three zones simultaneously enlarge and differentiate. The zona glomerulosa and zona fasciculata reach full diffentiation at about 3 years of age and the zona reticularis may not be fully differentiated until about 15 years of age (5).

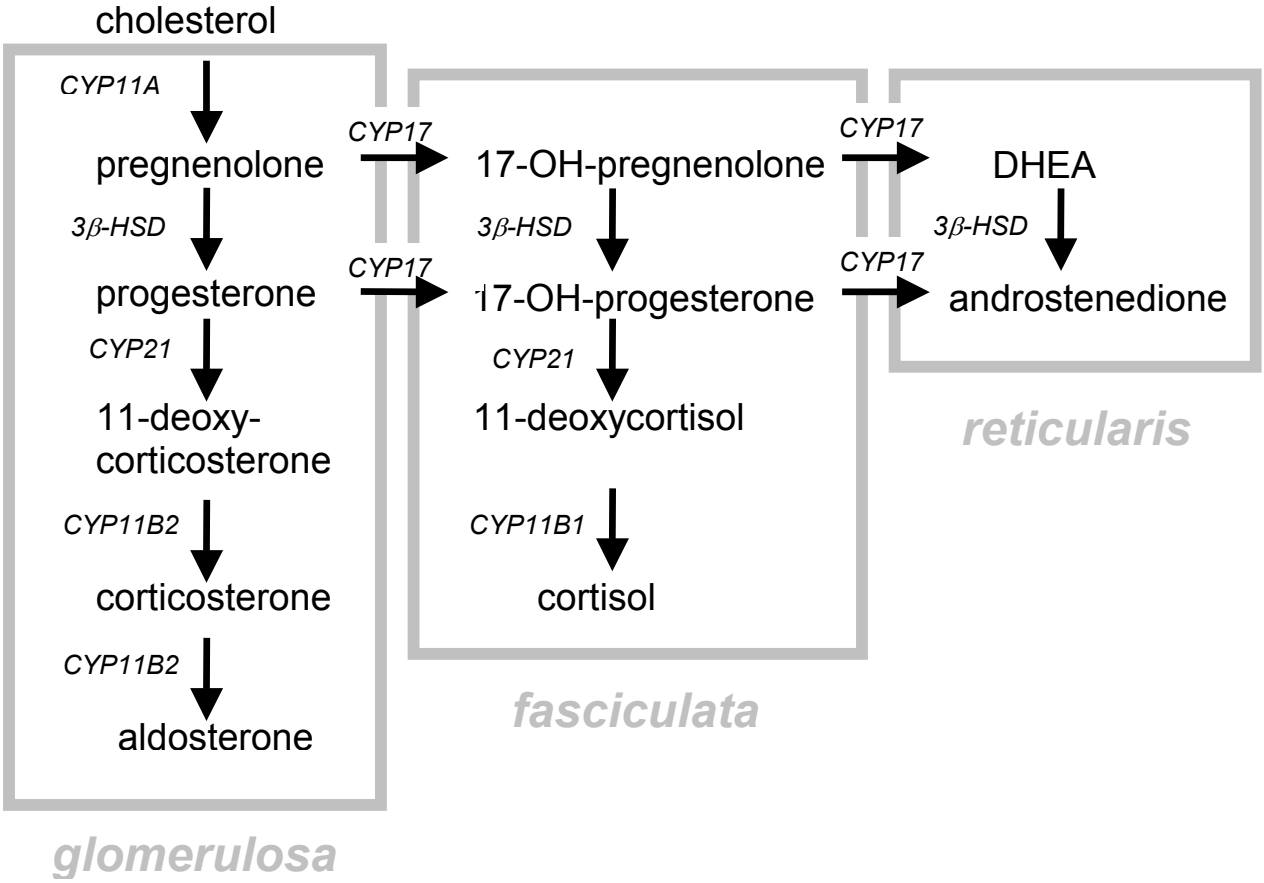

Figure 2. A schematic representation of steroidogenesis in the three zones of the human adrenal cortex. Cholesterol side-chain cleavage enzyme system *(CYP11A)*; 3β-hydroxysteroid-dehydrogenase *(3β-HSD)*; 21-hydroxylase *(CYP21)*; 17-hydroxylase & 17,20-lyase *(CYP17)*; 11β-hydroxylase *(CYP11B1)*; 11β- & 18-hydroxylase *(CYP11B2)*.

Steroidogenesis in the zona fasciculata and zona reticularis is under primary control of the hypothalamic-pituitary unit, whereas steroidogenesis in the zona glomerulosa is mainly regulated by the renin-angiotensin system. In healthy, unstressed humans, cortisol is secreted according to a diurnal pattern under the stimulatory influence of corticotropin (ACTH) secreted by the pituitary gland (Figure 3). ACTH secretion, in turn, is under stimulatory influence of the hypothalamic corticotropin-releasing hormone (CRH). Both CRH and ACTH are subject to negative feedback control by cortisol. In the circulation cortisol is bound to carrier proteins, mainly cortisol-binding globulin (CBG) and albumin, leaving less than 10 percent in

the free, bioavailable form (7). It is generally believed that the biological activity of cortisol depends on the free fraction of the hormone (8).

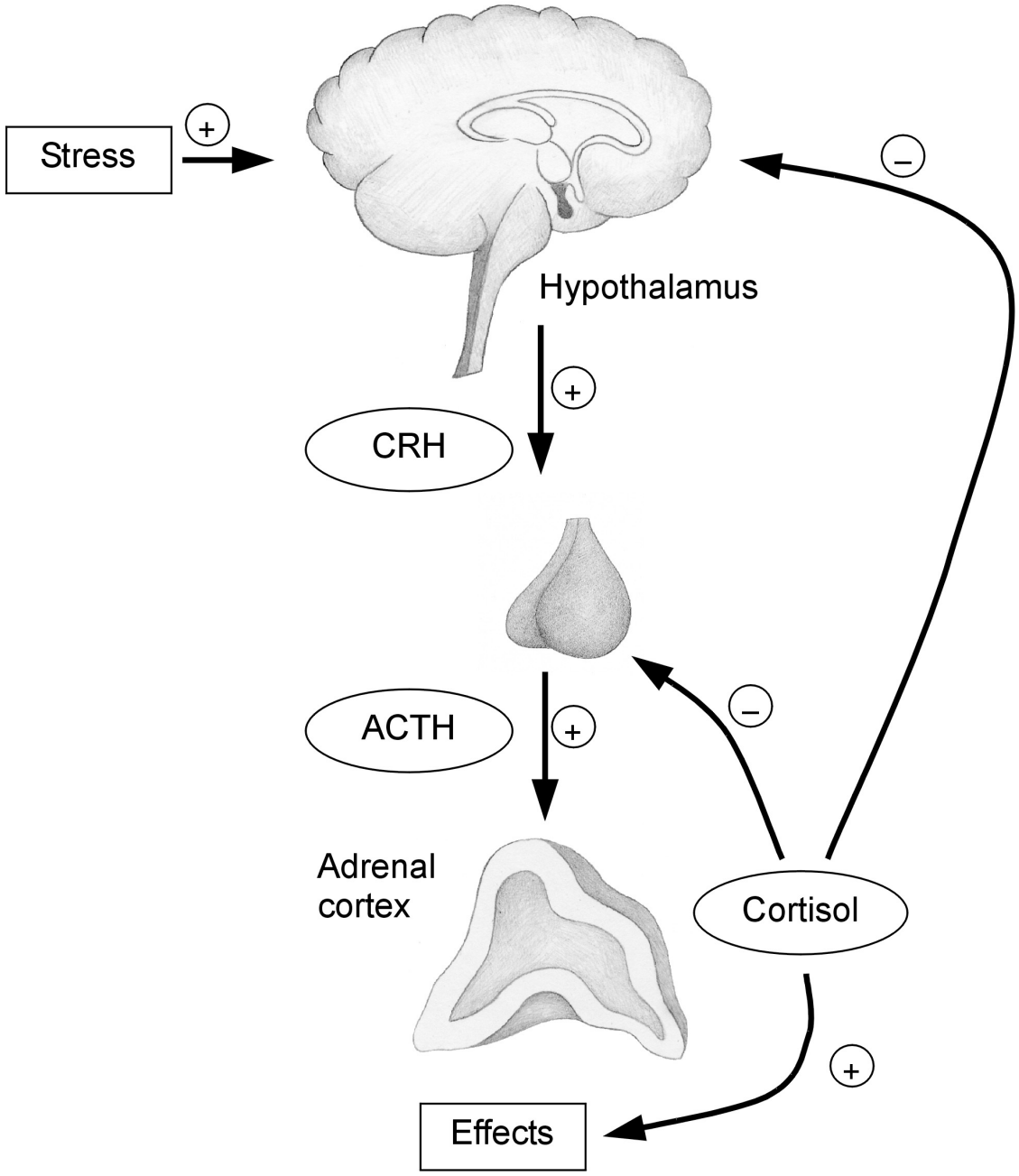

Figure 3. Simplified concept of the hypothalamic-pituitary-adrenal axis and its regulation.

In both adults and children, the physiological response to stress involves activation of the central nervous system with consequently stimulation of the hypothalamic-pituitary-adrenal (HPA) axis (9). Stressors are among other things cytokines, pain, tissue damage, surgery, hypoxemia, hypotension and hypoglycemia (10, 11). Activation of the HPA-axis will lead to increased levels of cortisol, loss of

the diurnal rhythm and reduced negative feedback control (12). In addition, CBG levels decrease rapidly in critically ill adults, resulting in increased levels of free, bioavailable cortisol, especially in the acute phase of critical illness (12-15).

Stimulation of the hypothalamic-pituitary adrenal axis, resulting in elevated serum cortisol levels, is an essential component of the general adaptation to critical illness. Even minor degrees of adrenal insufficiency, by some referred to as relative adrenal insufficiency, increase the mortality of critically ill patients. A clear definition of (relative) adrenal insufficiency in critical illness is, however, lacking and there is also much uncertainty about the most appropriate test to assess the integrity of the HPA-axis. Some authors advise a random total cortisol level (16) or free cortisol level (13-15), whereas others advise an ACTH-stimulation test (Synacthen®) (17, 18) or a CRH-test (19). The ACTH-stimulation test can be performed classically with a high dose (250 µg) of Synacthen® (17, 20) or alternatively with a low dose (1 µg) of Synacthen® (18, 21, 22), which has been assumed to have higher sensitivity and specificity in critical illness.

In critically ill children paradoxically lower cortisol levels in the most severely ill compared with the less severely ill have been reported (23-28). Data on bioavailable cortisol did, to our knowledge, not exist for acutely ill children. Therefore, it remained unclear, whether bioavailable cortisol levels in the most severely ill children were lowered as well. One of the explanations of low cortisol levels in children with meningococcal septic shock might be reduced activity of adrenal steroidogenic enzymes (Figure 2). During sepsis, adrenal steroidogenic enzyme function might be liable to circulating endotoxins, cytokines, and medication, whereas more intrinsically gene polymorphisms might predispose for decreased adrenal steroidogenic enzyme function as well (29, 30). Decreased steroidogenic enzyme function leads in general to lower serum levels of the downstream product in the presence of increased serum levels of its upstream precursor. Sepsis-induced adrenal insufficiency might also include functional loss of the zona glomerulosa and defects in the renin-aldosterone axis might thereby adversely affect cardiovascular homeostasis and thus outcome. Hyperreninemic hypoaldosteronism has been reported in critically ill adults (31), whereas lower plasma aldosterone concentrations were recently reported in children with meningococcal sepsis compared to other critically ill children (32). Data concerning the renin-aldosterone axis in relation to outcome in children with meningococcal sepsis were, however, lacking.

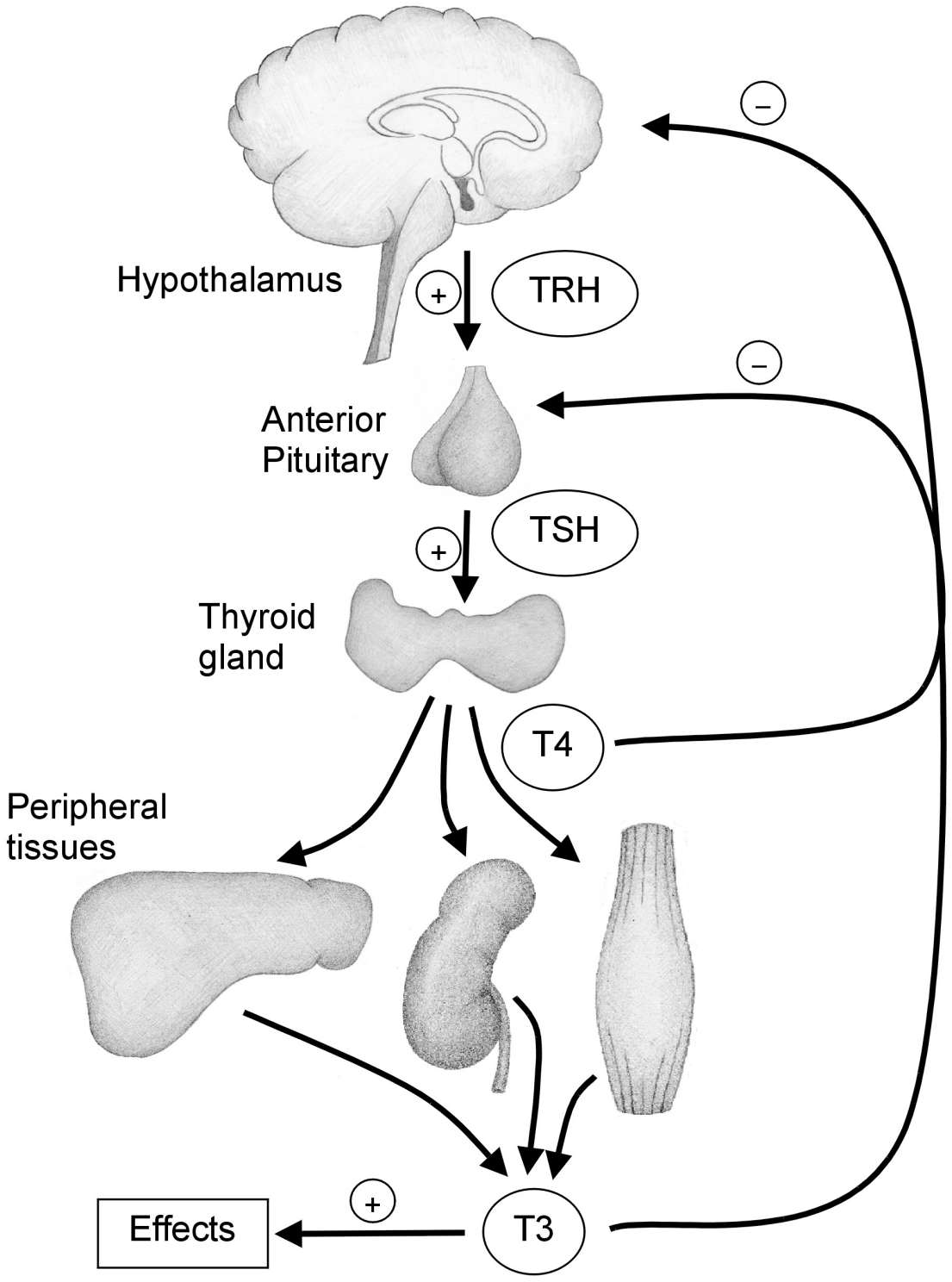

Figure 4. Simplified concept of the hypothalamic-pituitary-thyroid axis and its regulation. Under normal circumstances T4 is converted into active T3 in peripheral tissues, especially liver, kidney and skeletal muscle.

The hypothalamic-pituitary-thyroid axis

Thyroid hormone exerts a broad range of effects on development, growth and metabolism (33). Thyroxine (T4), the primary secreted product of the thyroid gland, is inactive until it is converted to the active hormone triiodothyronine (T3) *(see below*). T4 can therefore be considered as a pro-hormone. In healthy, unstressed humans, thyroidal secretory activity is regulated via the hypothalamic-pituitary unit (Figure 4). Thyrotropin releasing hormone (TRH), synthesized in the hypothalamus, governs the pituitary's release of thyrotropin or thyroid-stimulating-hormone (TSH). TSH stimulates the synthesis and release of thyroid hormones by the thyroid. Both TRH and TSH are subject to negative feedback control by T4 and T3, actually by the free levels of T4 and T3. Most of the thyroid hormones (T4 and T3) are bound to carrier proteins, mainly thyroxine-binding globulin (TBG), transthyretin and albumin, leaving less than 1 percent in the free, biologically available form (34). In addition, peripheral thyroid hormone metabolism plays an eminent role in the regulation of thyroid hormone bioactivity. The principal pathways of thyroid hormone metabolism are deiodination and conjugation, of which deiodination is the most important one (35). Under normal circumstances T4 is mainly deiodinated to active T3 by outer ring deiodination. Both T4 and T3 may also be inactivated by inner ring deiodination forming the inactive metabolites reverse-triiodothyronine (rT3) and 3,3'-diiodothyronine (T2), respectively (Figure 5).

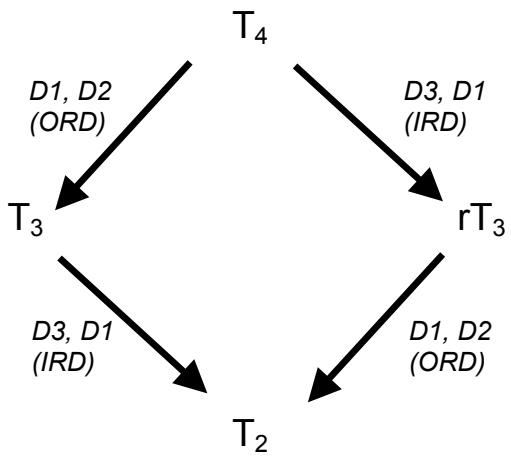

Figure 5. A schematic representation of thyroid hormone deiodination pathways. Under normal circumstances T4 is mainly denominated to active T3 by outer ring deiodination (ORD; by type 1 and type 2 deiodinase). Both T4 and T3 may also be inactivated by inner ring deiodination (IDR; by type 1 and type 3 deiodinase) forming the inactive substrates rT3 and T2.

Multiple alterations in the hypothalamus-pituitary-thyroid axis in critical illness have been recognized, the most prominent being the combination of high serum rT3 at the expense of low T3 levels, without compensatory elevated TSH levels, frequently coinciding with low T4 levels, forming the so called entity of "euthyroid sick

syndrome" or "non-thyroidal illness". In critically ill adults the degree of T3 suppression with concomitantly low TSH generally correlates positively with disease severity and duration, and negatively with outcome (36, 37). Based on studies in critically ill adults several factors have been proposed to contribute to the euthyroid sick syndrome (36): a) decreased concentrations of carrier proteins; b) inhibition of binding to carrier proteins, transport and metabolism by increased concentrations of non-estherified fatty acids (NEFAs) and bilirubin (38, 39); and c) changes in expression of transporters and deiodinases (40). Besides this, medication is known to influence serum thyroid hormones as well. Dopamine infusion may induce or aggravate the euthyroid sick syndrome in critical illness, by its suppressive effect on TSH secretion by the pituitary gland (41). Glucocorticoids and somatostatin *(see below)* may also suppress pituitary TSH release (42). An iatrogenic decrease of circulating T3 may, therefore, in addition to illness-induced changes, perpetuate the catabolic state of critically ill adults.

The euthyroid sick syndrome is well documented in children during and after cardiac surgery (43-53) and T3-repletion trials show promising results concerning improved post-operative cardiac function (50, 54, 55), although these trials did not show improved outcome and data on safety, and efficacy remain to be awaited (46, 56). Data in critically ill children, however, remain limited (57-59) and the relationship between thyroid hormone levels and mortality is less clear than in critically ill adults: a few studies in critically ill children showed an association between low levels of total T4 (TT4) (58) and total T3 (TT3) (60) with mortality, whereas others reported higher TT3 and TT4 levels in children who died compared to those who survived (23). However, in some other studies on critically ill children no relation was found between thyroid hormones and outcome (57, 59). Furthermore, dopamine infusion may induce or aggravate the euthyroid sick syndrome in critically ill children as well (61). This might be especially harmful in critically ill infants, as it has been shown that impaired thyroid function during early infancy increases the risk for irreversible neurological damage (62). Besides this so far little is known about factors influencing changes in thyroid hormone levels in relation to outcome in critically ill children.

The growth hormone-insulin-like growth factor-I (GH-IGF-I) axis

The GH-IGF-I axis is a complex of exquisitely controlled physiological processes that regulate key aspects of growth and metabolism (63). GH is produced in and secreted by the pituitary gland in reaction to many different stimuli. The regular stimulus originates from the hypothalamus and is the result of the balance between growth hormone releasing hormone (GHRH) and somatostatin, the former being stimulatory and the latter inhibitory. In addition, GH secretion is also subject to other stimuli of which GH releasing peptides (GHRP), such as ghrelin, opioid peptides, cytokines, hypoglycemia and low levels of NEFAs are stimulatory and dopamine is inhibitory (63, 64). Furthermore, GH secretion is subject of the negative feedback control of (free) IGF-I at the level of both the hypothalamus and the pituitary. Under normal

conditions GH release takes place in a pulsatile manner with the greatest GH-release (70%) during sleep. Circulating GH binds partially to GH binding protein (GHBP), which represents the extra-cellular domain of the GH receptor (GHR) and is derived from cleavage of the protein or alternative splicing of the mRNA (Figure 6) (65). Arriving at the tissues GH binds to the GHR and after dimerization of two GHRs tyrosine kinase is activated (Figure 7), which results in lipolysis, the direct action of GH, and hepatic production of IGF-I, IGF binding protein-3 (IGFBP-3) and acid-labile subunit (ALS), via which GH exerts its indirect growth-promoting actions. Most of the circulating IGF-I (>99%) is bound to IGFBPs, of which under normal conditions the larger part (75-90%) is bound to IGFBP-3 in a tertiary complex with ALS. IGFBP-1 is regulated by metabolic stimuli and has been claimed to be the major regulator of the free IGF-I level, as a substantial proportion is unsaturated under normal conditions. Binding of IGF-I to IGFBP-1 may therefore serve as a protection mechanism against he hypoglycemic effects of free IGF-I.

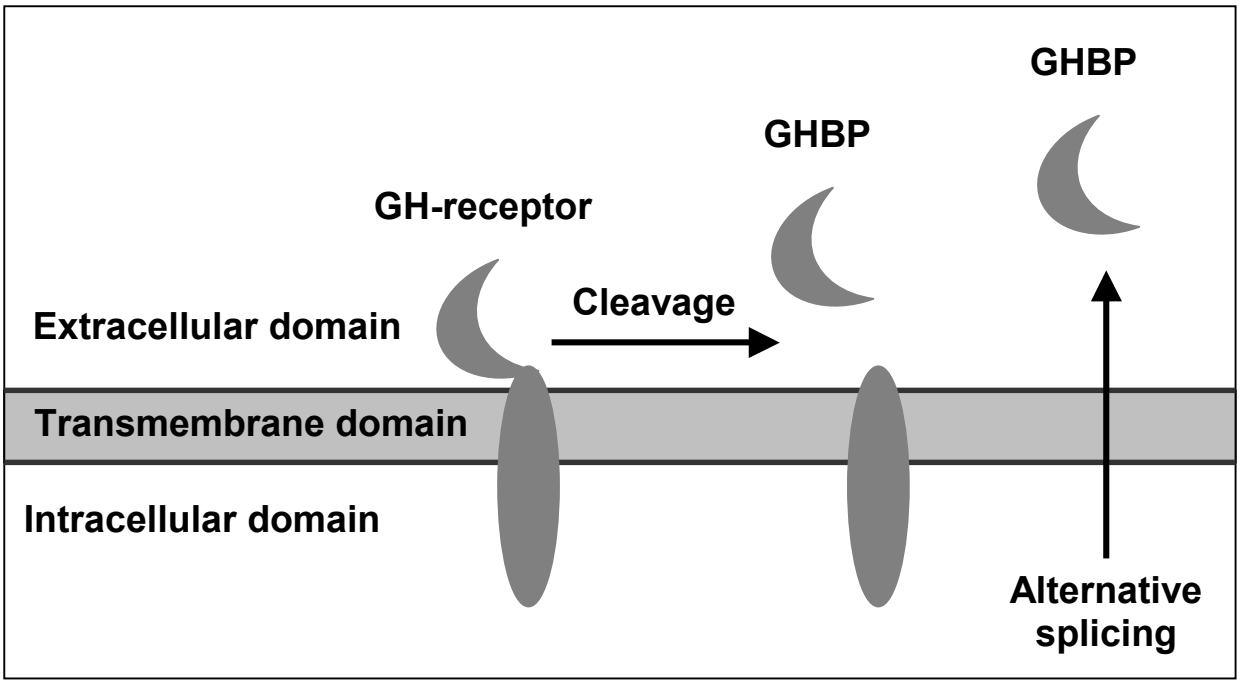

Figure 6. A general scheme showing GHBP generation. GHBP represents the extra-cellular domain of GHR and is derived by cleavage or by alternative splicing.

Studies on the acute phase of critical illness in adults show sustained to enhanced GH secretion, but low levels of IGFBP-3 and ALS (66, 67). These changes are generally interpreted as GH resistance and are presumed to be adaptive, as they promote the accessibility of metabolic substrates for vital organs and postpone anabolism. Despite many studies on GH/IGF-I function in critically ill adults, only very limited research has been performed in critically ill children (68) and after pediatric cardiac surgery (69, 70). In a pilot study in a small group of children with

meningococcal septic shock, de Groof et al. encountered very high levels of GH in nonsurvivors with concomitantly decreased IGF-I and IGFBP-3 levels, suggesting GH resistance (68). Data on the bioactivity of GH, and the serum levels of GHBP and IGF related components, such as ALS, were however lacking. In children after cardiac surgery, high levels of GH with low levels of IGF-I were also found, however, assessment of GH pulsatility are lacking (69, 70).

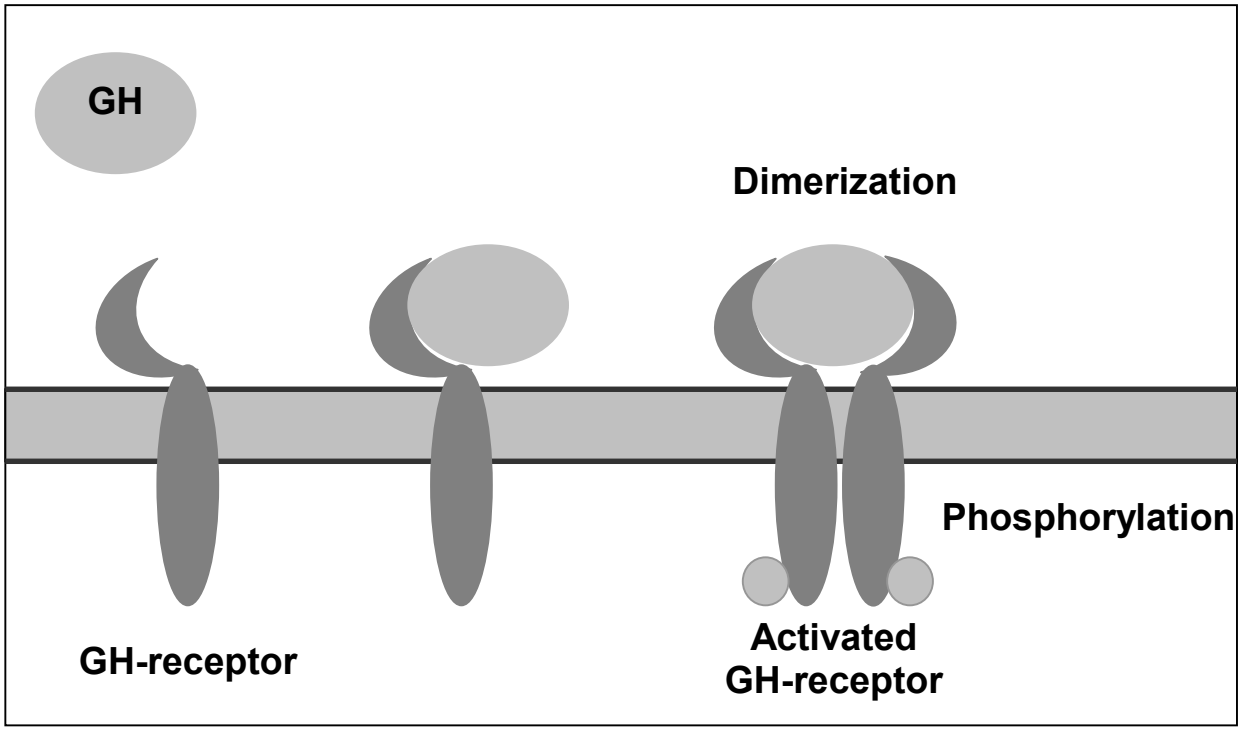

Figure 7. A schematic representation of the GH-GHR interaction that initiates intracellular signal transduction pathways. GH binds to GHR, but only after dimerization with two GHRs and phosphorylation the signal of GH is transduced.

Critical illness in children described in this thesis

Different types of acute life-threatening disease may cause critical illness, such as sepsis, respiratory insufficiency or hypoxia and severe neurological status. Furthermore, critical illness may also be initiated by trauma or surgical procedures, for instance open-heart surgery. This thesis will describe critical illness in children due to sepsis with petechiae or purpura, and in children undergoing open-heart operation.

Sepsis and septic shock with purpura

Sepsis is the rapidly evolving clinical picture of hypothermia or fever with tachycardia (=elevated heart rate) and tachypneu (= elevated respiratory rate) and is a generalized reaction to a microbial infection, usually caused by bacteria (71). In addition, when the clinical picture of sepsis deteriorates septic shock may develop.

Shock in general is a clinical picture comprising hypotension (= decreased blood pressure) and/or end-organ failure, for example decreased renal function. Sepsis and septic shock may coincide with coagulation abnormalities ranging from petechiae (=pin-point bleedings of the skin or mucous membranes) to purpura (=bleedings of the skin or mucous membranes). Sepsis and septic shock with coagulation abnormalities are mainly caused by *N. meningitidis* (72), but may also be caused by other microorganisms. In the clinical course of meningococcal sepsis, petechiae and /or purpura are a hallmark of disease and are assumed to be the first clear clinically detectable sign of disease. The children included in this study were admitted to the PICU with a clinical picture of meningococcal sepsis, i.e. sepsis with petechiae/purpura.

Open-heart operations

The development of the heart-lung machine (extracorporeal pump oxygenator or cardiopulmonary bypass (CPB)) in 1953 by Gibbons enabled cardiac surgeons to perform open-heart operations. During open-heart operations blood is diverted from heart and lungs through the CPB, which takes over the functions of heart and lungs, so that the heart can be arrested to enable surgery. In addition to CPB, patients' temperature is lowered to 34° – 28° (=mild to moderate systemic hypothermia) during bypass to minimize oxygen consumption by both body and brain. After adequate CPB is established, an aortic cross clamp (AOX = a clamp which prevents the blood to drain retrograde into the heart) and cardioplegia (= fluid which makes the heart stop beating) are applied, to ensure a dry and motionless heart. The time of cross clamping is called the ischemic time, since no blood is circulated though the heart itself. After surgical correction and closure of the heart, the cross clamp is removed, the heart is reperfused, temperature is normalized, the CPB is weaned and the patient is closed. The children included in this study underwent open-heart operations for congenital heart disease, such as ventricular and/or atrium septum defects, tetralogy of Fallot, heart valve defects or the construction of a (partial or total) Fontan circulation.

Design of the studies described in this thesis

All studies in this thesis are based on data of critically ill children with sepsis admitted to the PICU of the Erasmus MC-Sophia Children's Hospital between 1997 and 2004 and of children during and after open-heart surgery at the Thorax center of the Erasmus MC between 2001 and 2004. The inclusion and exclusion criteria and outline of the study protocol are shown in Appendix A.

Aims and outline of the thesis

The overall goals of the work presented in this thesis are to explore the endocrine adaptations of the acute stress response in critically ill children and to define endocrine parameters that can serve as prognostic factors for morbidity and mortality. We focused on three hypothalamic-pituitary-end-organ axes: the HPA-axis, the HPT-axis and the GH-IGF-I-axis.

HPA-axis in critically ill children

In **Chapter 2** we evaluate the HPA-axis in a large group of children with suspected meningococcal sepsis or septic shock on admission to the PICU. The purpose of this thesis was to describe differences in HPA-axis response in relation with severity of disease. Furthermore, we investigated serum bio-available cortisol levels, 21-hydroxylase and 11β-hydroxylase activity in relation with adrenal function and searched for factors associated with adrenal insufficiency and mortality.

Chapter 3 describes retrospectively the inhibiting influence of etomidate on adrenal function and steroidogenical enzyme function during the first 24h after PICU admission in children with meningococcal sepsis.

The HPT-axis in critically ill children

Chapter 4 describes thyroid function in relation with disease severity in a large group of children admitted to the PICU with sepsis or septic shock and assesses the influence of deiodination, sulfation, plasma binding of thyroid hormone and dopamine use on serum thyroid hormone levels.

Chapter 5 assesses the predictive value of thyroid function within the first 24h of admission on length of PICU stay in children who survived septic shock with purpura.

The GH-IGF-I axis in critically ill children

Chapter 6 thoroughly evaluates the GH-IGF-I axis in a large group of children with suspected meningococcal sepsis or septic shock on PICU admission. In addition to the study of GH profiles and growth factors, the purpose of this study was to assess the bioactivity of GH, serum levels of GHBP and IGF related components, such as acid-labile subunit (ALS).

Chapter 7 evaluates the GH-IGF-I axis in a large group of children before and after open-heart surgery and focuses on the relation between endocrine changes and influencing factors.

Chapter 8 discusses the findings of the studies described above and brings the data in perspective. **Chapter 9** briefly presents the conclusions of the described studies and presents new ideas concerning the future research. Finally, in **chapter 10** the findings presented in this thesis are summarized in Dutch.

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Appendix

Inclusion criteria

The general inclusion criteria were acutely ill children within 6h after admission to the PICU of the Erasmus MC - Sophia Children's Hospital with a clinical picture of ***meningococcal sepsis*** or children undergoing ***open-heart surgery*** at the Erasmus MC – Thoraxcenter.

Meningococcal sepsis was defined as sepsis with petechiae/purpura. Sepsis was defined as a temperature of <36.0 ºC or >38.5 ºC with tachycardia and tachypneu (73). In addition, children were considered to have septic shock, if they also had persistent hypotension (systolic blood pressure <75 mm Hg for children between 3-12 months, <80 mm Hg for 1-5 years, <85 mm Hg for 6-12 years, <100 mm Hg for children older than 12 years), or evidence of poor end-organ perfusion, defined as at least two of the following:

1. unexplained metabolic acidosis (pH < 7.3 or base excess < -5 mmol/l or plasma lactate levels > 2.0 mmol/l);
2. arterial hypoxia (PO_2 < 75 mmHg, a PO_2/FiO_2 ratio < 250 (torr) or transcutaneous oxygen saturation < 96%) in patients without overt cardiopulmonary disease;
3. acute renal failure (diuresis < 0.5 ml/kg/h for at least one hour despite acute volume loading or evidence of adequate intravascular volume without pre-existing renal disease); or
4. sudden deterioration of the baseline mental status

Children with the following ***open-heart surgical procedures*** were included: atrium and/or ventricular septum defects, total correction of tetralogy of Fallot, heart valve procedures, partial and total Fontan corrections and correction of aortopulmonary window.

Exclusion criteria

1. Age less than one week in term born children, age less than one month after the term date in ex-prematures (less than 36 weeks) and age more than 18 years.
2. Endocrine disorders.
3. Down's syndrome or other chromosomal abnormalities.
4. Severe psychomotor retardation.
5. End-stage renal disease requiring peritoneal or hemodialysis.
6. Immunosuppressive drug therapy, systemic corticosteroid treatment, radiation therapy or chemotherapy within the previous 6 months.
7. No informed consent, no arterial line or after removal of arterial line.

Study design

The studies described in **Chapters 2, 4, 5 and 6** were single center prospective observational studies and the study described in **Chapter 3** was a single center retrospective study. Of all children included in these studies, blood was drawn as soon as possible, within 6h after PICU admission and 12, 24 and 48h thereafter for determination of endocrine and immunological parameters. Next to the admission sample a 6-h GH profile was obtained, with samples taken every 30 minutes.

The study described in **Chapter 7** was a single center prospective observational study. Of all children described in this study blood was drawn before surgery (after induction), at the end of surgery and 12 and 24 h thereafter. Next to the blood sample at the end of surgery a 6h GH profile was obtained, with samples taken every 30 minutes. In addition, anthropometric measurements, such as length and weight, were collected before surgery.

Chapter 2

ADRENAL INSUFFICIENCY IN MENINGOCOCCAL SEPSIS: BIOAVAILABLE CORTISOL LEVELS AND IMPACT OF INTERLEUKIN-6 LEVELS AND INTUBATION WITH ETOMIDATE ON ADRENAL FUNCTION AND MORTALITY

Marieke den Brinker, Koen F. M. Joosten, Olivia Liem, Frank H. de Jong, Wim C. J. Hop, Jan A. Hazelzet, Marije van Dijk, and Anita C. S. Hokken-Koelega

Journal of Clinical Endocrinology and Metabolism 2005 sept; 90(9): 5110–5117
First Published Online June 28, 2005

Abstract

Context: Adequate adrenal function is pivotal to survive meningococcal sepsis. **Objectives:** The objective of the study was to evaluate adrenocortical function in meningococcal disease. **Design:** This was an observational cohort study. **Setting:** The study was conducted at a university-affiliated pediatric intensive care unit. **Patients:** Sixty children with meningococcal sepsis or septic shock participated in the study. **Main Outcome Measures:** The differences in adrenal function between nonsurvivors (n=8), shock survivors (n=43), and sepsis survivors (n=9) on pediatric intensive care unit admission were measured. **Results:** Nonsurvivors had significantly lower median cortisol to ACTH ratio than shock survivors and sepsis survivors. Because cortisol binding globulin and albumin levels did not significantly differ among the groups, bioavailable cortisol levels were also significantly lower in nonsurvivors than sepsis survivors. Nonsurvivors had significantly lower cortisol to 11-deoxycortisol ratios but not lower 11-deoxycortisol to 17-hydroxyprogesterone ratios than survivors. Using multiple regression analysis, decreased cortisol to ACTH ratio was significantly related to higher IL-6 levels and intubation with etomidate (one single bolus), whereas decreased cortisol to 11-deoxycortisol ratio was significantly related only to intubation with etomidate. Aldosterone levels tended to be higher in nonsurvivors than shock survivors, whereas plasma renin activity did not significantly differ. **Conclusions:** Our study shows that the most severely ill children with septic shock had signs of adrenal insufficiency. Bioavailable cortisol levels were not more informative on adrenal function than total cortisol levels. Besides disease severity, one single bolus of etomidate during intubation was related to decreased adrenal function and 11β-hydroxylase activity. Decreased adrenal function was not related to decreased 21-hydroxylase activity. Based on our results, it seems of vital importance to take considerable caution using etomidate and consider combining its administration with glucocorticoids during intubation of children with septic shock.

Introduction

Despite advances in management and therapy, the mortality rate of children with shock due to meningococcal disease continues to be considerable (1–4). Stimulation of the hypothalamic-pituitary adrenal axis is one of the most important hormonal reactions to critical illness. Cortisol has a vital supportive role in the maintenance of vascular tone, endothelial and vascular integrity, and the distribution of total body water (5). In previously reported pilot studies (6, 7), we showed that children dying from meningococcal septic shock had relatively low cortisol levels and extremely high ACTH levels.

It is generally believed that the biological activity of cortisol depends on the free fraction of the hormone, especially the fraction not bound to corticosteroid binding globulin (CBG), the bioavailable cortisol. Because CBG is the most important transport protein for cortisol, serum levels of cortisol may vary with CBG levels (8). Several authors demonstrated higher calculated or measured bioavailable cortisol levels during critical illness in adults than would have been expected on the basis of total cortisol levels, especially during the acute phase of critical illness (9–12). To our knowledge these data do not exist for acutely ill children.

Reduced activity of adrenal steroidogenic enzymes might lead to adrenal insufficiency in children with meningococcal sepsis. During sepsis, adrenal steroidogenic enzyme function might be liable to circulating endotoxins and/or cytokines, medication, or more intrinsically to polymorphisms. Mild forms of a decreased 21-hydroxylase function would become obvious only during extremely stressful conditions, such as sepsis, and would lead to lower cortisol levels and increased levels of its upstream precursor: 17-hydroxyprogesterone (17-OHP). The anesthetic drug etomidate is known to inhibit adrenal function by impeding mainly the enzyme 11β-hydroxylase. For this reason, etomidate has been withdrawn from long-term sedation regimens. Etomidate, however, is still a first-line anesthetic agent in the setting of rapid sequence intubation, in which one single bolus is used. This use of one single bolus is assumed to give only transient, clinically nonrelevant hormonal changes (13, 14).

In addition, adrenal insufficiency associated with meningococcal sepsis may also include functional loss of the zona glomerulosa and defects in the renin-mineralocorticoid axis may thereby adversely affect cardiovascular homeostasis and thus outcome. Hyperrenemic hypoaldosteronism has been reported in critically ill adults (15), whereas lower plasma aldosterone concentrations have been reported in children with meningococcal sepsis, compared with other critically ill children (16). Data concerning the renin-mineralocorticoid axis in relation to outcome in children with meningococcal sepsis are, however, lacking.

In this study, our aim was to evaluate whether low total cortisol levels correspond with low bioavailable cortisol levels in children with meningococcal

sepsis. In addition we aimed to assess whether reduced 21-hydroxylase or 11*β*-hydroxylase functions might underlie the adrenal insufficiency in children dying from meningococcal disease. Furthermore, we wanted to determine which factors were associated with adrenal insufficiency and mortality. We therefore evaluated adrenocortical function in a large group of children with meningococcal sepsis on admission to the pediatric intensive care unit (PICU).

Patients and Methods

Patients

The group consisted of 69 previously healthy children admitted to the PICU of Erasmus Medical Center–Sophia Children's Hospital between October 1997 and October 1999 and between October 2001 and January 2004, with a clinical picture of meningococcal sepsis, defined as sepsis with petechiae/purpura. Sepsis was defined as temperature lower than 36.0 ºC or higher than 38.5 ºC with tachycardia and tachypnea. In addition, children were assigned to have septic shock if they also had persistent hypotension or evidence of poor end-organ perfusion, as described previously (6, 17). Nine children who received corticosteroid therapy for suspected adrenal insufficiency before admission were excluded. It is important to note that administration of glucocorticoids during septic shock or after a single bolus of etomidate in the setting of rapid sequence intubation is not a routine procedure in The Netherlands. The medical ethics committee approved the study, and written informed consent was obtained from the parents or legal representatives of each patient before their participation in the study. Pilot data on total cortisol and ACTH levels of 27 children included between October 1997 and October 1999 have been published previously (6, 7).

Concomitant therapy and caloric intake

Concomitant therapy on admission included antibiotics (Cefotaxime; Sanofi-Aventis, Gouda, The Netherlands) and administration of fluids in all 60 children and inotropics in 51 children. At a median of 2 h 40 min before admission, 31 children were mechanically ventilated, of whom 23 had been intubated with one bolus of etomidate (median dose, 0.29 mg/kg) and eight with combinations of opiate agonists, propofol, ketamine, or midazolam. Mechanically ventilated children were sedated with benzodiazepines and/or morphine. On admission, patients received glucose iv at a rate of 4–6 mg/kg/min. They did not receive enteral or parenteral feeding until the second day.

Clinical parameters

Disease severity was determined using the Pediatric Risk of Mortality II (PRISM) score (6, 18) and the Sepsis-Related Organ Failure Assessment (SOFA) score (19)

and by measuring levels of established biomarkers, such as plasma IL-6, arterial lactate, and serum C-reactive protein (CRP). We recorded the interval between appearance of first petechia and PICU admission, respiratory support, drug use, blood pressure, and outcome. Blood pressure SD scores (Z-scores) were calculated based on published reference data (20).

Collection of blood samples

Arterial blood samples were obtained as soon as possible after admission. After clotting and centrifugation, serum and plasma were stored at –80 ºC until determination of CBG, 17-OHP, 11-deoxycortisol, and IL-6. All other laboratory parameters were determined immediately. The accuracy of cortisol and ACTH assays was guaranteed by continuously monitoring of intra- and interassay variabilities by estimating hormone concentrations in three serum pools in every assay. Whenever a pool was exhausted, samples from a new pool were estimated in parallel for at least five assays. A certified clinical chemistry laboratory (ISO 17025 and 9001) determined the other parameters.

Hormone analyses

Serum cortisol concentrations were measured by a competitive luminescence immunoassay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA) and plasma ACTH concentrations by an immunoradiometric assay (BioInternational, Gif sur Yvette, France). Because reference values of cortisol and ACTH for critical illness do not exist, we depicted nonstressed reference values for ACTH (<11 pmol/liter) and cortisol (between 200 and 800 nmol/liter at 0800 h) in tables and figures as a point of reference. 17-OHP was determined by an in-house RIA as described earlier (21). Values of 17-OHP below the assay's detection limit were set at the detection limit of 0.1 nmol/liter. Nonstressed reference values for 17-OHP were less than 5 nmol/liter for children younger than 12 yr of age and less than 10 nmol/liter for older children. Serum 11-deoxycortisol levels were obtained on admission and determined by RIA (22), using an antiserum from ICN Biomedicals (Costa Mesa, CA). Nonstressed reference values for 11-deoxycortisol were less than 50 nmol/liter. Serum CBG concentrations were measured by RIA (Biosource, Nivelles, Belgium). Because reference values for CBG were not available for children, we used reference values for adult men (between 442 and 1596 nmol/liter), except for girls 12 yr and older for whom reference values for women (between 615 and 2865 nmol/liter) were used. The within- and between-assay variation coefficients for the assays of cortisol, ACTH, and 17-OHP were less than 7% and for CBG and 11-deoxycortisol less than 14%. We calculated bioavailable cortisol, which represents the non-CBG-bound fraction, from total cortisol, CBG, and albumin levels using the formula based on binding equilibrium as described earlier (23, 24) with the association constants of CBG and albumin for cortisol (25). Plasma aldosterone

levels were determined by RIA (Diagnostic Products Corp.) and plasma renin activity by an in-house assay, as described elsewhere (26).

Other laboratory analyses

Arterial lactate and glucose were measured on a blood gas analyzer (ABL 625; Radiometer, Copenhagen, Denmark). Serum CRP was measured by an immunoturbidimetric assay, and serum albumin was measured by a bromocresol purple method, both on a Hitachi 912 analyzer (Roche Diagnostics, Mannheim, Germany). The reference values were less than 2.0 mmol/liter for lactate, less than 10 mg/liter for CRP, 2.6–11.0 mmol/liter for glucose, and 35–50 g/liter for albumin. Plasma IL-6 levels were analyzed using an ELISA (Sanquin, Amsterdam, The Netherlands).

Statistics

The results are expressed as medians unless specified otherwise. We used Mann-Whitney *U*, $_X^2$, or Fischer's exact test for group comparison and Spearman's correlation coefficients (r) to evaluate the relationship between different parameters. Multiple linear regression analysis was used to evaluate the relationships between various parameters and cortisol to ACTH and cortisol to 11-deoxycortisol ratios (both log transformed). Two-tailed $P < 0.05$ was considered statistically significant.

Table 1. Patients' characteristics on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

Variables	Nonsurvivors (n = 8)	Shock-survivors (n = 43)	Sepsis-survivors (n = 9)
Male gender (%)	6 (75)	26 (60)	5 (56)
Age (yr)	0.8 (0.5 – 1.9) [a]	5.0 (2.1 – 10.2)	4.8 (2.7 – 10.9)
Time first petechia – admission (h)	5.3 (3.8 – 7.6) [a]	7.3 (6.2 – 9.8)	7.6 (5.7 – 9.0)
PRISM score	34 (26 – 36) [a]	20 (16 – 27) [b]	9 (8 – 12)
SOFA score	16 (14 – 19) [a]	9 (7 – 11) [b]	2 (2 – 4)
IL-6 x 10^3 (pg/mL)	1195.5 (853.1– 1776.7) [a]	45.9 (4.8 – 126.9) [b]	0.4 (0.1 – 5.6)
Lactate (mmol/L)	7.3 (5.9 – 8.7) [a]	3.9 (2.6 – 5.4) [b]	2.1 (1.4 – 2.5)
CRP (mg/L)	31 (22 – 36) [a]	85 (58 – 131)	75 (64 – 215)
Glucose (mmol/L)	3.9 (2.5 – 6.5) [a]	6.9 (5.6 – 9.1)	8.2 (7.1 – 9.7)
Mechanical ventilation (%)	8 (100) [a]	23 (53) [b]	0 (0)
Intubated with etomidate (%)	7 (88) [a]	16 (37) [b]	0 (0)
Inotropic support (%)	8 (100) [b]	41 (95) [b]	2 (22)

All values are expressed as median (25 to 75 percentile). For reference values see *Patients and Methods*. [a] Significantly different, compared to all survivors, $P < 0.05$. [b] Significantly different, compared to sepsis-survivors, $P < 0.05$.

Results

Clinical parameters

The study group consisted of 60 children, 37 boys and 23 girls, admitted to the PICU without receiving glucocorticoid therapy before study enrollment. All children showed a clinical picture of meningococcal sepsis and blood cultures revealed *Neisseria meningitidis* in 50 of them. Children were divided according to presence of shock and survival into the following disease severity groups: (shock) nonsurvivors (n=8), shock survivors (n=43), and sepsis survivors (n=9). Nonsurvivors were significantly younger and had significantly shorter time from first petechia to admission than survivors (Table 1). Parameters of disease severity, such as PRISM and SOFA score, plasma IL-6, and arterial lactate levels were significantly higher in nonsurvivors, compared with survivors, and in shock survivors, compared with sepsis survivors, whereas CRP levels were significantly lower in nonsurvivors, compared with survivors. Arterial glucose levels were significantly lower in nonsurvivors, compared with survivors. Nonsurvivors were more often mechanically ventilated and had been more often intubated with etomidate than survivors, and shock survivors more often than sepsis survivors. Nonsurvivors and shock survivors received more often inotropic support than sepsis survivors, whereas systolic and diastolic blood pressure SD scores did not significantly differ between the groups (data not shown).

Table 2. Endocrine levels on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

Variables	Shock-nonsurvivors (n = 8)	Shock-survivors (n = 43)	Sepsis-survivors (n = 9)
Total cortisol (nmol/L)	615 (490 – 790) [a]	953 (696 – 1160) [b]	1276 (1044 – 1895)
ACTH (pmol/L)	274.1 (164.4 – 647.1) [a]	49.5 (13.4 – 109.7)	5.9 (3.8 – 70.8)
Total cortisol/ACTH ratio ($x10^3$)	2.6 (0.8 – 4.5) [a]	21.2 (5.4 – 90.3) [b]	195.3 (18.2 – 330.0)
CBG (nmol/L)	892 (466 – 1175)	700 (552 – 1108)	831 (668 – 1223)
Albumin (g/L)	40 (28 – 41)	33 (27 – 39)	34 (31 – 39)
Bio-available cortisol (nmol/L)	106 (30 – 220) [b]	173 (59 – 532) [b]	511 (356 – 843)
17-OHP (nmol/L)	2.8 (1.9 – 6.5) [a]	8.7 (3.9 – 10.3)	11.2 (3.4 – 15.3)
11-deoxycortisol (nmol/L)	143 (102 – 176)	70 (53 – 167)	73 (44 – 127)
11-deoxycortisol to 17-OHP ratio	48 (17 – 72) [a]	10 (7 – 29)	13 (6 – 17)
Cortisol to 11-deoxycortisol ratio	4.1 (3.1 – 4.8) [a]	16.0 (3.9 – 21.3)	17.3 (10.4 – 24.9)
Aldosterone (pg/ml)	569 (320 – 803)	296 (169 – 427)	269 (216 – 1475)
Plasma renin activity (ng AngI/ml/h)	15.9 (5.2 – 37.4)	19.5 (9.3 – 34.4)	19.1 (9.7 – 76.9)

All values are expressed as median (25 to 75 percentile). For reference values see *Patients and Methods*. [a] Significantly different, compared to all survivors, $P < 0.05$. [b] Significantly different, compared to sepsis-survivors, $P < 0.05$.

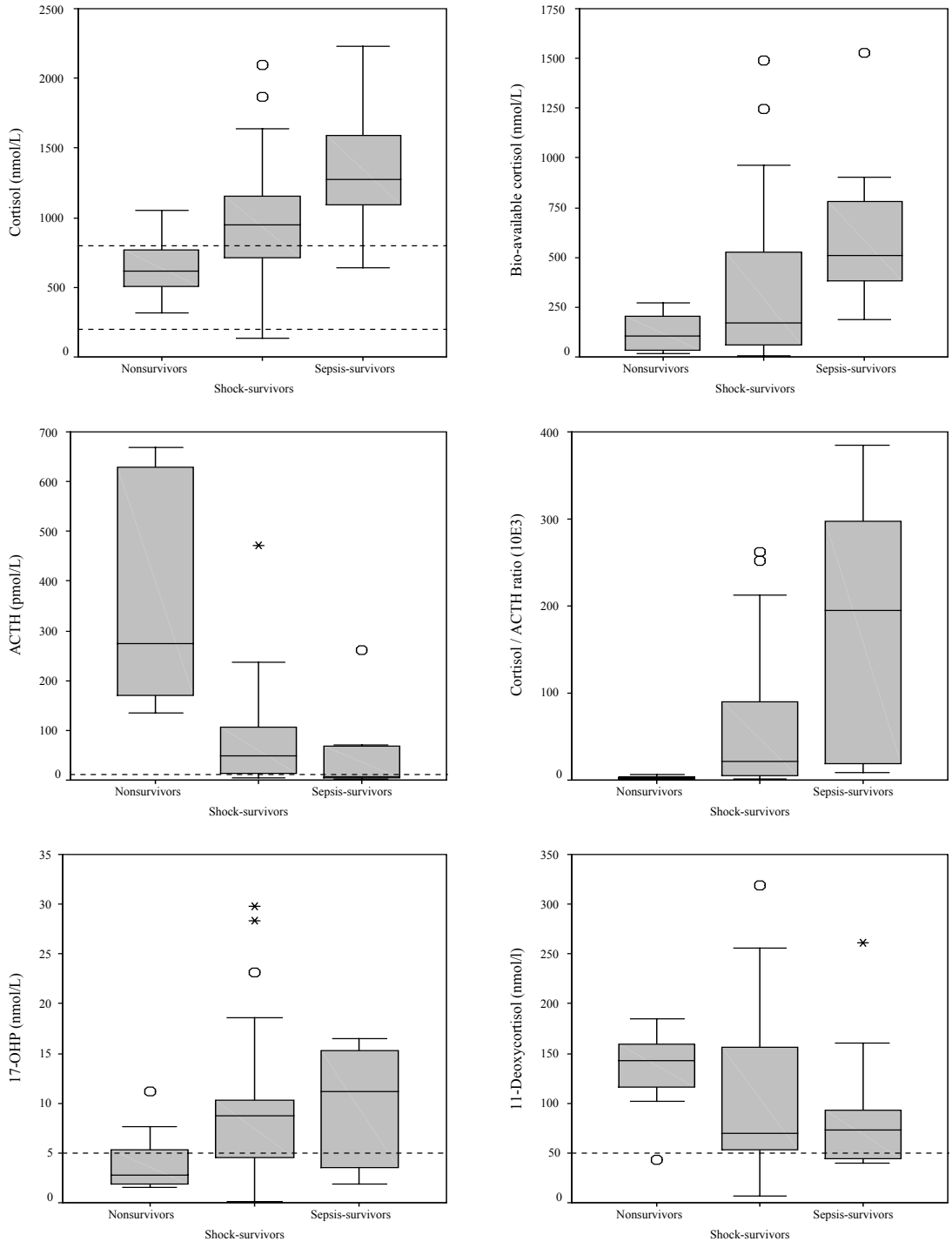

Figure 1. Serum cortisol levels, bio-available cortisol levels, ACTH levels, cortisol/ACTH ratios, 17-OHP levels and 11-deoxycortisol levels on admission. Nonsurvivors are depicted in the left box, shock-survivors in the middle box and sepsis-survivors.

Cortisol and ACTH levels

Total cortisol levels did not increase with increasing disease severity: nonsurvivors had significantly lower median serum cortisol levels than shock survivors as well as

sepsis survivors, and shock survivors had significantly lower cortisol levels than sepsis survivors (Table 2 and Figure 1). ACTH levels were significantly higher in nonsurvivors, compared with shock survivors and sepsis survivors. As a result, nonsurvivors had significantly lower median total cortisol to ACTH ratios, compared with survivors, and shock-survivors had significantly lower cortisol to ACTH ratios than sepsis survivors. Parameters of disease severity, such as PRISM and SOFA score, IL-6, and lactate levels, correlated positively with ACTH levels and negatively with serum cortisol levels as well as cortisol to ACTH ratios (Table 3). Arterial glucose levels correlated negatively with ACTH levels and positively with cortisol to ACTH ratio. Age and time from first petechia to admission did not correlate with cortisol levels, ACTH levels, or their ratio (data not shown).

CBG and bioavailable cortisol levels

On admission, serum CBG levels ranged from 223 to 1793 nmol/liter. CBG levels were within the normal range in the vast majority of children (86%), elevated in one girl (1.3 yr of age), and decreased in seven children, of which one girl was older than 12 yr. Serum CBG and albumin levels did not significantly differ among nonsurvivors, shock survivors, and sepsis survivors (Table 2), and CBG levels did not correlate with parameters of disease severity (Table 3). Median bioavailable cortisol levels were significantly lower in nonsurvivors and shock survivors, compared with sepsis survivors (Table 2 and Figure 1) and correlated negatively with parameters of disease severity (Table 3) and positively with CRP levels but not with age or time from first petechia to admission.

Table 3. Significant Spearman correlation coefficients of hormones of the HPA-axis with parameters of disease severity.

	PRISM	SOFA	Lactate	IL-6	CRP	Glucose
Cortisol	–0.48	–0.59	–0.41	–0.45	0.46	–
ACTH	0.49	0.60	0.54	0.66	–0.35	–0.30
Cortisol/ACTH	–0.58	–0.70	–0.62	–0.72	0.43	0.32
CBG	–	–	–	–	–	–
Bio-available cortisol	–0.35	–0.55	–0.41	–0.30	0.37	–
17-OHP	–	–	–	–	–	–
11-deoxycortisol	–	–	–	–	–	–0.29
11-deoxycortisol/17-OHP	–	0.38	–	0.39	–	–0.38
Cortisol/11-deoxycortisol	–0.40	–0.43	–0.35	–0.47	0.27	0.37
Cortisol/17-OHP	–	–	–	–	–	–
Aldosterone	–	–	–	0.33	–	–
Plasma renin activity	–	–	–	–	–	–

Non-significant correlation coefficients are represented with a dash.

17-OHP and 11-deoxycortisol levels and ratios with cortisol

On admission, serum 17-OHP ranged from values below the detection limit of 0.1 to 28.9 nmol/liter and serum 11-deoxycortisol levels ranged from 7 to 468 nmol/liter. Nonsurvivors had significantly lower 17-OHP levels than survivors, whereas median 11-deoxycortisol levels did not significantly differ between the groups (Table 2). Nonsurvivors had significantly higher 11-deoxycortisol to 17-OHP ratios and significantly lower cortisol to 11-deoxycortisol ratios than survivors, whereas these ratios did not significantly differ between all shock survivors and sepsis survivors and also between shock survivors and sepsis survivors who did not receive etomidate (data not shown). Most parameters of disease severity correlated positively with 11-deoxycortisol to 17-OHP ratios and negatively with cortisol to 11-deoxycortisol ratios but not with serum 17-OHP and 11-deoxycortisol levels (Table 3). Arterial glucose levels correlated negatively with 11-deoxycortisol levels and 11-deoxycortisol to 17-OHP ratios and positively with cortisol to 11-deoxycortisol ratios. Both serum 17-OHP and 11-deoxycortisol levels did not correlate with age and did not differ between girls and boys. Plasma ACTH levels and cortisol to ACTH ratios correlated significantly with 11-deoxycortisol levels (r=0.44 and r=0.39, respectively), whereas 17-OHP levels did not (data not shown).

Aldosterone levels and plasma renin activity

On admission, plasma aldosterone levels ranged from 129 to 1867 pg/ml and plasma renin activity from 3.3 to 97.2 ng angiotensin I per milliliter per hour. Aldosterone levels tended to be higher in nonsurvivors, compared with shock survivors (*P*=0.057) but did not significantly differ between shock survivors and sepsis survivors. Plasma renin activity did not significantly differ among nonsurvivors, shock survivors, and sepsis survivors. Aldosterone levels correlated significantly with age (r=0.69) and IL-6 levels (r=0.33) but not with other parameters of disease severity (Table 3), plasma renin activity, or ACTH levels (data not shown). Plasma renin activity correlated significantly with systolic blood pressure SD score (r = – 0.39) but not with diastolic blood pressure SD score, any parameter of disease severity, or age. Aldosterone levels and plasma renin activity did not significantly differ between children who received inotropics and those who did not.

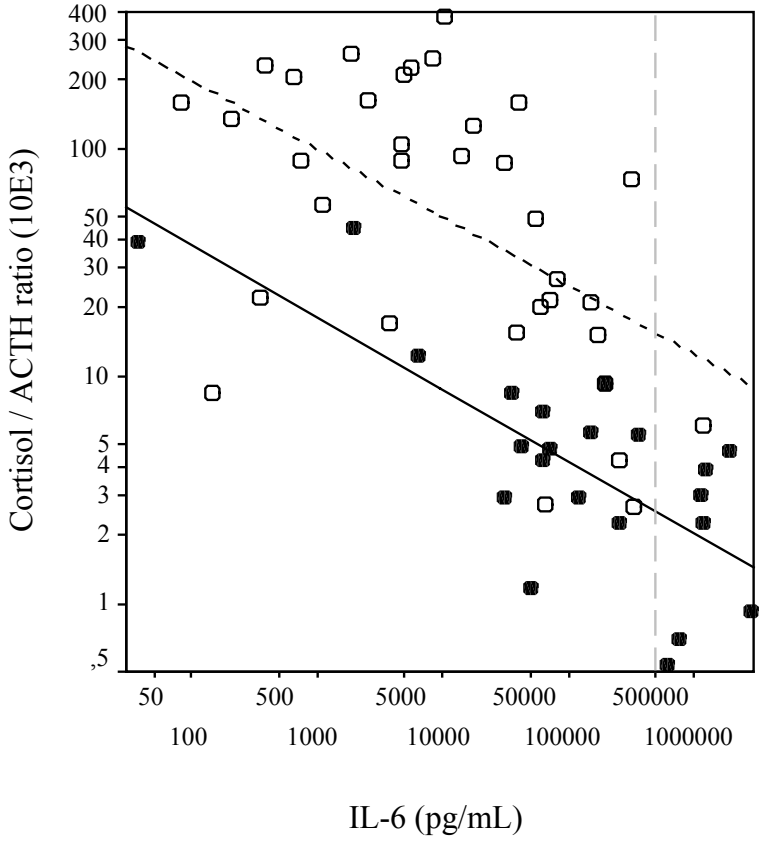

Figure 2. Relation between cortisol/ACTH ratios and IL-6 levels, in children who received etomidate (●, continuous line) and children who did not receive etomidate (○, dotted line). After adjustment for IL-6 levels, using multiple regression, mean cortisol/ACTH ratios were significantly higher in case of etomidate use ($p<0.001$). The vertical gray dotted line (IL-6 level of 500,000 pg/L) discriminates survivors (left) from nonsurvivors (right).

Multivariate analysis

In univariate regression analyses, the cortisol to ACTH ratio was significantly related to IL-6, CRP, 17-OHP, 11-deoxycortisol levels, age, mechanical ventilation, and intubation with etomidate but not to gender and time from first petechia to admission. Using multiple regression analysis, we found cortisol to ACTH ratios to be significantly related to IL-6 levels and intubation with etomidate before admission. These two variables in combination explained 65% of the variation in cortisol to ACTH ratio on admission, whereas IL-6 alone explained 45%. The cortisol to ACTH ratios decreased by 19% for every doubling of IL-6 levels and by 83% when etomidate was administered (Figure 2). In contrast, analyzing the relation between cortisol to ACTH ratios and IL-6 levels, we found no significant difference between children who were intubated without etomidate and those who were not intubated (ANCOVA, P=0.774). Children who were intubated on admission, independently of intubation with etomidate, had significantly higher disease severity parameters, such as PRISM, SOFA, IL-6, and lactate levels, than children who were not intubated. In univariate regression analyses, the cortisol to 11-deoxycortisol ratio was significantly

related to IL-6, CRP, ACTH levels, mechanical ventilation, and intubation with etomidate but not to age, gender, time from first petechia to admission, or 17-OHP levels. Using multiple regression analysis, we found only intubation with etomidate to be significantly predictive for cortisol to 11-deoxycortisol ratios, explaining 78% of the variation in cortisol to 11-deoxycortisol ratio on admission. The mean decrease of cortisol to 11-deoxycortisol ratios was 84% when children were intubated with etomidate (Figure 3).

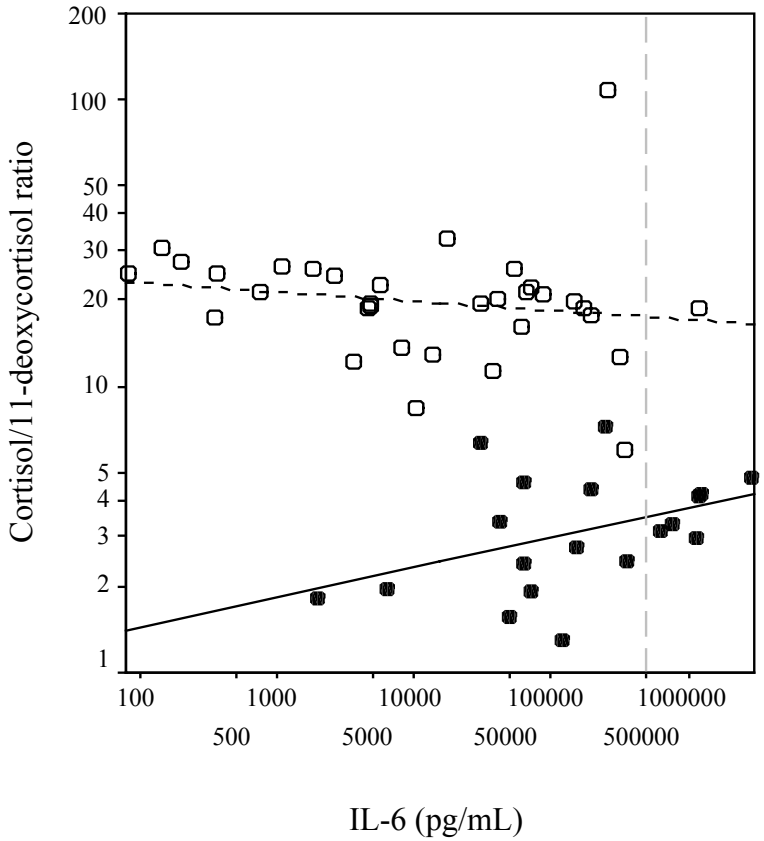

Figure 3. The cortisol/11-deoxycortisol ratio in relation to IL-6 levels, in children who received etomidate (●, continuous line) and children who did not receive etomidate (○, dotted line). Mean cortisol/11-deoxycortisol ratios were significantly higher in case of etomidate use ($p<0.001$). The vertical gray dotted line (IL-6 level of 500,000 pg/L) discriminates survivors (left) from nonsurvivors (right).

Discussion

In this study we found that the most severely ill children with meningococcal septic shock had signs of adrenal insufficiency on PICU admission, indicated by low cortisol and very high ACTH levels. Low total cortisol levels proved to correspond with low bioavailable cortisol. Serum CBG levels were normal in the vast majority of the children. We did not find signs of a reduced 21-hydroxylase activity because the 11-deoxycortisol to 17-OHP ratio was not reduced. We found, however, a reduction

in the cortisol to 11-deoxycortisol ratio, indicating that the 11*β*-hydroxylase activity was significantly reduced, particularly in the nonsurvivors. Nonsurvivors had significantly reduced cortisol to ACTH ratio, compared with the survivors. The cortisol to ACTH ratio was negatively related to IL-6 levels and various other disease severity scores. Using multiple regression analysis, it turned out that a decreased cortisol to ACTH ratio was significantly related to higher IL-6 levels and intubation with etomidate, whereas a lower cortisol to 11-deoxycortisol ratio was only significantly related to intubation with etomidate.

First of all, we examined whether total cortisol levels corresponded with bioavailable cortisol levels. Serum CBG and to a much lesser extent albumin are the most important serum proteins for transport of cortisol, thereby roughly determining the biologically active cortisol concentration. We found normal CBG and albumin levels in the vast majority of the children with meningococcal disease on PICU admission, indicating that the low total cortisol levels do correspond with low bioavailable cortisol levels in these children. This is in contrast to studies in critically ill adults reporting decreased serum CBG and albumin levels with concomitantly elevated free cortisol fractions especially during the acute phase of critical illness (9, 10, 12). A possible explanation for this difference might be that children in our study received plasma products from healthy adult donors as volume suppletion, which might have prevented a decline in CBG levels. From our study we can conclude that bioavailable cortisol levels were not more informative on adrenal function than total cortisol levels in these children on PICU admission.

The extremely elevated ACTH levels in combination with the decreased cortisol levels in nonsurvivors represent an inadequate adrenal response. We therefore investigated whether the enzymes 21-hydroxylase and 11*β*-hydroxylase were not or less active in this condition by measuring 11-deoxycortisol to 17-OHP and cortisol to 11-deoxycortisol ratios. It turned out that with increasing disease severity there were more signs of decreased 11*β*-hydroxylase activity, as depicted by lower cortisol to 11-deoxycortisol ratios, whereas we found no signs of decreased 21-hydroxyase activity. The median 11-deoxycortisol to 17-OHP ratio was significantly higher in nonsurvivors than survivors, which may have resulted from accumulation of 11-deoxycortisol levels by decreased 11*β*-hydroxylase activity. In search of factors influencing adrenal function, we found, besides disease severity, mechanical ventilation and in particular intubation with one single bolus of etomidate to be significantly related to decreased adrenal function at the level of 11*β*-hydroxylase. As Figure 2 shows, IL-6 levels higher than 500,000 pg/ml was discriminating for mortality. Etomidate was found to reduce 11*β*-hydroxylase activity independently of disease severity (Figure 3).

Our study suggests that the addition of one single bolus of etomidate to the existing overwhelming immune reaction in the most severely ill children might have increased the risk for mortality. For the less severely ill children, the addition of etomidate appeared not so disastrous. It is, however, difficult to identify the relative

contribution of the disease severity and the intubation with etomidate because the most severely ill children were more likely to be intubated than the less severely ill children. In our study the group of intubated children without etomidate was too small to differentiate whether etomidate was given, depending on the disease severity. We assume, however, it was not of influence because all children were intubated in the setting of a rapid sequence intubation. *In vitro* studies have shown that etomidate interferes with mainly two steroidogenic enzymes: the cholesterol (P450) side-chain cleavage enzyme system and 11*β*-hydroxylase (21, 22, 27). Already 20 yr ago etomidate has been withdrawn from the long-term sedation regimen due to high death rates; however, it still is a first-line anesthetic agent in the setting of rapid sequence intubation, in which one single bolus is used. This use of one single bolus had been assumed to give only transient, not clinically relevant, hormonal changes (14, 28). Although our study was not designed to study the direct effect of etomidate administration on adrenal function and mortality, our data suggest that in the most severely ill children with septic shock, the risk of death might have been increased by one single bolus of etomidate during intubation. This should be further investigated.

Our present study indicates that adrenal insufficiency should be considered in all children with severe sepsis and septic shock but particularly so when they received a bolus of etomidate during intubation. Based on our results and awaiting the final results future studies, it seems of vital importance to take considerable caution using etomidate and consider combining its administration with glucocorticoids during intubation of children with septic shock.

Various other mechanisms might be important in the pathogenesis of relative adrenal insufficiency during sepsis, such as impairment of enzymes of the steroidogenical pathway before 21-hydroxylase, ACTH receptor insensitivity, decreased levels of cholesterol, decreased (adrenal) blood flow during severe shock, and adrenal hemorrhage. All these factors might adversely influence cortisol production in the most severely ill children (29–32).

In our study, aldosterone levels tended to be higher in nonsurvivors, compared with shock survivors and correlated positively but weakly with IL-6 levels. However, because aldosterone levels were inversely correlated with age, this trend was apparently influenced by the younger age of nonsurvivors, compared with survivors. Plasma renin activity related negatively to age-matched systolic blood pressure values, suggesting an adequately renin response. However, plasma renin activity did not correlate with aldosterone levels, suggesting an inadequate aldosterone response.

In summary, our study shows that the most severely ill children with meningococcal septic shock had signs of adrenal insufficiency on PICU admission. Bioavailable cortisol levels were not more informative on adrenal function than total cortisol levels. Decreased adrenal function was strongly inversely related to IL-6 levels and at least partly to a decreased 11*β*-hydroxylase activity but not to a decreased 21-hydroxylase. In addition to IL-6 levels, one single bolus of etomidate

during intubation was related to a decreased adrenal function and 11*β*-hydroxylase activity. Based on our results, it seems of vital importance to take considerable caution using etomidate during intubation of children with septic shock and consider combining its administration with glucocorticoids.

Acknowledgments

We thank M. Maliepaard (research nurse) for her technical assistance, C. L. Vermont, M.D., Ph.D., and E. D. de Kleijn, M.D., Ph.D., and their colleagues as well as E. M. N. Bannink, R. D. van Beek, V. H. Boonstra, M. van Dijk, D. A. M. Festen, D. van der Kaay, and A. S. Slingerland (all M.D.s) for assisting to collect samples on weekends and the nursing staff of the pediatric intensive care for their support. We also thank F. Boomsma, Ph.D., for determining aldosterone levels and plasma renin activity.

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Chapter 3

THE INFLUENCE OF ETOMIDATE ON ADRENAL FUNCTION IN CHILDREN WITH MENINGOCOCCAL SEPSIS

Marieke den Brinker, Anita C.S. Hokken-Koelega, Jan A. Hazelzet,
Frank H. de Jong, Wim C.J. Hop, Koen F.M. Joosten

Submitted for publication

Abstract

Objective: To investigate the influence on adrenal function of one single bolus of etomidate used for intubation in children with meningococcal sepsis. **Design:** Retrospective study conducted between 1997 and 2004. **Setting:** University-affiliated paediatric intensive care unit (PICU). **Patients and participants:** Sixty children admitted to the PICU with meningococcal sepsis, not treated with steroids. **Interventions:** Adrenal hormone concentrations were determined as soon as possible after PICU admission, 12 and 24 hours thereafter. To assess disease severity, PRISM score and selected laboratory parameters were determined. **Measurements and main results:** On admission, before blood was drawn, 23 children had been intubated with etomidate, 8 without etomidate and 29 were not intubated. Children who were intubated had significantly higher disease severity parameters, whereas none of these parameters significantly differed between children who were intubated with or without etomidate. Children who received etomidate had significantly lower cortisol, higher ACTH and 11-deoxycortisol levels than those who did not receive etomidate. Arterial glucose levels were significantly lower in children who were intubated with etomidate than in children who were not intubated. When children were intubated with etomidate, cortisol levels were 3.2 times lower for comparable 11-deoxycortisol levels. Eight children died, 7 of them had received etomidate. Within 24h cortisol/ACTH ratios increased significantly in children who had received etomidate, but not in children who had not receive etomidate, resulting in comparable cortisol/ACTH ratios 24h after admission. **Conclusions:** Our data imply that even one single bolus of etomidate negatively influences adrenal function and thereby might increase risk of death.

Introduction

Stimulation of the hypothalamic-pituitary adrenal axis is one of the most important hormonal reactions to critical illness (1). We have previously shown that children dying from meningococcal septic shock had relatively low cortisol levels and extremely high ACTH levels, indicating inappropriate adrenal function (2, 3). The anaesthetic drug etomidate is known to inhibit adrenal function by mainly impeding the enzyme 11β-hydroxylase, the last step in the biosynthesis of cortisol, resulting in increased levels of 11-deoxycortisol in relation to cortisol levels (4) (Figure 1). Since long-term etomidate use resulted in increased mortality, etomidate has been withdrawn from long-term sedation regimens (5). Etomidate, however, remained a first-line anaesthetic agent in the setting of rapid sequence intubation, because it has a favourable cardiopulmonary profile. One single bolus of etomidate was assumed by many intensive care physicians to give only transient, clinically non-relevant hormonal changes (6-8). This assumption was mainly based on small studies in healthy adults undergoing elective surgery and may not be applicable in children with septic shock, who are at risk for adrenal insufficiency. Recently, Malerba *et al.* reported the use of etomidate to be independently related to relative adrenal insufficiency in critically ill adults (9). This stimulated us to evaluate retrospectively whether etomidate impaired adrenal function in our cohort of children with meningococcal sepsis.

Materials and Methods

Patients

The group consisted of 69 previously healthy children (42 boys and 27 girls) consecutively admitted to the PICU of the Erasmus MC-Sophia Children's Hospital, with a clinical picture of meningococcal sepsis, defined as sepsis with petechiae and/or purpura as described previously (2, 10, 11). Blood cultures revealed *Neisseria meningitidis* in 58 children. Nine children who received corticosteroid therapy for suspected adrenal insufficiency before admission were excluded. The lack of research staff to ensure adequate 24h stand-by service necessitated two study periods: from October 1997 to October 1999 and from October 2001 to January 2004. The study was approved by the local medical ethics committee and conducted according to declaration of Helsinki.

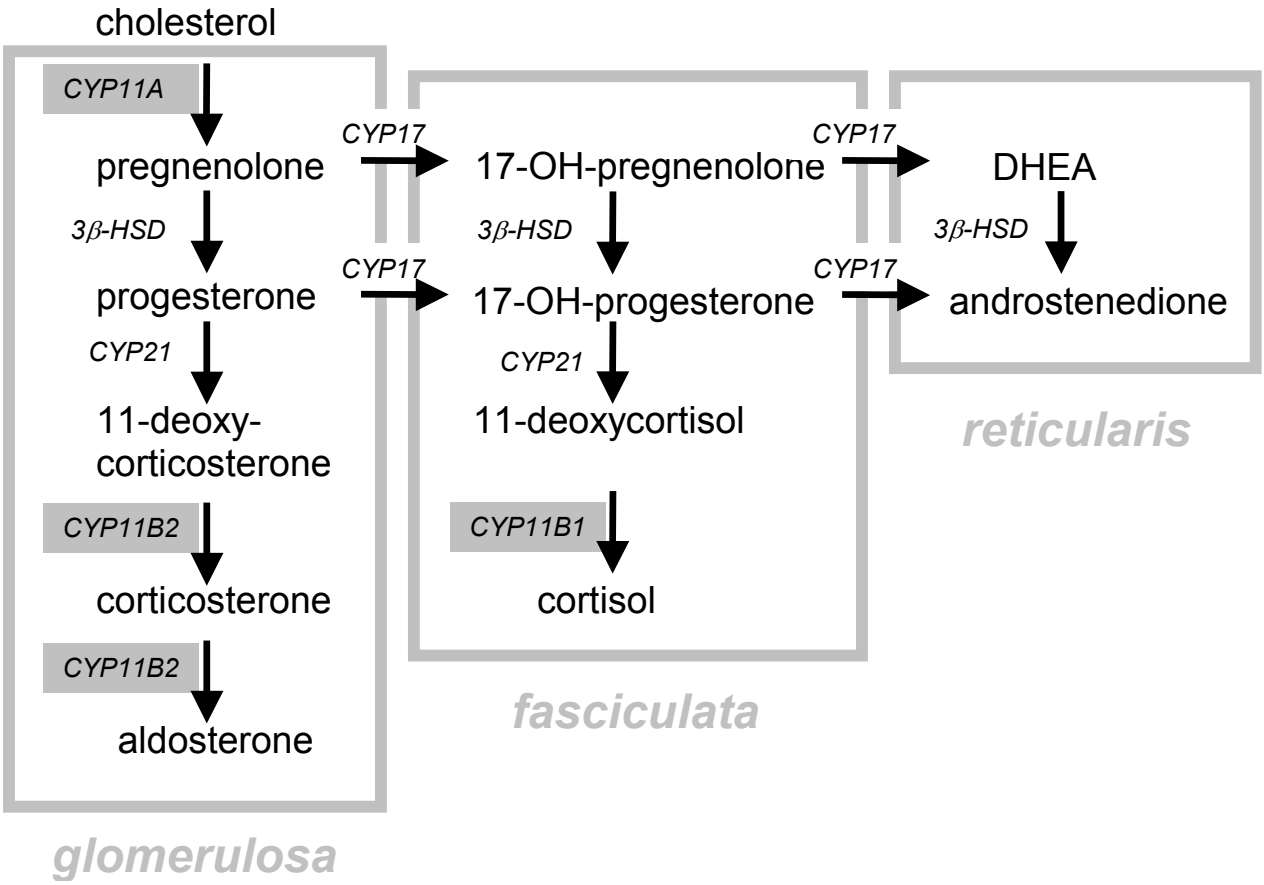

Figure 1. A schematic representation of steroidogenesis in the human adrenal gland and the effect of etomidate. Etomidate inhibits 11β-hydroxylase (CYP11B1), 11β- & 18-hydroxylase (CYP11B2) and cholesterol side-chain cleavage enzyme system (CYP11A) *(shaded enzymes)* with decreasing effectiveness. Decreased CYP11B1 activity will lead to lower levels of cortisol and increased levels of the upstream precursor 11-deoxycortisol. Decreased CYP11B2 will lead to lower aldosterone and higher 11-deoxycorticosterone levels, whereas decreased CYP11A will lead to a generally decreased steroidogenesis. 3β-HSD, 3β-hydroxysteroid-dehydrogenase; CYP21, 21-hydroxylase; CYP17, 17-hydroxylase & 17,20-lyase.

Concomitant therapy

Concomitant therapy on admission included antibiotics (Cefotaxim) and administration of fluids in all 60 children and inotropics in 51 children. On admission 31 children were mechanically ventilated, whereas 29 children were not. Mechanically ventilated children were intubated at median 2h and 40min (range, 5min to 7h) before study enrolment with etomidate (n=23) or with combinations of opiate agonists, propofol, ketamine or midazolam (n=8). The median dose of the etomidate bolus was 0.29 mg/kg (range, 0.20 to 0.67 mg/kg) and was significantly higher in children who died compared with those who survived (0.46 vs. 0.29, *P*=0.038). The sedatives and doses used for rapid-sequence intubation depended on the physicians' choice. After admission 4 more children were intubated with

etomidate. Mechanically ventilated children were all intubated for their clinical status only and were sedated with benzodiazepines and/or morphine. On admission, patients received intravenous glucose at a rate of 4–6 mg/kg/min.

Clinical parameters

Disease severity was determined using the Pediatric Risk of Mortality score (PRISM II) (12) during the first 6 hours of admission. We recorded etomidate use, respiratory and inotropic support, quantified with vasopressor score of Wernovsky on admission (13).

Collection of blood samples and analysis

Arterial blood samples were obtained as soon as possible after admission, 12 and 24 hours thereafter for determination of cortisol, ACTH, glucose, lactate and IL-6 (2, 3). Serum 11-deoxycortisol levels obtained on admission and determined by radioimmunoassay with antiserum from ICN Biomedicals (Costa Mesa, CA).

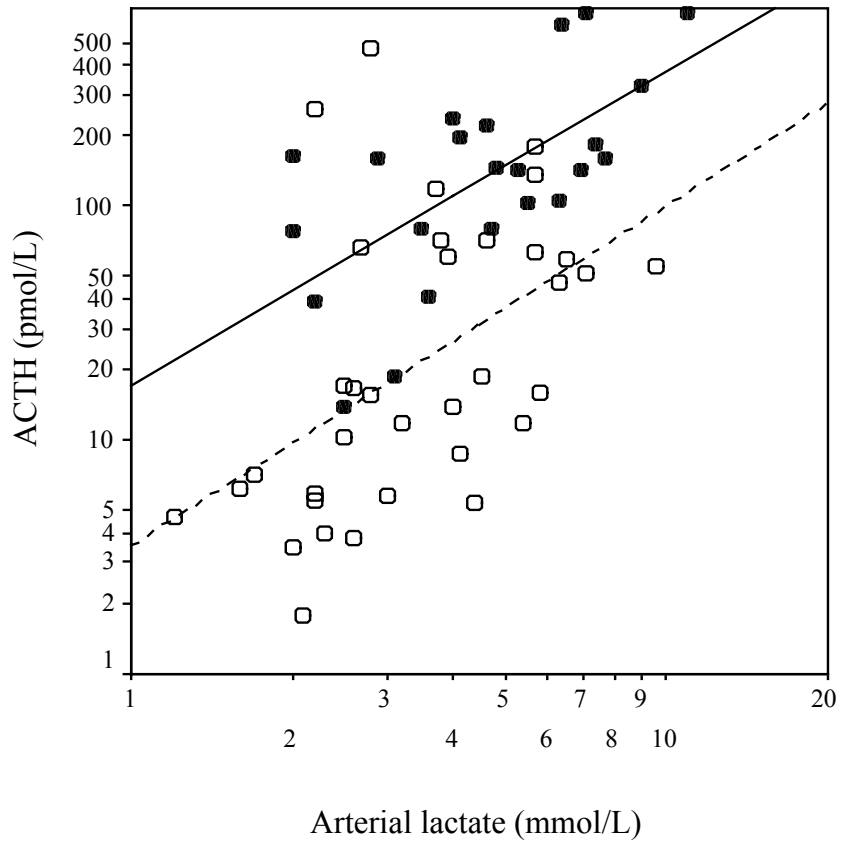

Figure 2. Relation between ACTH and arterial lactate levels, depending on etomidate use. After adjustment for arterial lactate levels, using ANCOVA, mean ACTH levels were a factor 4.1 higher in case of etomidate use (P<0.001). Children who received etomidate (●, continuous line) and children who did not receive etomidate (○, dotted line).

Table 1. Patients' characteristics and adrenal function on admission according to intubation and etomidate use.

	Reference values	Intubated with Etomidate (n=23)	Intubated without Etomidate (n=8)	Not intubated (n=29)
Age (yr)		3.7 (0.9 – 9.4)	4.0 (0.8 – 7.6)	4.9 (2.3 – 10.4)
Sex (M/F)		19/4 [a, b]	2/6	16/13
Septic shock (%)		23 (100) [b]	8 (100)	20 (69)
Vasopressor score		40 (15 – 60) [b]	35 (6 – 105) [b]	5 (0 – 18) [a, c]
PRISM score		25 (20 – 33) [b]	26 (18 – 35) [b]	17 (9 – 20) [c]
Survival (%)		16 (70) [b]	7 (88)	25 (100) [c]
IL-6 x 10^3 (pg/mL)	< 0.01	135.0 (40.0 – 853.1) [b]	63.2 (15.1 – 70.5)	8.3 (1.1 – 80.9) [c]
Lactate (mmol/L)	< 2.0	4.7 (3.1 – 6.9) [b]	4.0 (2.6 – 6.8)	3.0 (2.2 – 4.6) [c]
Glucose (mmol/L)	2.6–11.0	5.7 (4.1 – 7.7) [b]	7.8 (5.5 – 14.2)	7.7 (6.7 – 8.7) [c]
Cortisol (nmol/L)	200–800*	620 (502 – 782) [a, b]	1173 (818 – 1263)	1089 (971 – 1346)
ACTH (pmol/L)	< 11*	146.1 (79.3 – 222.0) [a, b]	53.7 (10.8 – 116.0)	14.0 (5.6 – 64.2)
Cortisol/ACTH (kM/M)		4.7 (2.3 – 8.4) [a, b]	20.7 (3.5 – 112.7)	89.4 (21.0 – 205.2)
11-deoxycortisol (nmol/L)	<50*	181 (137 – 248) [a, b]	49 (36 – 69)	62 (44 – 88)
Cortisol/11-deoxycortisol		3.2 (2.1 – 4.3) [a, b]	21.6 (16.7 – 25.8)	19.2 (13.6 – 24.5)

All values are expressed as median (25 to 75 percentile). * Non-stressed reference values (for cortisol morning values at 8:00 AM) [a] Significantly different compared to children who were intubated without etomidate, $P<0.05$. [b] Significantly different compared to patients who were not intubated, $P<0.05$. [c] Significantly different compared to patients who were intubated, independently of etomidate use, $P<0.05$.

Statistics

The results are expressed as medians unless specified otherwise. We used Mann-Whitney U, chi-square or Fischer's exact test, Spearman's correlation coefficient (r) and analysis of covariance (ANCOVA). The graph of the course of cortisol/ACTH ratios was constructed using mixed model analysis of variance. Two-tailed P-values of <0.05 were considered statistically significant.

Results

Clinical parameters

Children who had been intubated before admission, independently of etomidate use, had significantly higher disease severity parameters – such as PRISM, IL-6, lactate – and vasopressor score than children who were not intubated, whereas none of these parameters significantly differed between children who were intubated with or without etomidate (Table 1). Arterial glucose levels were significantly lower in children who were intubated with etomidate compared to children who were not intubated (*P*=0.023) and tended to be lower than in children who were intubated without etomidate (*P*=0.082).

Adrenal function on admission

On admission, cortisol levels were significantly lower and ACTH levels significantly higher with concomitantly lower cortisol/ACTH ratios in children who were intubated with etomidate compared to those who did not receive etomidate, independently of intubation (Table 1). Furthermore, children who were intubated with etomidate had significantly higher 11-deoxycortisol levels with concomitantly lower cortisol/11-deoxycortisol ratios compared to children who did not receive etomidate, independently of intubation. Compared to non-stressed values, 11-deoxycortisol levels were elevated in 95% of the children who received etomidate and in 65% of the children who did not receive etomidate (*P*=0.019), independently of intubation. Neither time from intubation to admission nor etomidate dose per kg body weight correlated significantly with serum levels of cortisol, ACTH, 11-deoxycortisol or their ratios on admission (data not shown).

ANCOVA revealed ACTH levels to be significantly related with intubation with etomidate and disease severity, as depicted by lactate levels (R^2=0.54), whereas age and gender were not. When children were intubated with etomidate, ACTH levels were a factor 4.1 higher for comparable lactate levels (Figure 2). In contrast, we found no difference in the relation of ACTH with lactate between children who were intubated without etomidate and those who were not intubated (ANCOVA, *P*=0.222). A similar model was found for ACTH levels with IL-6 levels and etomidate (R^2=0.55), in which ACTH levels were a factor 3.3 higher if children were intubated

with etomidate. ANCOVA revealed 11-deoxycortisol levels and intubation with etomidate to be significantly related to cortisol levels (R^2=0.57), whereas age and gender were not. Cortisol levels were a factor 3.2 lower, for comparable 11-deoxycortisol levels, if children were intubated with etomidate (Figure 3). In contrast, we found no difference in the relation of cortisol with 11-deoxycortisol, between children who were intubated without etomidate and those who were not intubated (ANCOVA, *P*=0.303).

Adrenal function time course

Within 5h after study enrolment 4 other children were intubated with etomidate. Seven children received glucocorticoid treatment for suspected adrenal insufficiency after study enrolment; 6 of them (intubated with etomidate) within 5h and one (intubated without etomidate) after 55 h. Eight children died due to hemodynamic failure at median 11h after PICU admission (range, 8 – 43 h), 7 of them had received etomidate. Within 24h cortisol/ACTH ratios increased significantly in children who had received etomidate, but not in children who did not receive etomidate, resulting in comparable cortisol/ACTH ratios 24h after admission (Figure 4).

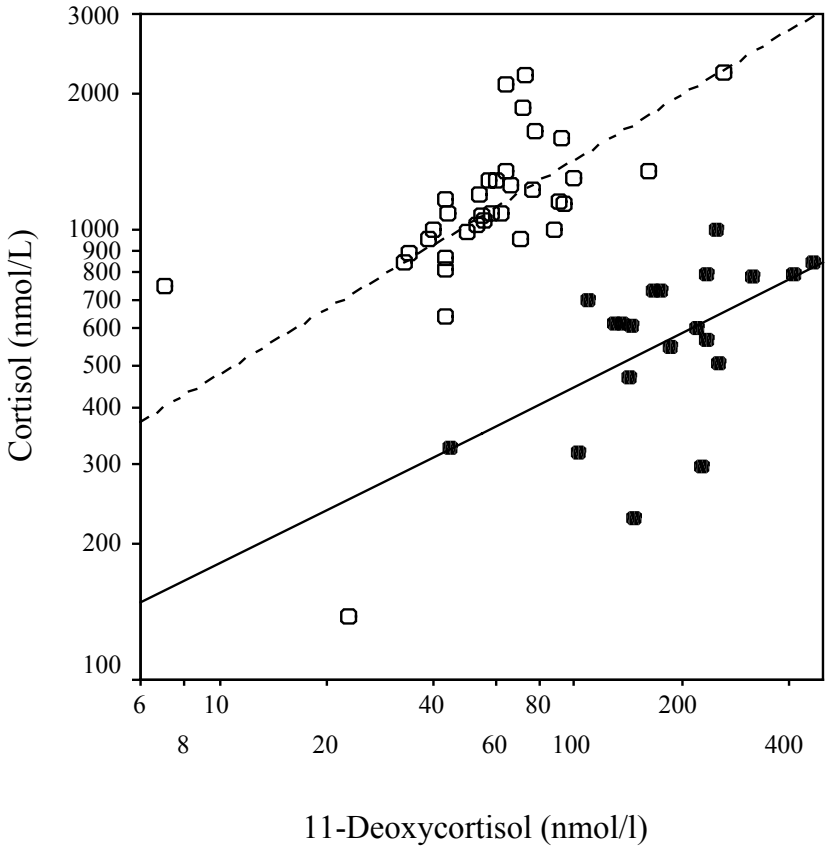

Figure 3. Relation between cortisol and 11-deoxycortisol levels, depending on etomidate use. After adjustment for 11-deoxycortisol levels, using ANCOVA, mean cortisol levels were a factor 3.2 lower in case of etomidate use (*P*<0.001). Children who received etomidate (●, continuous line) and children who did not receive etomidate (○, dotted line).

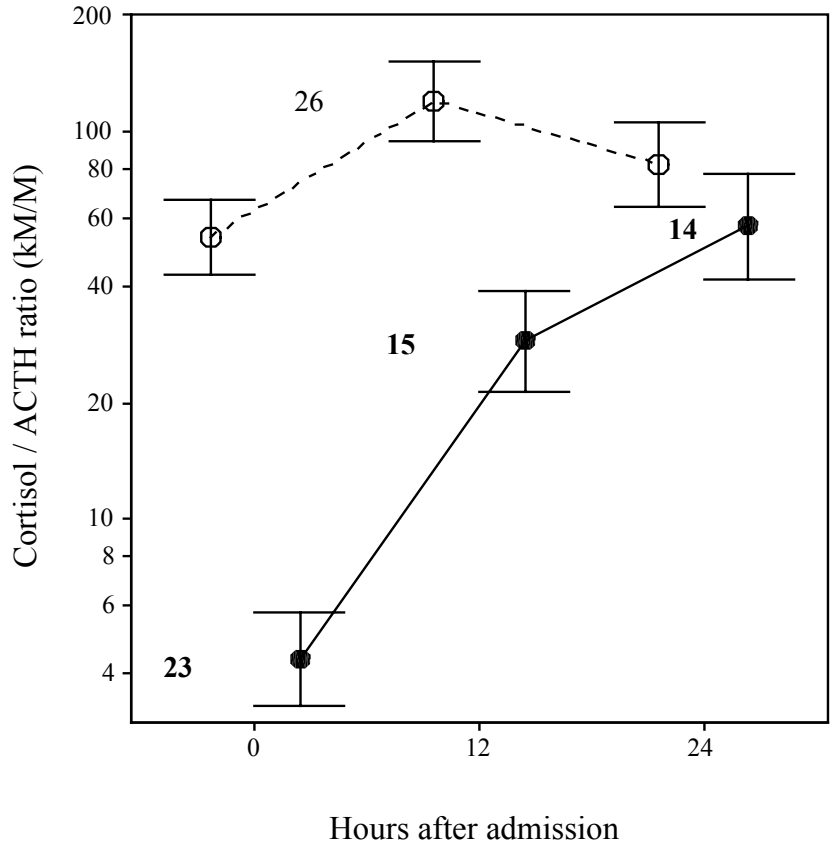

Figure 4. Cortisol/ACTH ratios according to patients' actual etomidate use during the first 24h of admission. The two profiles differed between the groups along time (*P*<0.001). Data shown are geometric means with standard errors. Children who received etomidate (●, continuous line) and children who did not receive etomidate (○, dotted line). Within group difference between successive time point (*a*, *P*<0.05). Between group difference at time points (*b*, *P*<0.05). Numbers alongside data-points indicate numbers of children.

Discussion

This retrospective study showed major differences in ACTH, cortisol and 11-deoxycortisol levels between children intubated with one single bolus of etomidate compared to children who did not receive etomidate, independently of intubation.

Although our study was not designed to investigate the direct relation between etomidate administration and adrenal function, we found significantly more signs of impaired adrenal function, as shown by the combination of significantly lower cortisol with much higher ACTH levels, in children who received etomidate compared to those who did not, even after correction for disease severity (Figure 2). Cortisol levels were 3.2 times lower and ACTH levels were 4.1 times higher in children who received etomidate compared with those who did not. Serum 11-deoxycortisol (the precursor of cortisol that exerts no endocrine actions) was significantly higher and cortisol/11-deoxycortisol lower in children who received etomidate compared to those who did not, indicating impaired 11β-hydroxylase activity (CYP11B1, Figure 1).

This is in accordance with *in vitro* and *in vivo* studies that show etomidate to interfere mainly with 11β-hydroxylase, and at higher concentrations also with 11β- & 18-hydroxylase (CYP11B2) and cholesterol side-chain cleavage enzyme system (CYP11A) (4, 14-17). Despite the fact that etomidate is known to suppress adrenal function in a dose-dependent manner, we did not find such a relation, due to lack of study power (14). However, the dose of etomidate was higher in children who died compared with those who survived. Because we studied retrospectively the effect of etomidate in an uncontrolled setting, it is difficult to report on relevant clinical deterioration, such as persistent hypotension or the course of glucose levels. We found nevertheless significant lower glucose levels on admission in children receiving etomidate compared to those who did not. Furthermore, concerning mortality, in this study 7 of the 8 children who died during admission received etomidate. These numbers are too small to draw conclusions in this complex clinical situation, because many other variables will be of influence to survive septic shock, but the risk of death might be increased when etomidate is used. Significant but transient adrenocortical suppression 24h after a single bolus of etomidate has been described (9, 18). We found no different cortisol/ACTH ratios between the studied groups more than 24h after intubation, whereas these differences were still present after 12 h, suggesting also a 12-24h adrenal cortical suppression. Future investigations using corticotropin test should be done to prove the duration of this adrenocortical suppression.

In summary, our data imply that even one single bolus of etomidate negatively influences adrenal function and thereby might increase risk of death. As recently stated (8), considerable caution should accompany the administration of etomidate in patients with septic shock. The potential role for concomitant steroid replacement should be elucidated.

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Chapter 4

EUTHYROID SICK SYNDROME IN MENINGOCOCCAL SEPSIS: THE IMPACT OF PERIPHERAL THYROID HORMONE METABOLISM AND BINDING PROTEINS

Marieke den Brinker, Koen F. M. Joosten, Theo J. Visser, Wim C. J. Hop, Yolanda B. de Rijke, Jan A. Hazelzet, Venje H. Boonstra, and Anita C. S. Hokken-Koelega

Journal of Clinical Endocrinology and Metabolism 2005 Oct; 90(10): 5613-5620
First Published Online August 2, 2005

Abstract

Context and Objectives: The objective of this study was to elucidate the influence of disease severity, deiodination, sulfation, thyroid hormone binding, and dopamine use on thyroid function in euthyroid sick syndrome. **Setting:** The study was performed at a university-affiliated pediatric intensive care unit (PICU). **Design:** This was an observational cohort study. **Patients:** Sixty-nine children with meningococcal sepsis were studied. **Main Outcome Measures:** Differences in thyroid function among nonsurvivors, shock-survivors, and sepsis-survivors on PICU admission were the main outcome measures. **Results:** The main study group consisted of 45 non-dopamine-treated children. All children had decreased total TT3/rT3 ratios without elevated TSH. T4 sulfate levels were decreased in 88%. Nonsurvivors had paradoxically higher TT3/rT3 ratios than shock-survivors (0.71 *vs.* 0.30); this ratio also correlated with shorter duration of disease (r = –0.43). TT4 and TBG levels declined with increasing disease severity. TBG levels correlated inversely with elastase levels (r = –0.46). Only TSH levels were significantly lower in 24 dopamine-treated children compared with non-dopamine-treated children (0.65 *vs.* 0.84), whereas other thyroid hormones did not significantly differ. Both higher TT3/rT3 ratios and lower TT4 levels were predictive for mortality, but this disappeared when IL-6 was entered into the regression model. **Conclusions:** All children with meningococcal sepsis showed signs of euthyroid sick syndrome. Alterations in peripheral thyroid hormone metabolism related inversely to the duration of disease and seemed to be enacted by profound induction of type 3 deiodinase rather than by down-regulation of type 1. Lower TT4 levels were related to increased turnover of TBG by elastase. Dopamine was found to suppress only TSH secretion, not other thyroid hormone levels, on PICU admission. Both the TT3/rT3 ratio and TT4 levels were predictive for mortality, but were not superior to IL-6.

Introduction

Meningococcal disease is one of the most severe infectious diseases in children. During critical illness, many endocrine changes take place. The initial phase is characterized by peripheral down-regulation of most hypothalamic-pituitary hormones, except cortisol (1). The changes in thyroid hormones during critical illness are called the euthyroid sick syndrome or nonthyroidal illness. Initially, serum total T3 (TT3) values decline, and rT3 values increase (2). When the condition deteriorates, total T4 (TT4) values may decrease as well, producing a combined low TT3-low TT4 state that typically does not result in compensatory higher levels of TSH, which generally remain normal. Alterations in peripheral thyroid hormone metabolism, such as deiodination, play an eminent role in the development of the euthyroid sick syndrome (3, 4). Altered sulfation may also lead to changes in peripheral thyroid hormones (5). Data on thyroid hormone sulfation in critically ill adults are scarce (6), whereas data in critically ill children do not exist.

Furthermore, because thyroid hormones are mainly bound to carrier proteins, a decline in serum thyroid hormones may be the result of decreased concentrations of or decreased binding to serum carrier proteins due to serum inhibitors, such as non-esterified fatty acids (NEFA) (2). Additionally, elastase, a serine protease released by activated neutrophils, has been shown to cleave T4-binding globulin (TBG) during inflammation, resulting in decreased affinity of TBG for thyroid hormones as well as increased clearance of TBG (7, 8). Besides this, medication such as dopamine is known to influence serum thyroid hormones as well, via suppression of TSH (9, 10).

Concerning the relationship between thyroid hormone levels and mortality, a few studies in critically ill children showed an association between low levels of TT4 (11) and TT3 (12) and mortality, whereas this relation was not found in some other studies (13, 14). We previously reported higher TT3 and TT4 levels in a small group of nonsurvivors of meningococcal septic shock compared with survivors (15). To date, little is known about factors influencing changes in thyroid hormone levels in relation to outcome in critically ill children.

We therefore evaluated thyroid function in relation to disease severity and assessed the influence of deiodination, sulfation, and thyroid hormone binding on thyroid hormone levels at pediatric intensive care unit (PICU) admission in a large group of acutely ill children with sepsis or septic shock, not treated with dopamine. In addition, we investigated the influence of dopamine on thyroid hormone levels in a separate group who did receive dopamine. Finally, we assessed the predictive value of thyroid hormone levels on mortality.

Patients and Methods

Patients

The group consisted of previously healthy children primary admitted or referred to the PICU of Erasmus Medical Center-Sophia Children's Hospital between October 1997 and February 2000 and between October 2001 and January 2004, suffering from meningococcal sepsis, defined as sepsis with petechiae/purpura. Sepsis was defined as temperature of less than 36.0 ºC or more than 38.5 ºC with tachycardia and tachypneu. In addition, children were determined to have septic shock if they had persistent hypotension or evidence of poor end-organ perfusion, as described previously (15). Children were not eligible for the study if they had endocrine or chromosomal abnormalities or had received radiation or chemotherapy within the previous 6 months. The local medical ethics committee approved the study, and written informed consent was obtained from the parents or legal representatives.

Clinical parameters

To access disease severity, we determined the pediatric risk of mortality (PRISM II) score (15, 16) and the sepsis-related organ failure assessment (SOFA) score (17) and analyzed plasma IL-6, lactate, and C-reactive protein (CRP) levels. We recorded the interval between appearance of first petechia and PICU admission, respiratory support, and medication use.

Sample collection and laboratory assays

Arterial blood samples were collected on admission; after centrifugation, serum/plasma was stored at –80 ºC until determination of thyroid hormones, IL-6, and elastase-α-1-antitripsine complex (elastase). All other laboratory parameters were determined immediately in a certified laboratory of clinical chemistry (ISO 17025 and 9001).

Serum levels of TT4, T4 sulfate (T4S), T3, rT3, and TBG were determined by RIA, free T4 (FT4) by single-step analog binding assay and TSH, as described previously (18, 19). Thyroid hormone SD scores (Z-scores) were calculated with data from a control group comprised of healthy age-matched children and considered normal between –2 and +2.

Serum CRP was determined by immunoturbidimetric assay, and serum NEFA by calorimetric assay (NEFA-C kit, WAKO Diagnostics, Richmond, VA), both on a Hitachi 912 analyzer (Roche, Indianapolis, IN). Arterial lactate and glucose were determined on blood gas analyzer (ABL 625, Radiometer, Copenhagen, Denmark). The reference values were less than 10 mg/liter for CRP, 35–50 g/liter for albumin, 0.2–1.2 mmol/liter for NEFA, less than 2.0 mmol/liter for lactate, and 2.6–11.0 mmol/liter for glucose. Plasma IL-6 levels were determined with ELISA (Sanquin, Amsterdam, The Netherlands), with a detection limit of 10 pg/ml. Plasma elastase

levels were determined by RIAs (Sanquin, Amsterdam, The Netherlands; normal range, <100 ng/ml) in patients included between October 2001 and January 2004.

Caloric intake

On PICU admission, children received glucose iv (4–6 mg/kg/min), but no enteral or parenteral feeding until the second day.

Statistics

Results, analyzed with SPSS 11.5 (SPSS, Inc., Chicago, IL), are expressed as medians unless specified otherwise. We used Mann-Whitney *U*, χ^2, or Fisher's exact test, when necessary, and Spearman's correlation coefficient (r). For backward multiple linear regression analysis and logistic regression analysis, data were log transformed when necessary. A value from two-tailed *t* test of $P < 0.05$ was considered statistical significant.

Table 1. Patients' characteristics of the main, non-dopamine-treated study group on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

Variables	Nonsurvivors (n=8)	Shock-survivors (n=30)	Sepsis-survivors (n=7)
Age (yrs)	1.1 (0.5 – 9.4) [a, b]	4.3 (0.1 – 16.1) [c]	6.1 (2.3 – 12.2) [c]
Male gender (%)	7 (88%)	19 (63%)	4 (57%)
Time 1st petechia – admission (h)	5.7 (2.4 – 10.5) [a, b]	7.9 (4.2 – 26.5) [c]	7.6 (4.9 – 15.7) [c]
PRISM score	32 (23 – 43) [a, b]	21 (8 – 35) [a, c]	9 (5 – 13) [b, c]
SOFA score	15 (13 – 19) [a, b]	9 (3 – 17) [a, c]	2 (0 – 6) [b, c]
IL-6 x 10^{-3}(pmol/L)	1165.1 (68.5 – 2934.1) [a, b]	50.3 (0.3 – 337.1) [a, c]	0.4 (0.08 – 10.5) [b, c]
Lactate (mmol/L)	6.8 (4.6 – 10.9) [a, b]	3.7 (1.1 – 7.1) [a, c]	2.2 (1.2 – 3.8) [b, c]
CRP (mg/L)	34 (21 – 136) [b]	90 (20 – 326) [c]	75 (20 – 252)
Cortisol (nmol/L)	675 (320 – 3440) [a]	992 (138 – 5467)	1276 (999 – 2200) [c]
Elastase (ng/mL)	-	366 (181 – 1269)	317 (182 – 404)
Albumin (g/L)	40 (34 – 41)	34 (29 – 39)	33 (31 – 40)
NEFA	0.29 (0.22 – 0.42) [a, b]	0.68 (0.52 – 1.22) [c]	0.63 (0.53 – 0.68) [c]
NEFA/albumin (M/M)	0.48 (0.41 – 1.13) [a, b]	1.41 (0.92 – 2.24) [c]	1.10 (1.09 – 1.33) [c]

Values are expressed as median (range). [a] Significantly different compared to sepsis-survivors, $P < 0.05$. [b] Significantly different compared to shock-survivors, $P < 0.05$. [c] Significantly different compared to nonsurvivors, $P < 0.05$.

Results

In the study period, 69 previously healthy children were admitted to the PICU with meningococcal sepsis. The total group was divided into the main study group (n=45), consisting of non-dopamine-treated children, and a dopamine-treated group (n=24). The latter group was separately analyzed and is presented at the end of *Results*.

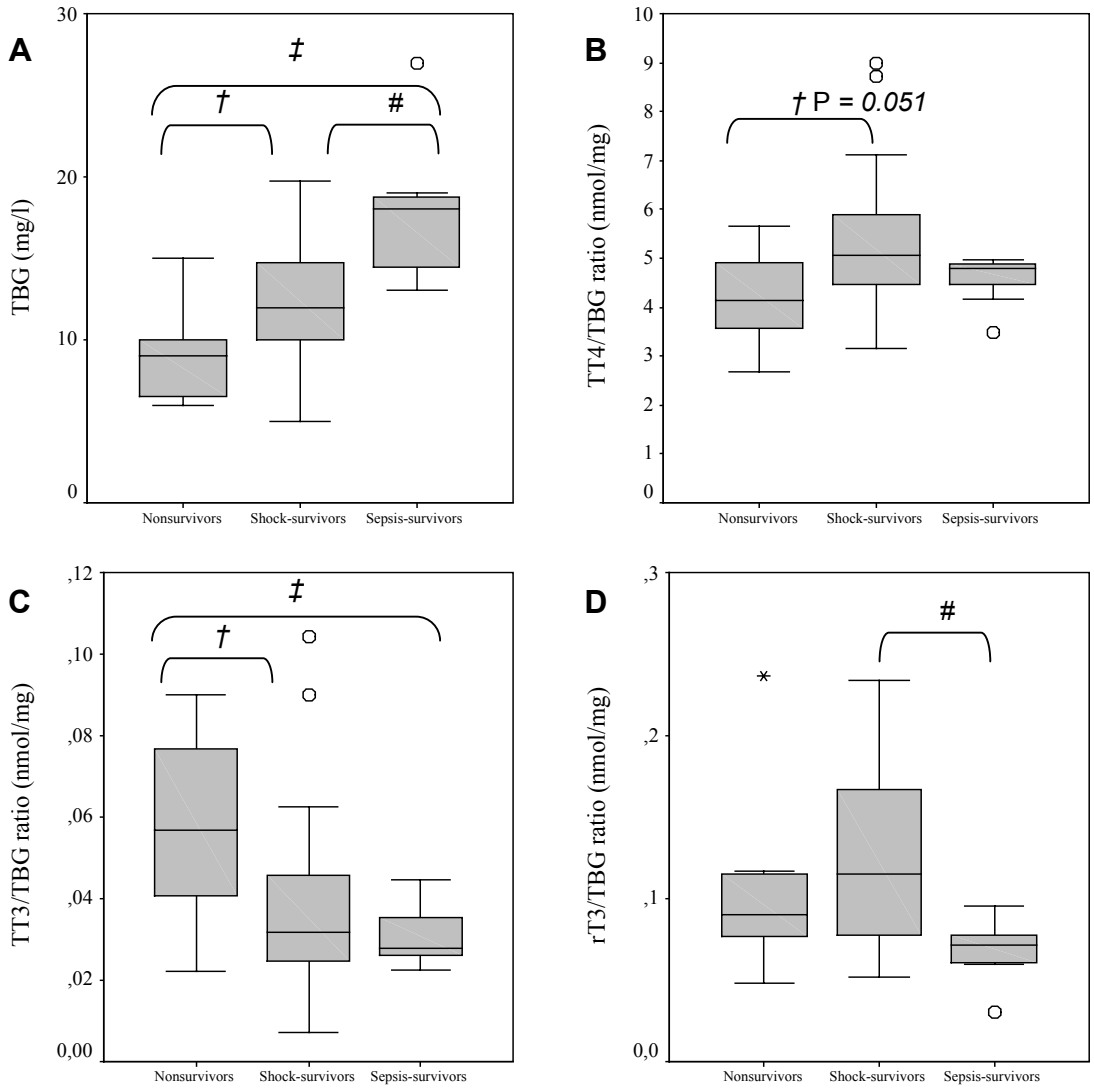

Figure 1. Serum TBG levels (A) and TT4/TBG ratios (B), TT3/TBG ratios (C) and rT3/TBG ratios (D) of the main, non-dopamine-treated study group on admission. Nonsurvivors are depicted in the left box, shock-survivors in the middle box and sepsis-survivors in the right box. Box-whisker-plots: the boxes indicate 25 to 75 percentile with the median and the attached whiskers the complete range with exclusion of outliers (○) and extremes (*). Significant differences between nonsurvivors and shock-survivors (†), between shock-survivors and sepsis-survivors (#) and between nonsurvivors and sepsis-survivors (‡).

Non-dopamine-treated group

Clinical parameters

The main study group (non-dopamine-treated) consisted of 30 boys and 15 girls. Children were divided according to the presence of shock and survival into the following disease severity groups: nonsurvivors (n=8), shock-survivors (n=30), and sepsis-survivors (n=7). Concomitant therapy before study enrollment included antibiotics and administration of fluids in all children, inotropics, not dopamine, in 36

children with septic shock and in one child with sepsis, and glucocorticoids in seven children with septic shock. Twenty-three children with septic shock were mechanically ventilated and sedated with benzodiazepines.

Nonsurvivors were significantly younger than survivors and had significantly shorter time from first petechia to admission than shock-survivors (Table 1). Parameters of disease severity were significantly higher in nonsurvivors compared with shock-survivors and sepsis-survivors for PRISM and SOFA scores, plasma IL-6, and arterial lactate, whereas CRP was significantly lower in nonsurvivors compared with shock-survivors. IL-6 levels correlated strongly with PRISM score (r=0.80; *P*<0.001), and CRP levels correlated strongly with time from first petechia to admission (r=0.52; *P*<0.001).

Table 2. Thyroid hormone levels and SD-scores of the main, *non-dopamine-treated* study group on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

Variables	Nonsurvivors (n=8)	Shock-survivors (n=30)	Sepsis-survivors (n=7)
TT4 (nmol/L)	38 (25 – 46) [a, b]	56 (49 – 73) [a, c]	86 (68 – 92) [b, c]
Free TT4 (pmol/L)	15 (13 – 21)	18 (15 – 20)	19 (14 – 20)
T4S (pmol/L)	52	22 (18 – 31)	27 (19 – 43)
TT3 (nmol/L)	0.49 (0.34 – 0.61)	0.40 (0.30 – 0.52)	0.45 (0.42 – 0.61)
rT3 (nmol/L)	0.75 (0.55 – 1.21) [b]	1.40 (1.11 – 1.73) [c]	1.17 (1.00 – 1.28)
TSH (mU/L)	0.88 (0.52 – 1.14)	0.84 (0.59 – 1.18)	0.80 (0.65 – 1.61)
TT3/rT3	0.71 (0.33 – 0.80) [b]	0.30 (0.20 – 0.44) [c]	0.41 (0.36 – 0.58)
TBG (mg/L)	9 (6 – 10) [a, b]	12 (10 – 15) [a, c]	18 (14 – 19) [b, c]
TT4 SD-score	–7.0 (–8.1 to –5.3) [a, b]	–3.5 (–4.8 to –2.4) [a, c]	–1.6 (–2.8 to –0.4) [b, c]
Free T4 SD-score	–1.1 (–1.8 to 0.6)	–0.3 (–1.2 to 0.3)	–0.1 (–1.4 to 0.1)
T4S SD-score	–2.3	–9.2 (–12.7 to –4.7)	–6.0 (–14.2 to 2.4)
TT3 SD-score	–3.9 (–4.3 to –3.7)	–4.0 (–4.2 to –3.7)	–3.7 (–4.0 to –3.4)
rT3 SD-score	1.3 (0.0 to 3.5) [a, b]	4.3 (2.8 to 6.1) [c]	4.5 (4.1 to 5.6) [c]
TSH SD-score	–1.5 (–2.5 to –1.1)	–1.6 (–2.2 to –1.0)	–2.1 (–1.7 to –0.5)
TT3/rT3 SD-score	–4.5 (–7.9 to –4.0) [b]	–7.7 (–13.2 to –5.9) [c]	–7.3 (–11.7 to –6.4)
TBG SD-score	–5.2 (–6.8 to –3.3) [a, b]	–2.9 (–4.4 to –2.3) [a, c]	–1.8 (–2.0 to –1.0) [b, c]

Values are expressed as median (25 to 75 percentile). [a] Significantly different compared to sepsis-survivors, $P < 0.05$. [b] Significantly different compared to shock-survivors, $P < 0.05$. [c] Significantly different compared to nonsurvivors, $P < 0.05$.

TT4, FT4, TT3, rT3 and TSH levels

Compared with age-matched reference values, the vast majority of the children in the main, non-dopamine-treated study group had low serum TT4 levels (80%), normal FT4 levels (89%), low TT3 levels (100%), high rT3 levels (89%), and low TT3/rT3 ratios (100%), whereas none had elevated TSH levels. TT4 levels were lower with increasing disease severity; nonsurvivors had significantly lower TT4 levels than survivors, and shock-survivors had significantly lower TT4 levels than

sepsis-survivors (Table 2), whereas FT4, TT3 and TSH levels did not significantly differ among the three groups. Median rT3 levels were significantly lower in nonsurvivors than in shock-survivors, whereas rT3 levels were significantly higher in shock-survivors than in sepsis survivors. As a result, the TT3/rT3 ratios were significantly higher in nonsurvivors compared with shock-survivors and tended to be lower in shock-survivors than in sepsis-survivors (*P*=0.057).

Parameters of disease severity correlated negatively with TT4 levels, but not with FT4, TT3, rT3, or TSH levels or TT3/rT3 ratios (Table 3). Time from first petechia to admission correlated positively with rT3 levels and negatively with TT3/rT3 ratios. Serum cortisol levels correlated positively with TT4 levels, but not with FT4, TT3, rT3, or TSH levels or TT3/rT3 ratio, whereas age did not correlate with any thyroid parameter.

T4S levels

On admission, serum T4S levels were measured in 34 non-dopamine-treated children (one nonsurvivor, 26 shock-survivors, and seven sepsis survivors). T4S levels were decreased in the majority of the children (88%) compared with age-matched reference values and did not significantly differ between shock-survivors and sepsis-survivors (Table 2).

T4S levels correlated positively with time from first petechia to admission, but not with age or any parameter of disease severity (Table 3). Furthermore, T4S levels tended to correlate with rT3 levels (r=0.32; *P*=0.069), but not with other thyroid hormone values or ratios.

TBG and albumin

Serum TBG levels were decreased in 62% of the children, and serum albumin levels were decreased in 54% compared with age-matched reference values, whereas none of the children had elevated TBG or albumin levels. TBG levels were lower with increasing disease severity; nonsurvivors had significantly lower TBG levels than survivors, and shock-survivors had significantly lower TBG levels than sepsis-survivors (Figure 1), whereas albumin levels did not significantly differ among the three groups (Table 1). TT4/TBG ratios tended to be lower in nonsurvivors than in shock-survivors (*P*=0.051), but did not differ from sepsis-survivors (Figure 1). TT3/TBG ratios were significantly higher in nonsurvivors compared with shock-survivors and sepsis survivors, whereas rT3/TBG ratios were not significantly different in nonsurvivors compared with shock-survivors or sepsis survivors.

TBG levels correlated negatively with elastase levels and parameters of disease severity, but not with age or time from first petechia to admission (Table 3). TT4/TBG ratios correlated positively with FT4 levels (r=0.46; *P*<0.001).

Table 3. Significant ($P < 0.05$) Spearman's correlation coefficients of thyroid hormones with clinical and laboratory parameters of the main, *non-dopamine-treated* study group on admission.

	Age	Time*	PRISM	SOFA	IL-6	Lactate	CRP	Cortisol	Elastase	TBG	NEFA/ Albumin
TT4	-	-	-0.62	-0.69	-0.72	-0.51	0.41	0.50 ‡	-	0.76	0.39
FT4	-	-	-	-	-	-	-	-	-	-	0.50
T4S	-	0.47	-	-	-	-	-	-	-	-	-
TT3	-	-	-	-	-	-	-	-	-	0.31	-0.41
rT3	-	0.49	-	-	-	-	0.64	-	-	-	0.40
TT3/rT3	-	-0.43	-	-	-	-	-0.43	-	-	-	-0.55
TSH	-	-	-	-	-	-	-	-	-	-	-
TBG	-	-	-0.54	-0.57	-0.52	-0.54	0.35	0.45 †	-0.46	-	-

Time* = time 1st petechia – admission. After exclusion of those children who received glucocorticoid therapy correlation coefficients became stronger between cortisol and TBG levels (r=0.57, †) and between cortisol and TT4 levels (r=0.59, ‡).

Elastase

Plasma elastase levels were measured in shock-survivors and sepsis-survivors and were elevated in all (Table 1). Median elastase levels did not significantly differ between shock-survivors and sepsis survivors, but elastase levels correlated positively with parameters of disease severity, such as IL-6 levels (r=0.66; *P*<0.001) and SOFA score (r=0.44; *P*<0.021). Furthermore, elastase levels correlated negatively with TBG levels (Table 3).

NEFA levels and NEFA/albumin molar ratios

Serum NEFA levels were decreased in 5%, normal in 83%, and elevated in 13% of the children. NEFA levels were significantly lower in nonsurvivors than in survivors (Table 1). NEFA/albumin molar ratios ranged from 0.4–3.9 (median, 1.4) and were significantly lower in nonsurvivors compared with survivors. The NEFA/albumin molar ratios correlated positively with FT4 (r=0.50) and TT4/TBG (r=0.37; *P*<0.022) and negatively with TT3/rT3 ratios (r=–0.55; Table 3).

Dopamine-treated group

Next to the main study group, in which none of the children received dopamine, 24 children with meningococcal septic shock received dopamine median, 3.7 h; (range, 0.25–8.7 h) before sampling. The dopamine-treated group consisted of two nonsurvivors and 22 shock-survivors, but no sepsis survivors. Because all children of the dopamine-treated group experienced septic shock, we analyzed both the nonsurvivors and shock-survivors together, comparing the dopamine-treated group with the non-dopamine-treated group. The mortality rate did not significantly differ between the dopamine-treated and the non-dopamine-treated group (by Fisher's exact test, *P*=0.29). The children with septic shock of the dopamine-treated group did not significantly differ in age (*P*=0.55), time from first petechia to admission (*P*=0.30), PRISM score (*P*=0.97), SOFA score (*P*=0.99), or lactate (*P*=0.32), IL-6 (*P*=0.56), or CRP levels (*P*=0.77) compared with children with septic shock of the non-dopamine-treated group. Median TSH levels were, however, significantly lower in the dopamine-treated children with septic shock than in the non-dopamine-treated group (0.65 *vs.* 0.84 mU/liter; *P*=0.025), and this applied also to the TSH/FT4 ratios (0.028 *vs.* 0.056; *P*=0.005). However, median TT4 (56 *vs.* 52 nmol/liter; *P*=0.86), FT4 (16.9 *vs.* 17.6 nmol/liter; *P*=0.87), TT3 (0.34 *vs.* 0.42 nmol/liter; *P*=0.10), rT3 (1.31 *vs.* 1.22 nmol/liter; *P*=0.41), and TBG (11 *vs.* 10 mg/liter; *P*=0.58) levels did not significantly differ between the dopamine-treated children with septic shock and the non-dopamine-treated group. Furthermore, the duration of dopamine use before admission did not correlate with TSH levels (*P*=0.24), TSH/FT4 ratios (*P*=0.35), or any other thyroid parameter on admission.

Total study group

Multivariate analysis

We investigated the contribution of thyroid hormone values on mortality with logistic regression analysis on the total group of 69 children. The odds for mortality against survival increased by a factor 3.7 for every doubling of the TT3/rT3 ratio and by 1.4 for every 10 nmol/liter lower TT4 level on admission. However, when admission values of IL-6 were added to the model, none of the thyroid hormone levels remained significantly related to mortality, and the odds for mortality against survival increased by a factor of 6 for every doubling of IL-6 levels.

We investigated the simultaneous contribution of pathophysiologically important factors to thyroid hormone levels on admission with multiple regression analysis on the total group of 69 children. Using multiple regression analysis, we found TT4 levels to decrease by 33% for every 10 mg/liter lower TBG level and by 4% for every halving of TSH levels. These two variables together explained 62% of the variation in TT4 levels, whereas TBG levels alone explained 58% of the TT4 variation. None of the additional variables investigated in this analysis (age, gender, IL-6 and cortisol levels, and NEFA/albumin molar ratios) were significantly related to TT4 levels. The relation between TT4 levels with TBG and TSH levels was not affected by dopamine administration before admission (P=0.63).

We found TT3/rT3 ratios to decrease by 5% for every additional hour between first petechia to admission and by 29% for every point increase in NEFA/albumin molar ratios (R^2=0.26). None of the additional variables investigated in the multiple regression analysis (age, gender, and IL-6, cortisol, and TT4 levels) were significantly related to TT3/rT3 ratios. The relation between TT3/rT3 ratios with time from first petechia to admission and NEFA/albumin molar ratios was not significantly affected by dopamine administration before admission (P=0.19).

We found T4S levels to increase by 38% for every 10 g/liter lower albumin level (R^2=0.17). None of the additional variables investigated in the multiple regression analysis (age, gender, cortisol and FT4 levels, and time between first petechia and admission) were significantly related to T4S levels. The relation between T4S levels and albumin levels was not affected by dopamine administration before admission (P=0.31).

We found TBG levels to decrease by 2.3 mg/liter for every doubling in elastase levels (R^2=0.15). None of the additional variables investigated (age, gender, and time from first petechia to admission) was significantly related to TBG levels. The relation between TBG levels and elastase levels was not affected by dopamine use on admission (P=0.27).

Finally, we found only dopamine use to be significantly related to TSH levels, explaining 10% of the variation in TSH levels. None of the additional variables investigated in the multiple regression analysis (age, gender, time from first petechia to admission, or IL-6, cortisol, and FT4 levels) was significantly related to TSH levels.

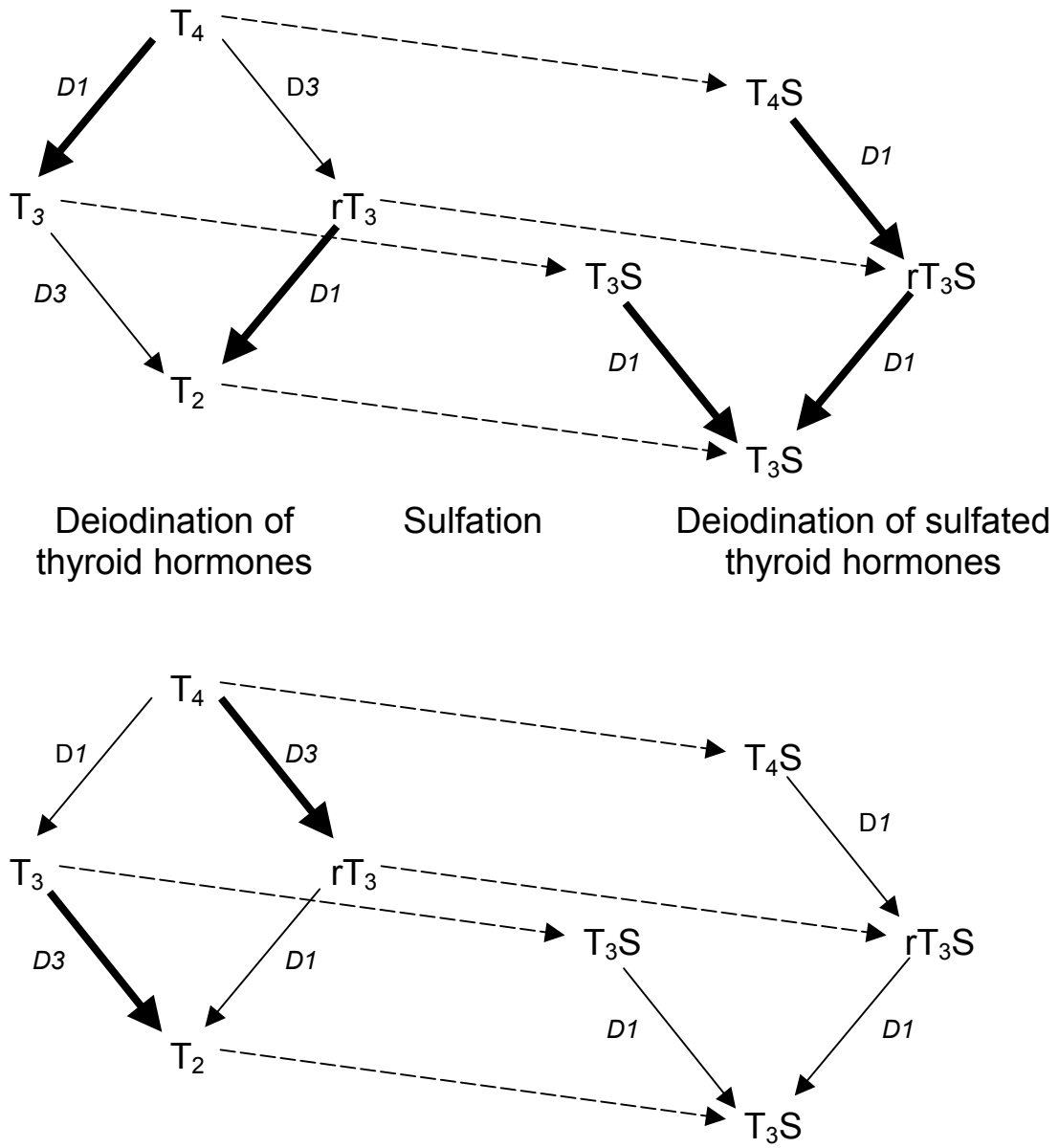

Figure 2. Peripheral thyroid hormone metabolism during normal homeostasis (A) and during critical illness (B). During critical illness deiodination is altered with reduced D1 activity (thin arrow) and increased D3 activity (thick arrow), which both result in increased rT3 levels at expense of TT3 levels (left side). Sulfated thyroid hormones, however, are exclusively deiodinated by D1 (right side) and reduced D1 levels will result in elevated levels of all sulfated thyroid hormones. Sulfation of thyroid hormones is indicated by dotted arrows.

Discussion

In this study all critically ill children with sepsis or septic shock had signs of euthyroid sick syndrome on PICU admission, depicted by low TT3 and TT4 levels and high rT3 levels without compensatory elevated TSH levels. In the main, non-dopamine-treated study group, nonsurvivors appeared to lack time to develop full-blown euthyroid sick syndrome, resulting in paradoxically higher TT3/rT3 ratios in

nonsurvivors than in shock-survivors. In survivors, both T4S levels and TT3/rT3 ratios were decreased, which suggests alterations in peripheral thyroid hormone metabolism with a profound induction of type 3 deiodinase (D3), rather than down-regulation of type 1 deiodinase (D1) in the initial phase of meningococcal sepsis (see below). Furthermore, lower TT4 levels were related to lower TBG levels, which may be due to increased degradation of TBG by elastase. In the dopamine-treated group, dopamine was found to only have a suppressive effect on pituitary TSH secretion, although apparently not long enough to suppress thyroid hormone levels at the time of PICU admission. In the total group, both higher TT3/rT3 ratios and lower TT4 levels were predictive for mortality, but their predictive value for mortality disappeared when IL-6 levels were entered in the model.

The peripheral inactivation of circulating thyroid hormones plays an important role in the initial phase of critical illness (1, 3, 5). Increased levels of biologically inactive rT3 at the expense of decreased TT3 levels are a hallmark of the euthyroid sick syndrome, which are enacted by altered deiodination, the main pathway of peripheral thyroid hormone metabolism (4) (Figure 2). We found decreased TT3/rT3 ratios in all children with meningococcal sepsis, suggesting altered deiodination. This is in agreement with studies in critically ill children and adults (11, 12, 20, 21). In contrast, however, we found significantly higher TT3/rT3 ratios in nonsurvivors than in shock-survivors. A possible explanation for this might be the more fulminant course of disease in nonsurvivors, represented by a shorter time between the appearance of first petechia and admission in those children. The appearance of petechiae is considered to be the first clearly detectable clinical sign of meningococcal sepsis. Because the TT3/rT3 ratios were significantly inversely related to time from appearance of first petechia to admission, this might suggest that the peripheral thyroid hormone metabolism in nonsurvivors lacked the time to develop the full-blown euthyroid sick syndrome before PICU admission. Similarly, the acute phase protein CRP, which is known to be induced within 6–8 h, correlated inversely with time from first petechia to admission as well, indicating that nonsurvivors had less time to produce CRP before admission than survivors. We cannot, however, rule out the possibility that nonsurvivors could not sufficiently adapt to conditions of acute critical illness.

Increased levels of cytokines (22–24) and glucocorticoids (3, 25), from either endogenous or exogenous sources, are both indicated as potential mediators in the alterations of thyroid function that occur in critical illness. In our study, major differences were seen in IL-6 levels, one of the key cytokines in the inflammatory response, between the disease severity groups. IL-6 levels were extremely high in nonsurvivors and much higher than in survivors, which is in agreement with studies in children (26) and adults (27) with life-threatening infections. Our study, however, does not support the postulation that cytokines, such as IL-6, are the potential mediators for altered peripheral deiodination during acute critical illness, because no significant relations were found between IL-6 and TT3 and rT3 levels or TT3/rT3

ratios. An explanation for this can be found in the different cortisol response in children during septic shock *vs.* adults. Although cortisol levels in critically ill adults are positively correlated with disease severity and outcome (20), in children with meningococcal sepsis, the opposite is true (15, 26, 28).

Next to deiodination, sulfation is another pathway of peripheral thyroid hormone metabolism, leading to the metabolites T4S, T3S, and rT3S (29). Data on thyroid hormone sulfation during critical illness are very scarce (6, 30). To our knowledge, we are the first to report T4S levels in the euthyroid sick syndrome. Contrary to our expectations, T4S levels were decreased in the vast majority of children with meningococcal sepsis compared with healthy age-matched children. Unpublished observations by H. van Toor and T. J. Visser revealed increased T4S levels in adults with the euthyroid sick syndrome. Recently, Peeters *et al.* (4) showed that the increased rT3 levels at the expense of TT3 levels in critical illness are not only due to down-regulation of D1, but also to induction of D3 (Figure 2). Because iodothyronine sulfates are exclusively and rapidly deiodinated by D1 (31), the combination of elevated rT3 levels with low T4S levels might be more suggestive of a profound induction of D3 rather than down-regulation of D1 in the initial phase of meningococcal sepsis. Decreased sulfation of TT4 or increased T4S uptake in tissues, however, cannot be excluded, although the latter is less likely, because tissue uptake of thyroid hormones in critically illness is generally decreased (2).

Because TT4, TT3, and rT3 are mainly bound to carrier proteins, differences in these hormone levels might also be based on decreased concentrations of or less binding to serum carrier proteins, such as TBG and albumin. Changes in serum levels of TBG, the most important carrier protein (32), explained 58% of the variation in TT4 levels, whereas increasing disease severity was correlated with both lower TBG and TT4 levels. Elastase levels were elevated and related positively to disease severity and inversely to serum TBG levels. This suggests that with increasing disease severity, TBG was increasingly cleaved by elastase, a serine protease that is released by activated neutrophils (33), resulting in lower TBG levels. Dilution and capillary leakage might be alternative processes resulting in lower TBG levels. Another, but less important, carrier protein for TT4, TT3, and rT3 is albumin. Although albumin levels were decreased in half the children, it is unlikely that albumin levels contributed to the changes in TT4, TT3, and rT3, because albumin levels did not significantly differ between the groups, whereas TT4, TT3, and rT3 levels did.

Besides decreased levels of binding proteins, less binding of thyroid hormones to their binding proteins by circulating inhibitors, such as NEFA, has been reported in the euthyroid sick syndrome (34). It has been reported that increased NEFA levels compete directly with TT4 for binding to TBG, especially when NEFA/albumin molar ratios exceed 5 (2, 35). Although the NEFA/albumin molar ratios in all of our patients were less than 5, they ranged widely from 0.4–3.9. Because the NEFA/albumin molar ratios were more strongly positively related to FT4

than to TT4/TBG levels, it cannot be ruled out that NEFA, even at relatively low NEFA/albumin molar ratios, might also be involved in the displacement of thyroid hormones from TBG. Furthermore, we found a significant inverse relation between NEFA/albumin molar ratios and TT3/rT3 ratios, suggesting that NEFA-induced displacement of thyroid hormones from their plasma binding proteins in addition to declined TBG levels might accelerate the inactivation of thyroid hormones in the euthyroid sick syndrome. Because nonsurvivors had significantly lower NEFA levels compared with survivors, this might also have contributed to the less decreased TT3/rT3 ratios in nonsurvivors.

Finally, in an additional group of children with meningococcal septic shock who received dopamine, we found significantly lower TSH levels and TSH/FT4 ratios than in those who did not receive dopamine, whereas other thyroid hormone levels did not significantly differ. The suppressive effect of dopamine infusion on the pituitary gland has been previously described in critically ill children and adults (9, 10). Dopamine directly inhibits anterior pituitary function through inhibitory dopamine receptors, resulting in diminished TSH release (36, 37). In children recovering from cardiac surgery, cessation of dopamine therapy resulted in an abrupt rise in TSH levels, followed by increases in TT4 and TT3 levels and in the TT3/rT3 ratio 24 h thereafter (9). Although our study was not designed to investigate the effects of dopamine, we found evidence for a suppressive effect on TSH levels, but not on TT4 or TT3 levels in critically ill children on PICU admission. An explanation for this might be the relatively short duration of dopamine administration at the time of sampling (median, 3.7 h) compared with the previously published data on dopamine administration of at least 21 h (9, 10).

In conclusion, our study shows that all critically ill children with sepsis or septic shock have signs of euthyroid sick syndrome on PICU admission. Alterations in peripheral deiodination were related to duration of illness and seemed to be enacted by a profound induction of D3 rather than down-regulation of D1. Low TT4 levels were related to increased cleavage of TBG by elastase. Dopamine-treated children showed only a reduction of TSH levels and no difference in other thyroid hormone levels compared with the non-dopamine-treated children at the time of PICU admission. Values of TT3/rT3 and TT4 were predictive for outcome, but this disappeared when IL-6 was entered in the model.

Acknowledgments

We thank M. Maliepaard (research nurse) for her technical assistance; C. L. Vermont (M.D., Ph.D.), E. D. de Kleijn (M.D., Ph.D.), and their colleagues as well as E. M. N. Bannink, R. D. van Beek, M. van Dijk, D. A. M. Festen, D. C. van der Kaay, and A. S. Slingerland (all M.D.s) for helping to collect samples on weekends; and the nursing staff of the PICU for their support. We also thank H. van Toor and Y. B. de Rijke (Ph.D.) for providing age-matched reference values of thyroid parameters.

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Chapter 5

THYROID FUNCTION AND OUTCOME IN CHILDREN WHO SURVIVED MENINGOCOCCAL SEPTIC SHOCK

Marieke den Brinker, Bertien Dumas, Theo J. Visser, Wim C. J. Hop, Jan A. Hazelzet, Dederieke A. M. Festen, Anita C. S. Hokken-Koelega, Koen F. M. Joosten

Intensive Care Medicine 2005 Jul;31(7):970-976

Abstract

Objective: To investigate the time course of thyroid function, factors that affect it, and its relationship to outcome in children surviving meningococcal septic shock. **Design and setting:** Observational cohort study in a university-affiliated pediatric intensive care unit (PICU). **Patients and participants:** We divided the 44 children admitted to the PICU who survived meningococcal septic shock into those with short-stay (<7 days, n=33) or long-stay (≥7 days, n=11). **Measurements and results:** Serum thyroid hormone concentrations were determined on PICU admission and after 24 and 48 h. The Pediatric Risk of Mortality score and selected laboratory parameters were used to assess disease severity. On admission all children showed signs of euthyroid sick syndrome: low total triiodothyronine (TT3) and high reverse triiodothyronine (rT3) without compensatory elevated thyrotropin (TSH). Admission rT3 levels and the TT3/rT3 ratio were correlated with C-reactive protein levels and with time from first petechia to admission. Short-stay children only had higher TT3 and lower interleukin 6 levels at admission than long-stay children; after 48 h they showed higher total thyroxin, free thyroxin, TT3, and TSH and lower rT3 than long-stay children. All changes in thyroid parameters within the first 24 h were related to length of PICU stay. In children receiving dopamine TSH levels and TT3/rT3 ratios remained unchanged, whereas both values increased in those who did not receive dopamine or in whom dopamine was discontinued. **Conclusions:** All children surviving meningococcal septic shock showed signs of euthyroid sick syndrome on admission. Thyroid hormone level changes in the first 24 h were prognostic for length of PICU stay.

Introduction

Septic shock with purpura is a life threatening critical illness in adults and children. We have previously shown that critical illness leads to thyroid function changes of the sort called the euthyroid sick syndrome, characterized by reduced total triiodothyronine (TT3) and elevated reverse triiodothyronine (rT3) levels without compensatory elevation in thyrotropin (TSH) levels. These changes are less pronounced in children who die than in those who survive (1, 2).

Thyroid hormones play an important role in the protein catabolism and the lack of capacity to synthesize proteins hinders the restoring from muscle wasting and thereby preventing the body from recovery (3). In adults thyroid hormones have been found to be an interesting parameter for evaluating disease severity and predict outcome (4). Furthermore, the magnitude of the TT3 decrease within 24 h reflects the severity of illness (4, 5). The sickest patients show the greatest abnormalities in thyroid hormone levels, while patients with complications show more prolonged depression and delayed recovery (6). In addition to disease severity, medication such as dopamine is known to depress thyroid axis as well (7–10). Studies in children have shown that thyroid function is depressed in critically ill children on admission, but prediction of mortality differs between reports. We previously reported higher total thyroxin (TT4) and TT3 levels in children dying from meningococcal septic shock (1, 2); some have found lower TT4 (11) and TT3 (12) levels to be related to mortality while others found no relationship between thyroid hormones and mortality (13, 14). Little is known at present about the course of thyroid function during pediatric intensive care unit (PICU) stay in relation to length of PICU stay.

The aim of our study was to evaluate thyroid function in children who survive septic shock with purpura in relation to length of PICU stay and to investigate factors influencing thyroid function.

Materials and methods

This observational cohort study was conducted between 1997 and 2002 at the Erasmus Medical Center – Sophia Children's Hospital, in Rotterdam. Part of the study from 1997 to 2001 consisted of a randomized, placebo-controlled dose-finding study of protein C concentrate (Ceproxin, provided by Baxter, Vienna, Austria) in the treatment of pediatric septic shock patients with petechiae and/or purpura (15).

Patients

A total of 54 children aged between 1 month and 18 years and surviving septic shock with petechiae/purpura requiring intensive care treatment were enrolled in this study within 6 h after PICU admission. The group consisted of children primarily admitted

or referred to our PICU. Ten children died after a median PICU stay of 12 h (range 8–42), and 44 survived and remained in the PICU for a median of 4.0 days (range 1.5–44.7 days; 27 boys, 17 girls; median age 4.9 years, range 0.1–16.1). Six children were discharged on day 2 and 11 on day 3. There were 33 children with a PICU stay shorter than 7 days (median 3.2) and 11 with a median stay of 7 days or longer (median 10.8); age and gender did not differ significantly between the groups (Table 1).

Shock was defined as persistent hypotension or evidence of poor end-organ perfusion, defined as at least two of the following: unexplained metabolic acidosis, arterial hypoxia in patients without overt cardiopulmonary disease, acute renal failure or sudden deterioration in baseline mental status as described previously (1, 16). The Medical Ethics Committee of the Erasmus Medical Center approved the study protocol. Informed consent was obtained from the parents of all children.

Table 1. Clinical and laboratory parameters on admission according to long and short PICU stay.

	Long-stay (n=11)	Short-stay (n=33)	P-value
Age (year) [a]	2.5 (0.1 – 15.7)	5.0 (0.3 – 16.1)	Ns
Gender (male) (%)	6 (55%)	21 (64%)	Ns
Time between first petechia-admission (h) [a]	6.6 (3.0 – 12.8)	4.5 (0.0 – 22.9)	Ns
PRISM [a]	24 (12 – 46)	20 (9 – 35)	Ns
IL-6 (pg/ml) [b]	97075 (41041 – 252893)	13521 (38 – 337951)	0.019
Lactate (mmol/l) [b]	4.4 (2.0 – 9.6)	3.3 (1.7 – 6.5)	Ns
CRP (mg/l) [b]	77 (20 – 161)	90 (18 – 326)	Ns

Results are depicted as median (range) (*a*) or as geometric mean (range) (*b*) and tested with Mann-Whitney-U test.

Clinical parameters

The Pediatric Risk of Mortality (PRISM II) score was calculated on the basis of the most abnormal values of 14 physiological parameters during the first 6 h of admission. A higher score indicates a higher risk of mortality (17). We recorded the period between appearance of first petechia and PICU admission, respiratory and inotropic support, and the length of PICU stay. To access severity of disease we analyzed lactate, C-reactive protein (CRP), and interleukin 6 (IL-6) levels. A PICU dependency of less than 7 days was defined as a short stay and one of 7 days or longer as a long stay.

Sample collection

Arterial blood samples were collected on admission and after 24 and 48 h for determination of TT4, free thyroxin (fT4), TT3, rT3, TSH, CRP, lactate, and IL-6.

Blood was collected in glass tubes, centrifuged, and stored at –80 °C until assay determination of thyroid hormones and IL-6. All other laboratory parameters were determined immediately. The admission samples were taken prior to the administration of study medication.

Hormonal assays

Plasma concentrations of TT4, TT3 , and rT3 were measured by established radio immunoassay procedures, as described previously (18, 19). Serum TSH concentrations were measured by an ultrasensitive immunometric assay (AmerliteTSH-30; Ortho-Clinical Diagnostics, Strasbourg, France) or with the immunoradiometric DYNOtest TSH assay (Brahms Diagnostica, Berlin, Germany). The within-assay coefficient of variation (CV) of the Amerlite TSH assay was 4–8% and the between-assay CV 4–11%. The within–assay CV of the Brahms TSH assay was 2–5% and the between–assay CV 2–14%. Serum fT4 levels were measured by a direct, labeled antibody, competitive immunoassay technique (Amerlite MAB fT4 Assay; Ortho-Clinical Diagnostics) or by Vitros ECi technology (Ortho-Clinical Diagnostics, Amersham, UK). The within-assay CV of the Amerlite fT4 assay was 4–8% and the between- assay CV 4–9%. The within-assay CV of Vitros was 3–7% and the between-assay CV 5–10%. Adult reference values of our laboratory are presented in Fig. 1 and Table 2.

Other laboratory assays

Arterial lactate was measured on a blood gas analyzer (ABL 625, Radiometer Copenhagen, Denmark). Serum CRP was determined by an immunoturbidimetric assay (normal <2 mg/l) and examined on a 912 analyzer (Roche Molecular Biochemicals, Mannheim, Germany), with a within-assay CV of less than 2% and between–assay CV less than 10%. Plasma IL-6 levels were analyzed using enzyme-linked immunosorbent assay (Sanquin, Amsterdam, The Netherlands) with a detection limit of 5 pg/ml.

Caloric intake

The patients were fed enterally and/or parenterally according to a standard feeding protocol; on PICU admission glucose was administered intravenously at a rate of 4–6 mg/kg per minute (glucose 5% or 10% solution). If enteral feeding could not be started on the second day, parenteral feeding was started. The initial daily dose of proteins and lipids was 1.0 g/kg (Aminovenös N-paed 10%, Fresenius, ‘s-Hertogenbosch, The Netherlands; Intralipid 20%, Pharmacia & Upjohn, Woerden, The Netherlands). If clinically possible, enteral and/or parenteral nutrition was adjusted on days 3 and 4 to normal needs for healthy children.

Statistics

Clinical parameters were expressed as medians and laboratory results as geometric means unless specified otherwise. Between–group comparisons were made using the Mann-Whitney U test for continuous data and c2 test for categorical data. Within-group comparisons were made using the Wilcoxon signed rank test. Graphs of the time course of thyroid hormone values were calculated using mixed-model analysis of variance on the two subsets of survivors based on duration of PICU stay, and data were logtransformed when necessary. Spearman's correlation coefficient (r) was used to evaluate the relationship between different parameters. Multiple regression analysis was used to evaluate the relationship between length of PICU stay and various variables. All reported p values are two-tailed, and values less than 0.05 are considered statistically significant.

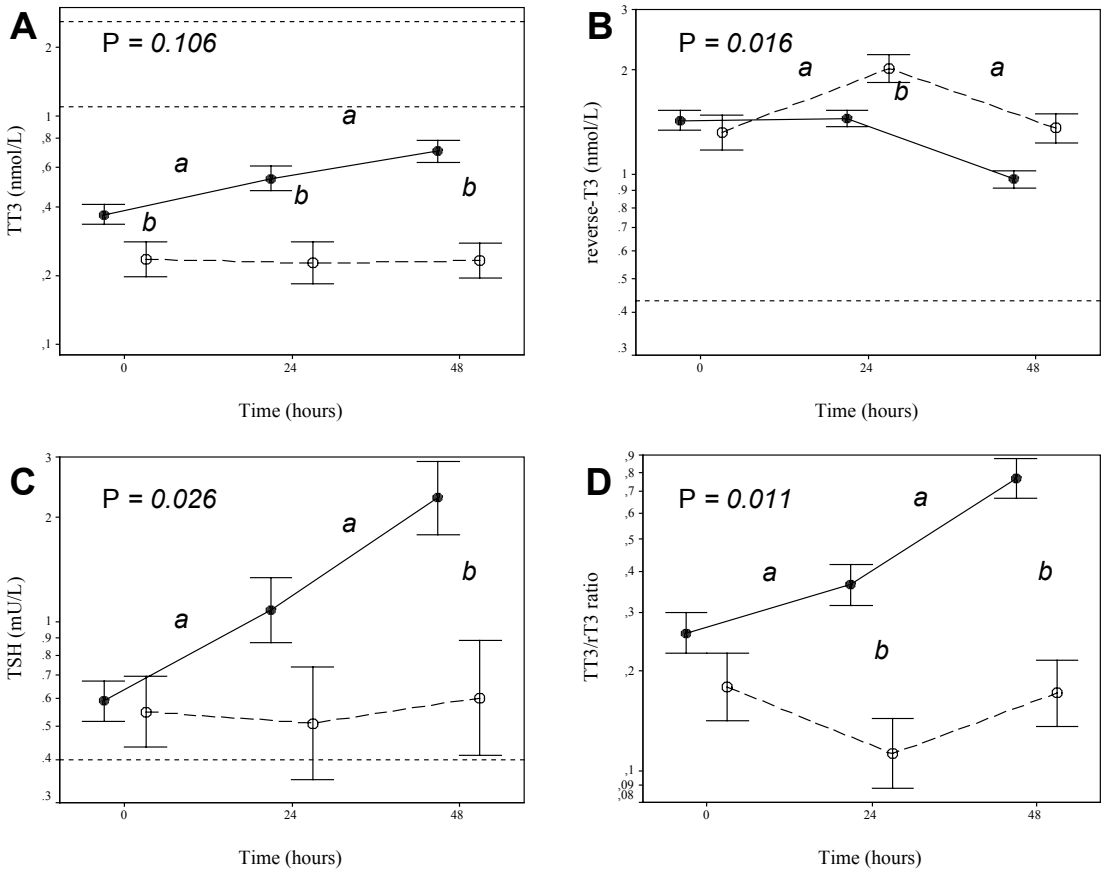

Figure 1. Time course of thyroid hormones according to long and short PICU stay. Data shown are geometric means with standard errors. P-values shown in figures represent the difference between groups of the two profiles along time. Short-stay children (●) and long-stay (○) children. Within group difference between successive time point (*a*, p<0.05). Between group difference at time points (*b*, p<0.05).

Results

Clinical parameters

Of all the parameters for monitoring disease severity only IL-6 was significantly lower in the short-stay than the long-stay group (13,520 vs. 97,074 pg/ml, p=0.019); PRISM score, arterial lactate, and CRP did not differ significantly (Table 1). Cultures of blood confirmed Neisseria meningitidis in 31 children. Concomitant therapy during the study period included antibiotics (cefotaxim) and administration of fluids and inotropics in all 44 children; 20 children received dopamine prior to inclusion (15 short-stay and 5 long-stay) and 3 thereafter (2 short-stay and 1 long-stay). Children receiving dopamine at any time during PICU admission did not differ significantly in age, time between first petechia and admission, PRISM score, IL-6, lactate, or CRP levels on admission, or duration of PICU stay. Twenty-seven children (9 long–stay and 18 short-stay) required mechanical ventilatory support and sedation with benzodiazepines and/or morphine. Twenty-four children were included in the protein C study and 20 in the sepsis-cohort study. Of the 24 children participating in the protein C study 19 received protein C in three different doses after admission; they were equally distributed between short and long-stay patients. Analysis of variance revealed no effect of protein C on any thyroid hormone level during the first 48 h.

Thyroid parameters on admission

On admission all children had lower TT3 levels and higher rT3 levels than age-matched reference values (data not shown) while all TSH levels remained within the normal range. TT4 levels were low in 50% of the children and within the normal range in the other 50%, while fT4 levels were normal in 50% and elevated in the other 50%. Low TT4 levels with concomitantly elevated fT4 levels occurred in 14% of the children. Only TT3 values were significantly higher in short-stay than in long-stay children (0.38 vs. 0.24 nmol/l, p=0.022; Fig. 1). On admission the rT3 level and TT3/rT3 ratio were significantly correlated with CRP levels (r=0.45 and r=–0.34, respectively). The TT3/rT3 ratio and CRP level were correlated with time from first petechia to admission (r=–0.30 and r=0.47, respectively; Fig. 2). No other thyroid hormone value was correlated with any parameter of disease severity.

Length of PICU stay was correlated positively with parameters of disease severity on admission, such as IL-6 levels (r=0.72) and lactate levels (r=0.36), and negatively with TT4 (r=–0.37) and TT3 levels (r=–0.35). Changes (Δ) in thyroid hormones from admission to 24 h are expressed as ratios (24 h/admission). Of these changes significant correlations with IL-6 levels on admission were found for: ΔTT4 (r=–0.42), ΔfT4 (r=–0.40), ΔTT3 (r=–0.44), ΔrT3 (r=0.50), ΔTT3/rT3 (r=–0.49), and ΔTSH (r=–0.41). Furthermore, changes in thyroid hormones were correlated with duration of PICU stay for: ΔTT4 (r=–0.49), ΔfT4 (r=–0.37), ΔTT3 (r=–0.54), ΔrT3 (r=0.52), ΔTT3/rT3 (r=–0.60), and ΔTSH (r=–0.49).

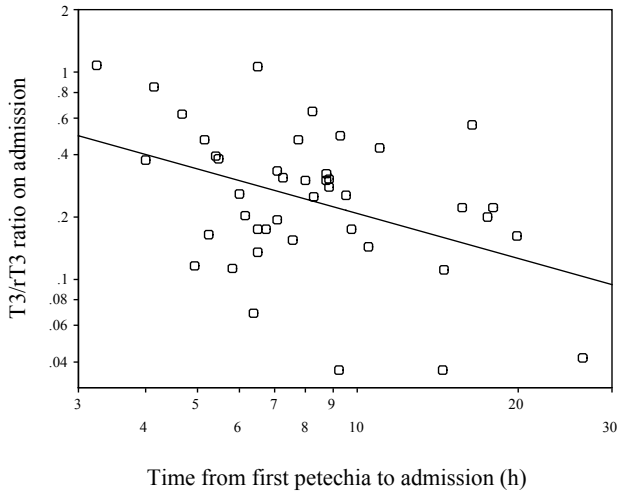

Figure 2. Relation between TT3/rT3 ratio and time from first petechia to admission: ^{10}Log (TT3/rT3) = 0.040 – 0.724 x ^{10}Log (Time first petechia - admission).

Time course of thyroid hormones

After 48 h short-stay children showed significantly higher TT4, fT4 , TT3 , TSH levels and TT3/rT3 ratio and lower rT3 levels than long-stay children. Short-stay children showed within 48 h an increase in TT3 and TSH levels and TT3/rT3 ratio and a decrease in rT3 levels; long-stay children showed a decrease in fT4 and rT3 after 24 h and no change in TT4, TT3, TSH levels and an increase after 24 h in TT3/rT3 ratio. Children who were discharged from the PICU on day 2 or 3 still had elevated rT3 levels, and all except one had still decreased TT3 levels on the day of discharge.

Effect of dopamine

Only TSH levels were significantly lower in children who received dopamine on admission than those who did not (0.4 vs. 0.7 mU/l, p=0.017). Multivariate analysis showed none of the following parameters to be significantly related to TSH levels on admission taking account of dopamine use prior to admission: age, gender, time between first petechia and admission, PRISM score, IL-6, lactate, and CRP levels.

Thyroid hormone levels were available in 13 of 23 children receiving dopamine before and after dopamine withdrawal (Table 2). At a median of 14 h after dopamine withdrawal the levels of TSH (X4.5), TT3/rT3 ratio (X2.1), and TT3 (X1.6) were still significantly increased whereas the rT3 level (X0.7) were still significantly decreased. In these 13 patients the before vs. after changes in TT4 levels and TT3/rT3 ratio were positively correlated with duration of dopamine use (r=0.56 and r=0.69, respectively) and time from dopamine withdrawal to next sample (r=0.63 and r=0.60, respectively). Corresponding changes in other thyroid parameters were not correlated with either one of these parameters. Median duration of dopamine use was significantly shorter in short-stay patients than in long–stay patients (39 vs. 111, p<0.001). Analysis of variance showed, that patients with dopamine at 24 h after

admission had significantly lower levels of TSH after 24 h than those who stopped dopamine or who had never received dopamine before that time. Taking account of this effect, we found no difference in TSH levels between short-stay and long-stay children. Analysis for the effect of dopamine use on TSH levels at 48 h resulted in similar findings (Fig. 3).

Table 2. Thyroid hormone levels prior to and after dopamine withdrawal.

Thyroid hormones	Reference values	Prior (n=13)	After (n=13)	*P*-value	Ratio * (n=13)
TT4 (nmol/l)	64 – 132	51 (29 – 89)	63 (25 – 131)	0.069	1.2
FT4 (pmol/l)	11 – 25	13 (8 – 19)	17 (9 – 36)	0.087	1.3
TT3 (nmol/l)	1.1 – 2.6	0.43 (0.18 - 1.03)	0.69 (0.10 – 1.80)	0.017	1.6
rT3 (nmol/l)	0.15 – 0.43	1.39 (0.50 - 3.02)	1.03 (0.66 – 1.95)	0.006	0.7
TT3/rT3	n.a.	0.32 (0.11 – 0.97)	0.67 (0.13 – 2.43)	0.002	2.1
TSH (mU/l)	< 4.5	0.5 (0.1 – 3.9)	2.2 (0.2 – 10.7)	0.011	4.5

Geometric mean (range), Wilcoxon signed rank test and ratio of geometric mean (*). Not available (n.a.).

Multivariate analysis

Multiple regression analysis revealed that both IL-6 and TT4 levels on admission were major factors significantly related to length of PICU stay. These two variables together explained 50% of the variance in length of stay. The length of stay increased 15% for every doubling of IL-6 levels and 16% for every 10 nmol/l lower level of TT4 on admission. None of the other variables investigated (age, gender, time from first petechia, dopamine use) contributed significantly to the model.

Multiple regression analysis with data available at admission and 24 h thereafter revealed the main factors predicting length of PICU stay to be PRISM score, admission level of TT4, change in TT4, and rT3 level within the first 24 h. These variables together explained 64% of the variance in length of PICU stay. The length of stay increased 35% for every 10 points higher PRISM score, 10% for every 10 nmol/l lower level of TT4 on admission, 20% for every 10 nmol/l decrease in TT4 levels within the first 24 h of admission, and 69% for every doubling of the change in rT3 levels within the first 24 h of admission. None of the other variables investigated (age, gender, dopamine use and changes in fT4, TT3 and TSH within the first 24 h) contributed significantly to the model. In contrast, multiple regression analysis of clinical parameters alone (age, gender, time from first petechia, dopamine use, IL-6 levels, PRISM score) revealed only IL-6 levels to be significantly related to length of PICU stay, explaining 39% of the variance in length of PICU stay.

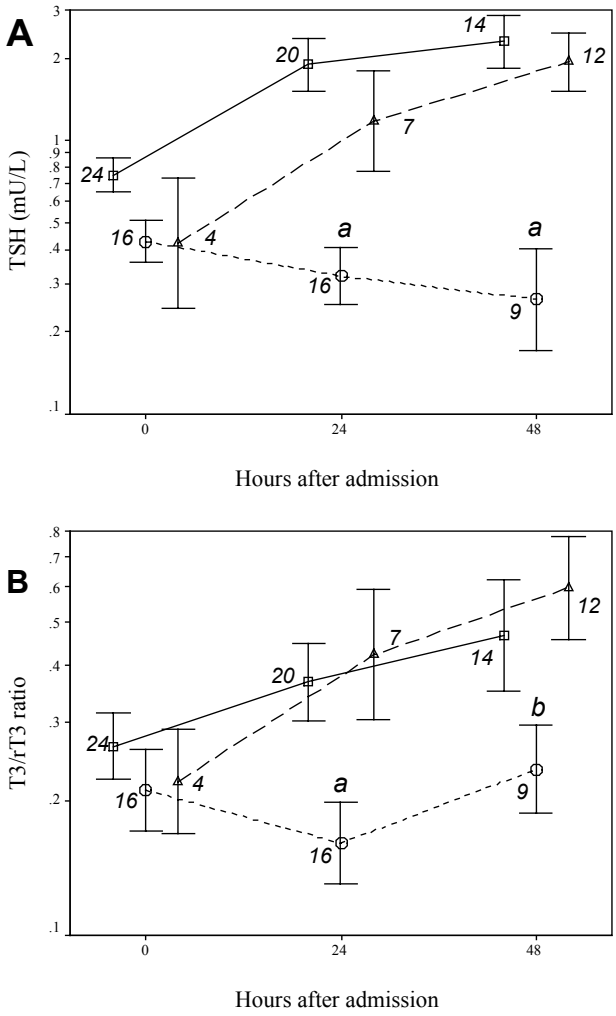

Figure 3. TSH levels and TT3/rT3 ratios according to patients' actual dopamine use at the various time points. Children without dopamine (□), with dopamine (○) and after dopamine withdrawal (Δ). Data shown are geometric means with standard errors. Between group differences were calculated with Student's T-Test. Numbers alongside data-points indicate numbers of patients. (*a*) between group differences of (○) with (□) and (Δ), P <0.05. (*b*) between group differences of (○) with (Δ), P <0.05.

Discussion

In this study all patients surviving from meningococcal septic shock showed features of the euthyroid sick syndrome, defined as decreased levels of TT3 and TT4, increased levels of rT3 and without compensatory increased levels of TSH. On admission short-stay children only had higher TT3 and lower IL-6 levels than long-stay children, while 48 h thereafter short-stay children showed higher TT4, fT4 , TT3 , and TSH levels, lower rT3 levels, and higher TT3/rT3 ratios than long-stay children. Parameters of disease severity, thyroid hormone levels on admission, and the changes over the first 24 h strongly predicted length of PICU stay. Dopamine was found to have an important effect on the course of thyroid levels.

On admission TT3 and IL-6 levels were the only laboratory parameters that differed significantly between short and long-stay children. Median IL-6 levels were more than four time lower in short-stay than long-stay children. IL-6 levels were not correlated with TT3 levels or other thyroid hormones on admission, in all patients and those with and without dopamine, which may indicate that thyroid function is not affected primarily by the initial response of the cytokine cascade due to infection. This finding is in accordance with a study in adults undergoing abdominal surgery (20), but in contrast to some studies of less sick children with respiratory infection (mean IL-6 levels 16.7 pg/ml) or after open-heart surgery (mean IL-6 levels 124.4 pg/ml) and healthy humans (21–23).

Interestingly, rT3 levels and TT3/rT3 ratio were correlated significantly with CRP levels on admission and with time from first petechia to admission. As previously reported, CRP level on admission reflects the duration of illness (24, 25). This may indicate that the initial changes in thyroid function on admission were affected by the duration of illness prior to admission.

Previous studies in adult patients have shown that the magnitude of the TT3 decline within the first 24 h of admission reflects severity of illness (4, 5). We found that duration of PICU stay was related to changes in all thyroid hormone levels and to the TT3/rT3 ratio within the first 24 h. Moreover 64% of variance in the length of PICU stay was explained by TT4 levels on admission, changes in TT4, and rT3 levels within 24 h and PRISM score, while clinical parameters alone explained 39% of the variation. Measurement of thyroid hormones and changes within 24 h may therefore be a useful tool to predict length of PICU stay and thus to identify those children at risk for a long stay at the PICU.

Short-stay children showed significantly better thyroid function 48 h after admission than long-stay children, as revealed by increased TT3 and TSH levels and TT3/rT3 ratio and decreased rT3 levels. However, all but one of the children who were discharged from the PICU within 3 days after admission still had elevated rT3 and decreased TT3 levels and thus euthyroid sick syndrome despite early clinical improvement.

The suppressive effects of dopamine infusion on the pituitary gland have been reported in infants and children (8). During dopamine infusion receptors in the pituitary gland are highly stimulated, thereby inhibiting TSH release. In contrast, cessation of dopamine therapy results in an abrupt rise in TSH levels. Although our study was not designed to investigate the suppressive effects of dopamine, we also found evidence of this suppressive effect of dopamine in these critically ill children who were treated with dopamine. First, we found that in children treated with dopamine TSH levels and TT3/rT3 ratios did not significantly change during administration, whereas those who did not receive dopamine and those in whom dopamine was stopped within the first 48 h showed changes to normalization. Secondly, we found an increase of more than four times in TSH at a median of 14 h after dopamine withdrawal, which is in accordance with the findings of a study in

children after cardiac surgery which reported a ten times higher level of TSH 24 h after dopamine withdrawal (8). The duration of dopamine use has been reported to be related to thyroid function in adult patients (7), but we found no evidence of this relationship; this may have been due to the relatively short period of dopamine administration in children in comparison with critically ill adults.

In conclusion, all children surviving meningococcal septic shock showed signs of euthyroid sick syndrome on admission. Changes in thyroid function on admission were affected by the duration of illness prior to admission. Changes in thyroid hormone levels over the first 24 h of admission, especially the TT3 decrease and rT3 increase were prognostic of length of PICU stay.

Acknowledgements

We thank Mrs. M. Maliepaard, research nurse, for her technical assistance and Mrs. C.L. Vermont, E.D. de Kleijn, and their colleagues, and Mrs. E.M.N. Bannink, Mr. R.D. van Beek, Mrs. V.H. Boonsta, Mrs. M. van Dijk, Mrs. D. van der Kaay, and Mrs. A.S. Slingerland for helping to collect samples on weekends. We also thank the nursing staff of the pediatric intensive care for their support.

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Chapter 6

GROWTH HORMONE-INSULIN LIKE GROWTH FACTOR-I AXIS IN CHILDREN WITH MENINGOCOCCAL SEPSIS: THE SPECTRUM FROM SEPSIS TO FATAL SEPTIC SHOCK

Marieke den Brinker, Koen F.M. Joosten, Jaap van Doorn, Ellen M.N. Bannink, Joop A.M.J.L. Janssen, Wim C.J. Hop, Jan A. Hazelzet, Anita C.S. Hokken-Koelega

Submitted for publication

Abstract

Context and Objectives: To evaluated GH-IGF-I axis in meningococcal disease. **Design:** Observational cohort study. **Setting:** University-affiliated pediatric intensive care unit (PICU). **Patients:** Thirty-nine children with meningococcal sepsis or septic shock. **Main outcome measures:** Differences in GH-IGF-I axis between nonsurvivors (n=4), shock-survivors (n=29) and sepsis-survivors (n=6) on PICU-admission. **Results:** Most children showed elevated or normal GH levels with low levels of total IGF-I, total IGFBP-3 and ALS, which was most striking in nonsurvivors. Total GH correlated very strongly with bioactive GH levels (r=0.94). GHBP levels were within the normal range in the majority of children and related positively to total IGF-I (r=0.55), IGFBP-3 (r=0.70) and ALS (r=0.71) levels. Most children showed decreased intact/total IGFBP-3 ratio. Surprisingly, this ratio was higher with increasing disease severity. IGFBP-1 levels increased with increasing disease severity and correlated inversely with free IGF-I levels (r=–0.59). Using multiple regression analysis, mean-GH levels increased with higher IL-6 levels and younger age and decreased with dopamine use. **Conclusions:** Most children with meningococcal sepsis or septic shock had elevated or normal GH and low IGF-I, IGFBP-3 levels, indicating a GH resistance state. Serum bioactive GH tightly paralleled total GH levels. GHBP levels were normal and related positively with IGF-I, IGFBP-3 and ALS levels. In combination with elevated cytokine levels, which are known to induce the intracellular negative feedback loop of the suppressors of cytokine signaling (SOCS), this might suggest reduced post-GH-receptor (GHR) signaling rather than a decreased GHR function to play a role in this GH resistance state.

Introduction

Meningococcal septic shock is a life threatening critical illness in children and characterized by a sudden onset and rapid progression of disease. Despite advances in management and therapy, the mortality rate of children with shock due to meningococcal disease continues to be considerable (1). Critical illness leads to a biphasic spectrum of neuroendocrine and metabolic changes with substantial differences in the acute and chronic phase (2). Studies on the acute phase of critical illness in adults show sustained to enhanced GH secretion, but low levels of IGFBP-3 and ALS (3). These changes are generally interpreted as GH resistance and are presumed to be adaptive, as they promote the accessibility of metabolic substrates for vital organs and postpone anabolism. In a previous pilot study in a small group of children with meningococcal septic shock, we encountered very high levels of GH in nonsurvivors with concomitantly decreased IGF-I and IGFBP-3 levels, suggesting GH resistance (4). However, the bioactivity of GH, and the serum levels of GH binding protein (GHBP) and IGF related components, such as acid-labile subunit (ALS), were not investigated. A better understanding of the hormonal response in critically ill children is essential for proper clinical management of these children. Hence, in this present study we thoroughly evaluated the GH/IGF-I axis in a large group of children suffering from meningococcal sepsis or septic shock.

Materials and Methods

Patients

Seventy-one previously healthy children were admitted to the PICU of Erasmus MC – Sophia Children's Hospital, with a clinical diagnosis of meningococcal sepsis, defined as sepsis with petechiae/purpura. Sepsis was defined as temperature of <36.0 ºC or >38.5 ºC with tachycardia and tachypneu. In addition, children were assigned to have septic shock, if they also had persistent hypotension, or evidence of poor end-organ perfusion, as previously described (4). The lack of research staff to ensure adequate 24 hours stand-by service for inclusion of children and processing of the samples, necessitated two study periods: between October 1997 and October 1999 and between October 2001 to April 2004. GH profiles could be obtained in 39 of the 71 children on admission. Children in whom a GH profile was performed did not significantly differ in age, time from first petechia to admission or disease severity parameters – such as PRISM, SOFA, IL-6 and lactate levels – from those without GH profile (data not shown). Twelve of the 39 children participated in a randomized, double-blinded, placebo-controlled study. In this study children received either placebo or one of three dosages of protein C concentrate every 6 hours for the

first three days of admission followed every 12 hours with a maximum of 7 days (5). Pilot data on GH-profiles of these 12 children have been published previously (4). The other 27 children participated in an observational cohort study. The medical ethics committee of the Erasmus MC approved the study and written informed consent was obtained from parents or legal representatives.

Clinical parameters

Severity of illness was assessed by the Pediatric Risk of Mortality score II (PRISM) score (6), Sepsis-related Organ Failure Assessment (SOFA) (7) and by measuring levels of established biomarkers, such as plasma interleukin-6 (IL-6), arterial lactate, and serum C-reactive protein (CRP). The interval between appearance of first petechia and PICU-admission, respiratory support, medication and outcome were recorded.

Caloric intake

The patients were fed according to a standard feeding protocol; on admission at the PICU glucose was started intravenously at a rate of 4–6 mg/kg/min (glucose 5 or 10% solution). Enteral and/or parenteral nutrition was started on the second day, if clinically possible, and adjusted to the normal needs for healthy children on days 3 and 4.

Sample collection

Arterial blood samples were obtained as soon as possible after admission and subsequently 6-h GH-profiles, with samples taken every 30 min, were obtained. Serum and EDTA-plasma were stored at –80°C until determination of GH, IGF-I and related components and cytokines. All other laboratory parameters were determined immediately.

Assays

Serum GH levels were determined with an immunoradiometric assay (CIS-Bio International, Gif-sur-Yvette, France) or Immulite 2000 (Diagnostic Products Corporation, LA, CA, USA). Plasma total IGF-I levels were determined with an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). Plasma IGFBP-1 and IGFBP-3 levels were determined with specific RIAs (Utrecht Medical Center, Utrecht, The Netherlands) (8, 9). Serum ALS, growth hormone binding protein (GHBP), and bioactive (immunofunctional) GH levels were determined with specific ELISAs (Diagnostic System Laboratories (DSL), Webster, TX, USA) and dissociable free IGF-I with IRMA (DSL). Gender- and age-specific reference values (SD-scores or SDS) were available for total IGF-I, IGFBP-3, ALS and free IGF-I (9, 10). SD-scores between –2 and +2 were considered normal. Gender and age-specific reference values for GHBP were provided by DSL.

Levels of intact IGFBP-3, capable of binding [125I] radiolabeled IGF-I, were obtained with a ligand immunofunctional assay (LIFA), as described previously (11). Intact IGFBP-3 levels were expressed as a proportion of the corresponding total (RIA) IGFBP-3 levels, both determined in the retentate of the plasma ultrafiltrates. IGFBP-3 contents of series of plasma samples from 5 randomly selected children were also investigated with Western immunoblotting (12), using a specific polyclonal antibody at a dilution of 1:5000. In all cases, the results confirmed the values of intact/total IGFBP-3 as determined previously with LIFA (data not shown).

Serum insulin concentrations were determined with an immunoradiometric assay and serum cortisol concentrations with a competitive luminescence immunoassay (Immulite 2000, DPC) (13). Arterial lactate and glucose were determined on a blood gas analyzer (ABL 625, Radiometer Copenhagen, Denmark) and serum NEFA with a calorimetric assay (NEFA-C kit, WAKO Diagnostics) on a Hitachi 912 analyzer (Roche Diagnostics) in a certified clinical chemistry laboratory (ISO 17025 and 9001). The reference values were <2.0 mmol/L for lactate, 0.2–1.2 mmol/L for NEFA, 2.6-11.0 mmol/L for glucose (fasted) and <180 pmol/L for insulin (fasted). Plasma IL-6 levels were analyzed with an enzyme-linked immunosorbent assay (Sanquin, Amsterdam, the Netherlands).

Analysis of GH-profiles

GH profiles were analyzed using the PULSAR program, of which areas under the curve above zero (AUC_0) and mean-GH levels were derived. The AUC_0 were divided by 2 to rescale time and was similar when calculated by the trapezoidal method. Mean-GH levels were expressed as SD-scores, using gender- and Tanner stage-specific reference values (14). SD-scores between –2 and +2 were considered normal.

Statistics

Data were analyzed with SPSS 11.5. Results were expressed as medians unless specified otherwise. We used Mann-Whitney U, chi-square or Fisher's exact test, when necessary, and Spearman's correlation coefficient (r). Data were log-transformed for multiple linear regression analysis when necessary. Two-tailed P-values <0.05 were considered statistically significant.

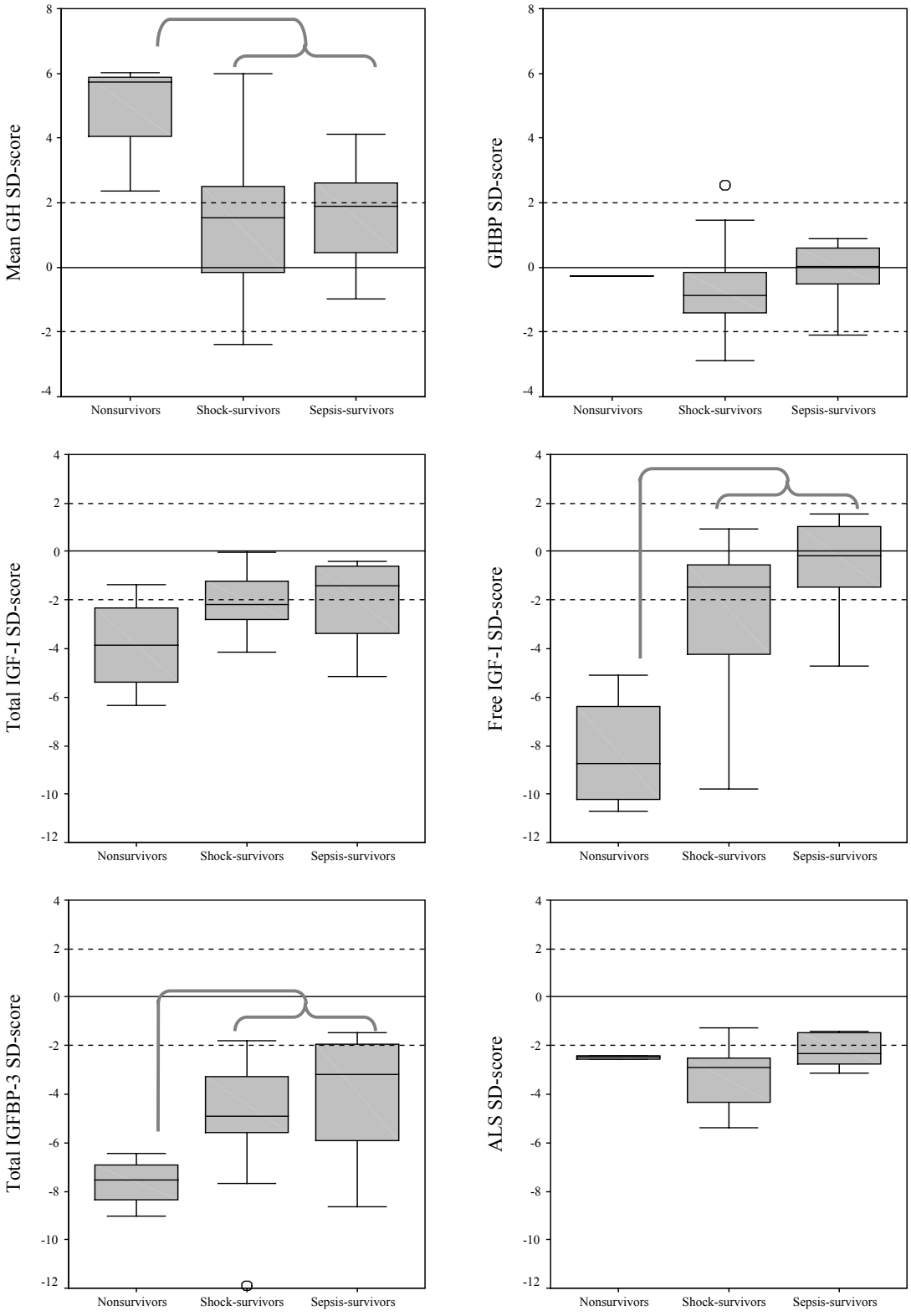

Figure 1. Mean-GH, GHBP, total and free IGF-I, IGFBP-3 and ALS SD-scores on admission. Nonsurvivors are depicted in the left box, shock-survivors in the middle box and sepsis-survivors in the right box. Box-plots indicate 25 to 75 percentile with the median and whiskers the range without outliers (○) and extremes (*). Normal values are between the dotted lines. Significant (P<0.05) differences are depicted with gray angle brackets.

Results

Clinical parameters on admission

The study group consisted of 39 children (22 boys). All children showed a clinical picture of meningococcal sepsis and blood cultures revealed *Neisseria meningitidis* in 35 of them. Children were divided according to presence of shock and survival into the following disease severity groups: (shock) nonsurvivors (n=4), shock-survivors (n=29) and sepsis-survivors (n=6). Concomitant therapy during the whole study period included antibiotics and administration of fluids in all children, administration of inotropics in children with septic shock and in one child with sepsis; and glucocorticoids in 5 children with septic shock (Table 1). Twenty-five children with septic shock required mechanical ventilatory support and sedation with benzodiazepines. Nonsurvivors were significantly younger and had significantly higher PRISM and SOFA scores, IL-6 and arterial lactate levels than survivors. Both nonsurvivors and shock-survivors had significantly lower cortisol levels than sepsis-survivors. Nonsurvivors died after median 13 h (range, 8 – 25 h). Shock-survivors had median PICU stay of 4.5 days (range, 1.1 – 14.7) and sepsis-survivors of 1.1 days (range, 0.5 – 2.3).

Table 1. Patients' characteristics on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

	Nonsurvivors (n=4)	Shock-survivors (n=29)	Sepsis-survivors (n=6)
Age (y)	1.1 (0.5 – 2.0) [a]	4.1 (0.3 – 15.7)	5.4 (2.3 – 12.2)
Male gender (%)	4 (100)	15 (52)	3 (50)
Time * (h)	6.9 (2.4 – 10.5)	7.4 (4.0 – 26.5)	6.8 (4.9 – 15.7)
PRISM score	32 (25 – 34) [a]	21 (9 - 46) [b]	9 (5 – 13)
SOFA score	14 (13 – 19) [a]	9 (3 - 17) [b]	2 (0 - 6)
Lactate (mmol/L)	5.5 (4.6 – 10.9) [a]	3.9 (1.1 – 9.6) [b]	2.1 (1.2 – 3.8)
IL-6 (pg/mL)	1571923 [a] (68517 – 2934110)	63233 [b] (205 – 362333)	366 (82 – 10494)
CRP (mg/L)	28 (24 - 136)	74 (18 - 326)	80 (63 – 252)
Cortisol (nmol/L) ‡	810 (620 - 1050) [b]	1020 (138 - 2096) [b]	1313 (999 - 2200)
Inotropics (%)	4 (100) [b]	29 (100) [b]	1 (17)
Dopamine (%)	0 (0)	10 (34)	0 (0)
Glucocorticoids (%)	1 (25)	4 (14)	0 (0)
Mechanical ventilation (%)	4 (100) [b]	21 (72) [b]	0 (0)

Data are expressed as median (range) or numbers. Between group difference compared with (*a*) all survivors or (*b*) sepsis-survivors, $P < 0.05$. Time * (h) = time from 1st petechia to admission in hours. ‡ Cortisol levels excluding glucocorticoid treated children.

GH-profiles, bioactive GH and GHBP levels

On admission, mean-GH levels were elevated in about one-half of the children (SDS>2; 51%), normal in 46% and decreased in one child. Nonsurvivors had significantly higher mean-GH levels, mean-GH SD-scores and AUC_0 than survivors, whereas these parameters did not significantly differ between shock-survivors and sepsis-survivors (Table 2; Figure 1). Mean-GH levels correlated positively with IL-6 levels and inversely with cortisol levels (Table 4).

Concentrations of bioactive GH and GHBP were determined in each first sample of the GH-profiles of 33 children (1 nonsurvivor, 26 shock-survivors and 6 sepsis-survivors). Bioactive GH and the ratio bioactive/total GH did not significantly differ between shock-survivors and sepsis-survivors (Table 2). The ratio bioactive/total GH was median 0.52 of the whole group. Bioactive GH levels showed a very strong correlation with total GH levels ($r=0.94$, $p<0.0001$) and were inversely related with cortisol levels (Table 4).

In the vast majority of children (85%) GHBP levels were within the normal range. GHBP levels were decreased in 4 children and elevated in one child. Median GHBP levels and SD-scores did not significantly differ between shock-survivors and sepsis-survivors (Table 2; Figure 1). GHBP levels did not correlate with IL-6 levels, cortisol levels (Table 4), time from first petechia to admission and mean-GH or bioactive GH levels (data not shown).

Table 2. GH-profiles, bioactive GH and GHBP of all children on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

	Nonsurvivors (n=4)	Shock-survivors (n=29)	Sepsis-survivors (n=6)
AUC_0 (mU/L)	776 (285 – 887) *	77 (32 – 115)	99 (68 – 195)
Mean-GH (mU/L)	131 (48 – 148) *	13 (6 – 19)	16 (11 – 32)
Mean-GH SDS	5.7 (3.2 to 6.0) *	1.5 (–0.2 to 2.5)	1.9 (0.1 to 3.0)
Bioactive GH (mU/L)	88 †	8 (4 – 17)	6 (5 – 11)
Bioactive / total GH ratio	0.74 †	0.53 (0.46 – 0.61)	0.39 0.37 – 0.58)
GHBP (pmol/L)	470 †	505 (413 – 593)	630 (380 – 1060)
GHBP SDS	–0.3 †	–0.9 (–1.4 to –0.1)	0.0 (–0.9 to 0.7)

All values are expressed as median (25 to 75 percentile). For reference values see methods. * Significant difference between nonsurvivors and all survivors, $P < 0.05$. † Data of one nonsurvivor available.

Total IGF-I, total IGFBP-3 and ALS levels

On admission, total IGF-I SD-scores were less than -2 in 44% of the children and normal in the others (Table 3; Figure 1). Total IGFBP-3 and ALS SD-scores were decreased in the vast majority of children (92% and 86%, respectively) and normal in the others. Nonsurvivors had significantly lower levels of total IGF-I, total IGFBP-3

and ALS than either shock-survivors or sepsis-survivors, whereas these parameters did not significantly differ between shock-survivors and sepsis-survivors. When corrected for gender and age, nonsurvivors exhibited lower levels for total IGF-I (p=0.074), IGFBP-3 (p=0.003) and ALS (p=0.030) than survivors.

Total IGF-I, total IGFBP-3 and ALS levels correlated positively with GHBP levels, but not with mean-GH or bioactive GH levels (Table 4). Total IGF-I levels correlated inversely with IL-6 levels, whereas total IGFBP-3 levels tended to do so (p=0.089) and ALS levels did not. Total IGF-I levels correlated strongly with total IGFBP-3 and ALS levels.

Table 3. Growth factors, glucose, insulin and NEFA on admission, divided in nonsurvivors, shock-survivors and sepsis-survivors.

	Nonsurvivors (n=4)	Shock-survivors (n=29)	Sepsis-survivors (n=6)
Total IGF-I (nmol/L)	1.1 (0.4 – 2.9) [a]	5.8 (4.6 – 8.3)	5.8 (2.6 – 23.7)
Total IGF-I SDS	-3.9 (-5.9 to –1.9)	-2.2 (-2.8 to –1.2)	-1.4 (-3.8 to –0.6)
Free IGF-I (pmol/L)	3 (3 – 6) [a]	20 (7 – 41)	30 (13 – 144)
Free IGF-I SDS	-8.7 (-10.6 to –5.8) [a]	-1.4 (-4.7 to –0.5)	-0.2 (-2.3 to 1.2)
Free/total IGF-I (%)	0.28 (0.21 – 0.94)	0.25 (0.15 – 0.57)	0.54 (0.43 – 0.60)
IGFBP-3 (nmol/L)	11 (9 – 12) [a]	20 (16 – 28)	26 (15 – 59)
IGFBP-3 SDS	-7.5 (-8.7 to –6.7) [a]	-4.9 (-5.6 to –3.2)	-3.2 (-6.6 to –1.8)
Intact/total IGFBP-3 ratio	0.7 (0.4 – 1.1) [b]	0.5 (0.2 – 0.7) [b]	0.1 (0.1 – 0.2)
ALS (nmol/L)	19 and 28 * [a]	55 (35 – 82)	82 (38 – 193)
ALS SDS	-2.6 and –2.4 *	-2.9 (-4.4 to –2.5)	-2.3 (-2.9 to –1.5)
IGFBP-1 (nmol/L)	33.4 (10.2 – 47.6) [a]	5.1 (3.2 – 8.1)	2.3 (0.7 – 6.1)
Glucose (mmol//L)	4.6 (1.6 – 10.8)	6.5 (5.5 – 10.1)	8.5 (7.6 – 11.4)
Insulin (pmol/L)	29 (29 – 336)	104 (40 – 242)	81.6 (52 – 203)
Insulin/Glucose (10E-6)	15 (6 –34)	15 (7 – 32)	10 (5 –19)
NEFA (mmol/L)	0.23 (0.20 – 0.28) [a]	0.82 (0.47 – 1.30)	0.65 (0.53 – 0.70)

All values are expressed as median (25 to 75 percentile). For reference values see methods. Between group difference compared with (*a*) all survivors or (*b*) sepsis-survivors, $P < 0.05$. * Data of two nonsurvivors available.

Free IGF-I levels

On admission, free IGF-I levels and SD-scores were significantly lower in nonsurvivors than in survivors (Table 3; Figure 1). In 3 nonsurvivors and 2 shock-survivors free IGF-I levels were even under the detection limit. Free IGF-I levels correlated inversely with the IL-6, IGFBP-1 levels, intact/total IGFBP-3 ratios ($r=–0.61$, $p<0.001$), and positively with levels of IGF-I, IGFBP-3 and ALS (Table 4). Free IGF-I levels correlated also inversely with mean-GH and bioactive GH levels.

Intact IGFBP-3 levels

Although, on admission, total IGFBP-3 levels tended to be lower with increasing disease severity, the intact/total IGFBP-3 ratio did not. This ratio was even higher in nonsurvivors and shock-survivors than in sepsis-survivors (Table 3). The intact/total IGFBP-3 ratio correlated positively with IL-6 levels (r=0.49), negatively with total IGF-I levels (r=–0.42) and ALS levels (r=–0.47).

IGFBP-1 levels

On admission IGFBP-1 levels were higher with increasing disease severity: IGFBP-1 levels were significantly higher in nonsurvivors than shock-survivors and tended to be higher in shock-survivors than in sepsis-survivors (Table 3; p=0.053). The IGFBP-1 levels correlated significantly with IL-6 levels (r=0.48), but not with insulin, glucose or cortisol levels (Table 4).

Glucose, insulin and NEFA levels

On admission the majority of children had glucose (79%), insulin (68%) and NEFA (73%) levels within the normal range for (fasted) children. Glucose levels were decreased in 2 children (both nonsurvivors) and increased in 6 (1 nonsurvivor, 4 shock-survivors, 1 sepsis-survivor). Glucose and insulin levels and their ratio did not significantly differ among the groups (Table 3). NEFA levels were decreased in 2 children (1 nonsurvivor, 1 shock-survivor) and elevated in 8 (all shock-survivors). NEFA levels were significantly lower in nonsurvivors than in survivors.

Multiple regression analysis

With multiple regression analysis we investigated the influence of disease severity and various factors on mean-GH levels on admission. We found mean-GH levels to be significantly related to IL-6 levels, dopamine use and age, whereas free IGF-I levels and gender did not contribute to this model. Mean-GH levels increased with 36% for every 10-fold increase of IL-6 levels and with 27% for every halving of age, whereas mean-GH-levels decreased with 66% when dopamine was administered (R^2=0.401). As both IL-6 and cortisol levels correlated with mean-GH levels, we performed an additional model in which cortisol levels were added. In that model mean-GH levels increased with 55% for every halving of cortisol levels, whereas IL-6 levels just lost significance (p=0.146).

Using multiple regression analysis, we found total IGF-I levels to decrease with 21% for every halving of age, with 44% for every halving of time between first petechia and admission, and with 21% for every 100-pmol/L lower GHBP levels, together explaining 67% of the variation of total IGF-I levels. None of the additional variables investigated in this analysis (IL-6 levels, gender or steroid therapy) contributed significantly to this model.

Table 4. Significant spearman correlation coefficients between GH-profile parameters, growth factors and parameters of disease severity on admission.

	GH Mean	GH Bioactive	GHBP	IGF-I Total	IGF-I Free	IGFBP-3 Total	ALS	IGFBP-1	Glucose	Insulin
IL-6	0.37 *	-	-	-0.38 #	-0.57 #	-	-	0.48 #	-	-
Cortisol ‡	-0.47 #	-0.54 #	-	-	0.36 *	-	-	-	-	-
Total IGF-I	-	-	0.55 #		0.73 #	0.83 #	0.79 #	-0.50 #	0.40 #	0.48 #
Free IGF-I	-0.38 *	-0.38 *	0.42 *	0.73 #		0.65 #	0.55 #	-0.59 #	-	-
Total IGFBP-3	-	-	0.70 #	0.83 #	0.65 #		0.91 #	-0.55 #	0.43 #	0.41 *
ALS	-	-	0.71 #	0.79 #	0.55 #	0.91 #		-0.39 *	0.56 #	0.54 #
IGFBP-1	-	-	-0.35 *	-0.50 #	-0.59 #	0.55 #	-0.39 *		-	-

Correlation is significant at $P < 0.05$ (*) or at $P < 0.01$ (#). Non-significant correlation coefficients are represented by a dash. Excluding glucocorticoid treated children (‡).

Discussion

In this study, we found in the majority of the acutely ill children with sepsis high or normal GH levels with low levels of total IGF-I, total IGFBP-3 and ALS on PICU admission, suggesting a GH resistance state. Nonsurvivors had extremely high GH profiles and the lowest growth factors, whereas the GH profiles and growth factors did not differ between shock-survivors and sepsis-survivors. Total GH correlated very strongly with bioactive GH levels. GHBP levels were within the normal range in the vast majority of children and correlated positively with IGF-I, IGFBP-3 and ALS levels, suggesting that the GHR was still functioning. Contrary to our expectations, the intact/total IGFBP-3 ratio increased with disease severity. IGFBP-1 levels increased with increasing disease severity and correlated inversely with free IGF-I levels, emphasizing its counter-regulatory role in critical illness.

We found normal to extremely elevated GH values in the majority of children. This is in accordance with data of critically ill adults in whom GH values show a large variation in GH values (3, 15) and the scarce data in critically ill children (4, 16). Not only nonsurvivors but also some survivors showed extremely elevated GH levels, which is a new finding compared with previously reported data in children (4). One explanation for the elevated GH levels might be the extreme cytokine load in reaction to endotoxins, comparable with experimental *in vivo* studies reporting elevated GH levels after endotoxin challenge (17, 18). We found elevated IL-6 levels to be one of the major factors related to elevated GH levels, besides age and dopamine use, which were both inversely related to GH levels. In addition, an interesting finding was the relation between decreased cortisol and elevated GH levels. Both cortisol and GH exert direct substrate mobilizing actions, while in the acute phase of critical illness anabolism is postponed (3, 19). As cortisol levels decreased with increasing disease severity, which has been previously reported (13, 20, 21), the extremely elevated GH levels might have been an ultimate attempt of the body to provide essential substrates for survival. Furthermore, nutritional factors might have had their influence as well (3, 19), as all the children in our study were fasted on admission.

Circulating GH consists of a mixture of molecular isoforms arising from both posttranscriptional and posttranslational modifications (22). GH bioactivity depends on many factors, including these isoforms and serum levels of GHBP. In our study, bioactive GH levels tightly paralleled total GH levels. As there was no relation between bioactive GH levels and GHBP levels, it suggests that for critically ill children GHBP does not play a major role in determining the bioactivity of serum GH. In addition, the median ratio of bioactive over total GH in our study did not differ from data in healthy volunteers (23).

Despite elevated or normal GH levels in the majority of children we found low levels of total IGF-I, total IGFBP-3 and ALS, suggesting a GH resistant state. GH

resistance may be associated with reduced GHR density, the latter assumed to be reflected by reduced levels of GHBP, the circulating part of the GHR (24). On the basis of this assumption, one would assume GHR density to be normal in the vast majority of the critically ill children as their GHBP levels were within the normal range. This is in contrast to studies in adults after abdominal surgery, reporting decreased GHBP levels (25). However, GHBP levels may not invariably reflect GH receptor status during the acute phase of critical illness due to sepsis (26). Studies in animals report conflicting data on GHBP levels and hepatic GHR density: one study in rats reports hepatic GHR density to fall after endotoxins injection in the presence of normal serum GHBP levels (27), whereas another study reports normal hepatic GHR density in the presence of elevated GHBP levels (28). In fact, the regulation of GHBP serum concentrations is complex and various factors, such as increased proteolytic cleavage of the GH-receptor or alternative splicing may be involved (29). On the other hand, the GHBP levels were related strongly and positively with IGF-I, IGFBP-3 and ALS levels, suggesting that the hepatic GHR is still functioning. It might well be that the post-GHR signaling was disturbed, for instance by the intracellular negative feedback loop of the suppressors of cytokine signaling (SOCS). Members of the SOCS protein family are rapidly induced upon exposure to cytokines, GH, growth factors and fasting and play an important role in the cellular response to GH (30, 31). In our study all children were fasted and had elevated cytokine levels and extremely so in the most severely ill. The combination of normal to elevated GH and low IGF-I levels with normal GHBP levels and elevated cytokines and a fasting condition might therefore be suggestive for post-GHR signaling induced GH resistance, particularly in the liver (31, 32).

Proteolyzed IGFBP-3 (30 kD) has been considered to have a reduced affinity for IGF-I. During critical illness increased IGFBP-3 protease activity has frequently been reported (4, 33, 34) and held responsible, at least partly, for the lowering of IGFBP-3 levels. As expected we found decreased intact/total (= intact / intact + proteolyzed) IGFBP-3 ratios in the majority of children, suggesting increased protease activity. Surprisingly, however, we found the highest intact/total IGFBP-3 ratio in the most severely ill children with the lowest levels of total IGFBP-3, thus very low levels of proteolyzed IGFBG-3. Moreover, the intact/total IGFBP-3 ratio correlated strongly and positively with IL-6 levels and PRISM. As serum protease activity and neutrophil protease levels in the acute phase of critical illness are generally elevated (35), these results seem in contrast to *in vitro* data showing the protease activity of neutrophil proteases to increase within hours and at higher concentrations of proteases (36). One explanation for the relatively high intact/total IGFBP-3 ratio, i.e. relatively low level of proteolyzed IGFBP-3, might be increased turnover of proteolyzed IGFBP-3 in the most severely ill children with septic shock.

We found IGFBP-1 levels to strongly correlate with parameters of disease severity, i.e. IL-6 levels, and found extremely high levels in nonsurvivors. This is in accordance with data in critically ill adults, in whom IGFBP-1 levels were predictive

for mortality (37, 38). In contrast to normal conditions (39), we could not find a relation between IGFBP-1 and insulin. The manifold metabolic alterations that occur during critical illness might have caused this. Hepatic production of IGFBP-1 is acutely regulated by metabolic stimuli, of which in particular hepatic substrate deprivation is stimulatory (38). IGFBP-1 has been suggested to fulfill a counter-regulatory role by binding free IGF-I in order to block the insulin like activity of free IGF-I, thereby preventing hypoglycemia, for which the brain is especially vulnerable (3). In our study, IGFBP-1 levels were inversely related to free IGF-I levels and nonsurvivors had extremely high IGFBP-1 levels and the lowest free IGF-I levels. As a consequence, glucose uptake into cells might have been further decreased in the already existing situation of minimal nutritional supply due to septic shock and fasting. Together with the low levels of alternative metabolic substrates, such as NEFAs, many cells might have been deprived from energy in a situation of very high metabolic demands.

In summary, our study shows that the majority of children with meningococcal sepsis had normal or elevated GH levels with decreased total IGF-I levels suggesting a GH resistance state on PICU admission, which was most striking in nonsurvivors. GH levels increased with increasing IL-6 levels and decreased with age and dopamine use. The bioactivity of serum GH turned out to parallel total GH levels. Surprisingly, GHBP levels were within the normal range and related positively to total IGF-I, IGFBP-3 and ALS levels. In the presence of extremely elevated cytokine levels, this might suggest reduced post-GHR signaling rather than decreased GHR function. IGFBP-1 levels increased with increasing disease severity and correlated inversely with free IGF-I levels, emphasizing its counter-regulatory role in critical illness.

Acknowledgements

We thank M. Maliepaard, research nurse, and G. Graat for their technical assistance, C.L. Vermont, E.D. de Kleijn (both MD, PhD) and their colleagues, and R.D. van Beek, V.H. Boonsta, M. van Dijk, D.A.M. Festen, D. van der Kaay and A.S. Slingerland (all MDs) for helping to collect samples on weekends. We also thank B. Dumas, O. Liem, H. Nooteboom, and F. van Veghel for helping to collect the GH profile samples. And finally, we thank the nursing staff of the pediatric intensive care for their support.

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Chapter 7

THE INFLUENCE OF AGE, DISEASE SEVERITY AND MEDICAL INTERVENTIONS ON THE GROWTH HORMONE-INSULIN-LIKE GROWTH FACTOR-I AXIS BEFORE AND AFTER PAEDIATRIC CARDIAC SURGERY

Marieke den Brinker, Anita C.S. Hokken-Koelega, Robert J. van Thiel, Ad J.J.C. Bogers, Wim Helbing, Marianne J. Wijers, Wim C.J. Hop, Daniëlle C. van der Kaay, Koen F.M. Joosten

Submitted for publication

Abstract

Objectives: To evaluate GH-IGF-I axis before and after paediatric cardiac surgery and to determine influencing factors. **Design:** Observational study. **Patients:** Forty-nine children undergoing cardiac surgery. **Measurements:** GH profiles after surgery and IGF-I, IGFBP-3 and IGFBP-1 levels before, at end of surgery, 12 and 24 hours thereafter, in infants (n=19), young children (n=20) and teenagers (n=10). **Results:** At start of surgery, IGF-I and IGFBP-3 SD-scores were lower than reference values and related to low weight SD-scores and cyanosis. At end of surgery, mean-GH levels were elevated and related inversely to high-dose glucocorticoid and dopamine administration, but not to IL-6 levels. Glucose levels were higher in patients who received glucocorticoids than in those who did not. At end of surgery, IGF-I and IGFBP-3 SD-scores related to the percentage plasma administered during surgery, and not to IL-6 or GH levels. At start of surgery, IGFBP-1 related to insulin levels, but at end of surgery to IL-6 levels. Twenty-four hours after cardiac surgery, IGF-I, IGFBP-3 SD-scores and IGFBP-1 levels returned to the initial values at start of surgery. **Conclusions:** At start of surgery low IGF-I related to an underweight and low IGFBP-3 to cyanosis. Glucocorticoids and dopamine had a significant influence on GH and percentage plasma on IGF-I and IGFBP-3 levels, whereas IL-6 levels had no significant influence. Glucocorticoid administration during surgery was associated with higher glucose levels. Already the day after surgery, IGF-I, IGFBP-3 and IGFBP-1 levels had returned to the initial values at start of surgery.

Introduction

Critical illness leads to a biphasic spectrum of neuroendocrine and metabolic changes with substantial differences in the acute and chronic phase (1). Studies in adults show, that in the acute phase of critical illness GH resistance characterizes the alterations in the GH/IGF-I axis: normal to enhanced GH secretion, but low levels of IGF-I and IGFBP-3 (1, 2). The changes in the acute phase of critical illness are presumed to be adaptive, as it provides metabolic substrates to vital organs and postpones anabolism. Despite many studies on GH-IGF-I function in critically ill adults, only very limited research has been performed in children. In a previous pilot study in a group of children with meningococcal septic shock, we encountered very high levels of GH in nonsurvivors with concomitantly decreased IGF-I and IGFBP-3 levels, suggesting GH resistance (3). High levels of GH with low levels of IGF-I were also found in children after cardiac surgery, however, pulsatile GH assessments were lacking (4, 5). Understanding of the hormonal response in children with critical illness or undergoing cardiac surgery might be essential for the clinical management of these children. We therefore thoroughly evaluated the GH-IGF-I axis in a large group of paediatric patients at start and after open-heart surgery and assessed the influence of age, disease severity and medical interventions.

Materials and Methods

Patients

The group consisted of 49 paediatric patients – aged 2 months to 18 years – with congenital heart disease who underwent open-heart surgery (cardiac surgery with cardiopulmonary bypass) in the Erasmus MC between October 2001 and April 2004. Paediatric patients were operated for left-right shunt (n=18), Tetralogy of Fallot (n=9), partial cavo-pulmonary connection (PCPC, n=4), total cavo-pulmonary connection (TCPC, n=2), left ventricle outflow tract obstruction (n=8), right ventricle outflow tract obstruction (n=3), mitral valve annuloplasty (n=2), anomalous left coronary artery from pulmonary artery together with aortopulmonary window (n=1) or combined correction of atrial septum defect and ventricular septum defect with tricuspid valve correction (n=1). Patients were not eligible for the study if they had endocrine or chromosomal abnormalities or received radiation or chemotherapy within the previous 6 months. The Medical Ethics Committee of the Erasmus MC approved the study and written informed consent was obtained from the parents or legal representatives of each child and of all children aged >12 years.

Anaesthesia, peri-operative management and caloric intake

All paediatric patients underwent elective cardiac surgery upon cardiopulmonary bypass support (CPB) and 45 of them underwent cardioplegic arrest. General anaesthesia and peri-operative management was performed as described earlier (6). Most patients received mild hypothermia (median 31°C) and one (aged 16.6 years) received deep hypothermia (22.5°C) during cardiac surgery. Thirty-two patients received one bolus of glucocorticoids during cardiac surgery, most of them methylprednisolone (30 mg/kg), one patient dexamethasone (1 mg/kg) and one hydrocortisone (2 mg/kg). The administration of glucocorticoids was not a standard procedure and depended on the anaesthetist's choice. We calculated equivalent doses methylprednisolone, expressed per body surface (mg/m^2) and per body weight (mg/kg), using the glucocorticoid equivalent potencies 30 / 5 / 1 for dexamethasone, methylprednisolone and hydrocortisone, respectively (7). Twenty-nine patients received inotropic support, which was initiated during cardiac surgery and lasted for median 18h; 21 of them received dopamine, 7 received dobutamine and one received both. Patients were fasted before surgery and received glucose intravenously (4–6 mg/kg/min) after surgery. Enteral and/or parenteral nutrition was initiated at the first post-operative day if clinically possible.

Clinical parameters

Anthropometrical measurements were taken the day before cardiac surgery. Weight SD-scores were calculated using Dutch age- and gender-specific reference values (8, 9) (www.growthanalyser.com). Patients were assigned to have congestive heart failure according to the criteria of Van der Kuip *et al.* adjusted for age (10). To assess severity of illness, levels of established biomarkers were measured, such as plasma interleukin-6 (IL-6) and arterial lactate. The presence of cyanosis, duration of cardiopulmonary bypass (CPB), aorta cross clamp time (AOX), medication, fluid infusion and outcome were recorded. The percentage plasma of total volume (referred to as 'percentage plasma'), including the volume of the CPB circuit, added to the circulation during surgery was calculated to assess the influence of exogenous plasma on circulating levels of IGF-I and IGFBPs.

Sample collection

Arterial blood samples were obtained at start of surgery, at the end of surgery (after sternal closure), 12 and 24 hours thereafter. Six-hour GH-profiles were obtained starting at the end of surgery, with samples taken every 30 min. Before patients were connected to the CPB machine, a sample was drawn from the CPB circuit to assess the concentrations of IGF-I and IGFBPs in the CPB circuit. Serum and plasma were stored at -80° C until assay of GH, IGF-I, IGFBP-3, IGFBP-1 and cytokines. All other laboratory parameters were determined immediately.

Assays and reference values

Serum GH levels were determined on an Immulite 2000 (Diagnostic Products Corporation, L.A., CA, USA). GH profiles were analysed using the PULSAR program (11), of which areas under the curve above zero (AUC_0) and mean-GH levels were derived. The AUC_0 was divided by 2 to rescale time and was similar when calculated by the trapezoidal method. Mean-GH levels were expressed as SD-scores, using sex- and Tanner stage-specific reference values (12). Plasma IGF-I levels were determined with an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). Plasma IGFBP-1 and IGFBP-3 levels were determined with specific RIAs (Utrecht Medical Center, Utrecht, The Netherlands) (13, 14). Extended sex- and age-specific reference values (SD-scores) were available for IGF-I and IGFBP-3 (14, 15). SD-scores between –2 and +2 were considered normal. Serum insulin concentrations were determined with an immunoradiometric on an Immulite 2000 (DPC) (16). Plasma IL-6 levels were analysed with an enzyme-linked immunosorbent assay (Sanquin, Amsterdam, the Netherlands), with a detection limit of 10 pg/mL. Arterial lactate and glucose were determined on a blood gas analyser (ABL 725, Radiometer Copenhagen, Denmark) in a certified clinical chemistry laboratory (ISO 17025 and 9001). The reference values were <2.0 mmol/L for lactate and 2.6-11.0 mmol/L for glucose (fasted).

Statistics

Data were analysed with SPSS 11.5. Results were expressed as medians with range unless specified otherwise. IGF-I and IGFBPs were analysed with one-way ANOVA with post hoc Bonferroni analyses for group comparison at different time points and mixed model analyses for analysing the course and plotting the graphs. Paired t-tests were used to compare SD-scores with zero SD. For other parameters we used Kruskal-Wallis, Mann-Whitney U, chi-square or Fisher's exact test, or Wilcoxon signed rank test, when necessary. Backward multiple regression analyses were used to assess the relationship between GH, IGF-I and IGFBPs and pathophysiological important parameters. Data were log-transformed when necessary. Two-tailed P-values <0.05 were considered statistically significant.

Results

The study group consisted of 49 paediatric patients (24 boys). They were divided according to age: less than one year (referred to as 'infants'; n=19), between one and ten years (referred to as 'children'; n=20) and older than ten years (referred to as 'teenagers'; n=10). Data will be discussed in chronological order.

Table 1. Clinical and laboratory parameters at start of surgery, divided according to age.

	Infants (n=19)	Children (n=20)	Teenagers (n=10)	*P*-value
Age (years)	0.4 (0.2 – 0.8) [b c]	3.3 (1.0 – 9.1) [a c]	14.2 (10.3 - 17.6) [a b]	<0.001
Male gender (%)	10 (53)	7 (35)	7 (70)	0.180
Weight SD-score	–1.2 (–3.4 to 1.4)	–1.0 (–2.6 to 0.6)	–0.3 (–1.8 to 1.5)	0.264
BMI SD-score	–1.5 (–4.4 to 0.1)	–0.9 (–4.4 to 1.1)	–0.4 (–2.8 to 1.3)	0.062
Height SD-score	–0.5 (–2.7 to 1.5)	–0.2 (–1.8 to 1.7)	–0.7 (–2.4 to 2.7)	0.611
Congestive heart failure (%)	9 (47)	4 (20)	1 (10)	0.058
Cyanosis (%)	8 (42) [c]	3 (15)	0 (0) [a]	0.021
IL-6 (pg/mL)	10 (10 – 10)	10 (10 – 15)	10 (10 – 10)	0.484
Lactate (mmol/L)	1.8 (0.6 – 1.3)	1.0 (0.7 – 1.6)	0.9 (0.7 – 1.4)	0.077
Glucose (mmol/L)	4.5 (3.0 – 9.1)	4.6 (3.5 – 5.5)	4.9 (4.3 – 6.2)	0.092
Insulin (pmol/L)	15 (5 – 173)	14 (14 – 27) [c]	37 (14 – 77) [b]	0.005
Insulin / glucose (µM/M)	3.7 (1.2 – 42.3)	3.3 (2.7 – 5.3) [c]	6.2 (7.8 – 16.8) [b]	0.023

Data are expressed as median (range) or numbers (percentage). *P*-values represent Kruskal-Wallis, Pearson χ^2 or Fishers group comparisons, when significant differences were tested between groups: Significantly different compared to infants (a), children (b) and teenagers (c) at $P < 0.05$.

At start of cardiac surgery

Clinical and laboratory parameters at start of cardiac surgery are depicted in Table 1. Infants were significantly more often cyanotic than teenagers. Weight and BMI SD-scores were significantly lower than reference values (zero SD) in infants and children ($P \leq 0.001$), but not in teenagers. Height SD-scores did not significantly differ from zero SD. IL-6 levels were below the detection limit in all but one patient and arterial lactate levels were within the normal range in all. Glucose levels did not significantly differ among the age groups, but infants and children had significantly lower insulin levels and insulin/glucose molar ratio than teenagers.

IGF-I and IGFBP-3 SD-scores were significantly lower than reference values (zero SD) in all patients ($P<0.013$ and $P<0.003$, respectively) (Figure 1). IGF-I SD-scores did not significantly differ among the groups, whereas IGFBP-3 SD-scores were significantly lower in infants compared to children and teenagers. Multiple regression analysis showed that IGF-I SD-scores was only inversely related to weight SD-scores (Table 2), whereas not to IL-6 levels, congestive heart failure or preoperative cyanosis. IGFBP-3 SD-scores were inversely related to preoperative cyanosis, and not to weight SD-scores, congestive heart failure, IL-6 or lactate levels.

IGFBP-1 levels varied considerably (median 8.2 nmol/L, range 0.7 – 19.3 nmol/L). Both infants and children had significantly higher IGFBP-1 levels than teenagers (Figure 1). Before surgery, IGFBP-1 levels were inversely related to insulin levels, weight SD-scores and age (Table 2), and not to glucose levels, presence of cyanosis or congestive heart failure.

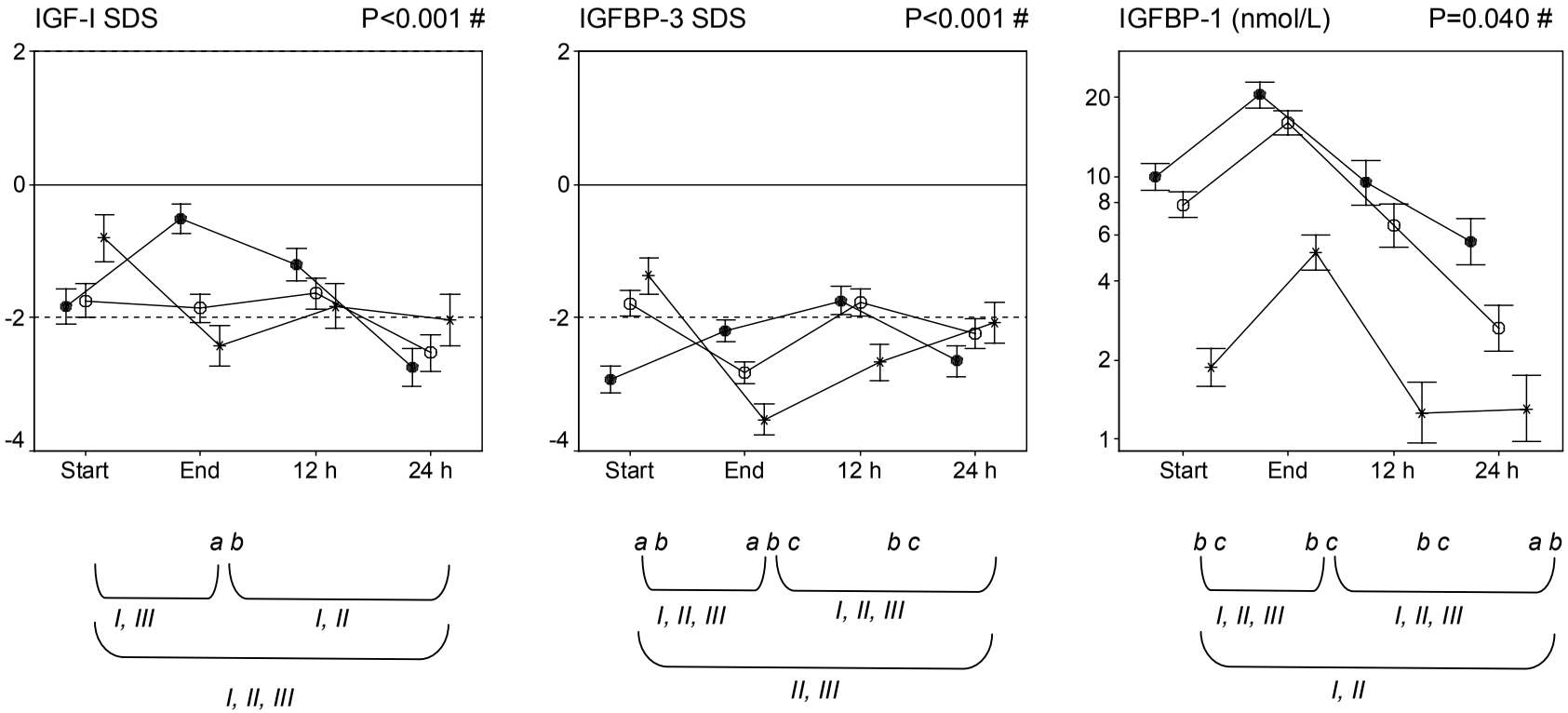

Figure 1. Time course of IGF-I SD-scores, IGFBP-3 SD-scores and IGFBP-1 levels, divided into infants (●), young children (○) and teenagers (*). Data shown are geometric means with standard errors. (#) *P*-values shown on top of the figures represent the difference between the three profiles over time by ANOVA. Between group differences at successive time points are depicted below the figures and represent differences between: infants and young children *(a)*, infants and teenagers (*b)* and young children and teenagers *(c)* (all at *P*<0.05). Within group differences are also depicted below the figures and represent differences between the start and end of surgery and between end of surgery and 24h thereafter (*I*, *II* and *III* for groups ●, ○, and *, respectively, *P*<0.05).

Table 2. Multiple regression analyses with depending variables IGF-I SD-score, IGFBP-3 SD-score and IGFBP-1 level at start of surgery.

Dependent variable	Independent variable	B	P-value	R^2
IGF-I SD-score	Weight SD-score	0.382	0.012	0.13
IGFBP-3 SD-score	Cyanosis (Yes=1, No=0)	1.036	0.003	0.17
IGFBP-1 level	Age (^{2}Log (years))	-0.090	<0.001	0.74
	Insulin level (^{2}Log (pg/mL))	-0.185	<0.001	
	Weight SD-score	-0.077	0.006	

During and at the end of cardiac surgery

During cardiac surgery the depth of hypothermia, as well as ECC and AOX time did not significantly differ between the groups (Table 3). During surgery, infants and children received significantly more often dopamine than teenagers and tended to receive higher equivalent doses of methylprednisolone. As shown in Table 3, infants and children received significantly more total volume with significantly higher percentage plasma during surgery than teenagers. The concentrations of IGF-I and IGFBP-3 in the CPB circuit were higher in infants and children than in teenagers. Interestingly, glucose levels were significantly higher and the insulin/glucose molar ratios significantly lower in both infants and children compared to teenagers, whereas insulin levels did not significantly differ between the age groups. In addition, glucose levels were significantly higher in patients who received glucocorticoids compared to those who did not (9.5 vs. 7.4 mmol/L, P=0.001), whereas insulin and IL-6 levels did not significantly differ (P=0.337 and P=0.438, respectively).

At the end of surgery, mean-GH levels did not significantly differ between the age groups (Figure 2). When compared to reference values, mean-GH SD-scores were significantly higher than zero SD in infants and children ($P \leq$ 0.001), but not in teenagers, whereas mean-GH SD-scores did not significantly differ among the groups. Using multiple regression analysis, we found mean-GH levels to decrease with age, dopamine and glucocorticoid use (Table 4), whereas IL-6 levels and gender had no influence.

Table 3. Parameters on cardiac surgery and laboratory parameters at end of surgery, divided according to age.

		Infants (n=19)	Children (n=20)	Teenagers (n=10)	*P*-value
ECC time (min)		98 (43 – 158)	68 (22 – 273)	78(25 – 210)	0.217
AOX time (min)		47 (0 – 113)	43 (0 – 109)	39 (0 – 112)	0.633
Hypothermia (°C)		29.0 (27.0 – 36.3)	31.4 (24.8 – 36.0)	31.7 (22.5 – 34.9)	0.278
Steroid therapy (%)		13 (68)	15 (75)	4 (40)	0.154
Equivalent MP dose (mg/m^2) †		468 (0 – 687)	638 (0 – 1098)	0 (0 – 1053)	0.077
Equivalent MP dose (mg/kg) †		26 (0 – 37)	29 (0 – 47)	0 (0 – 31)	0.070
Dopamine therapy (%)		14 (74) [c]	8 (40) [c]	0 (0) [a b]	0.001
WI score ‡		33.8 (0.0 – 146.0) [c]	30.0 (0.0 – 141.5)	1.4 (0.0 – 37.5) [a]	0.046
Percentage plasma [#]		25 (14 – 37) [b, c]	15 (0 – 35) [a, c]	0 (0 – 28) [a, b]	<0.001
Total volume (ml/kg) [##]		150 (87 – 272) [c]	72 (39 - 154) [c]	53 (32 - 156) [a, b]	<0.001
CPB circuit	IGF-I levels (nmol/L)	4.8 (2.7 – 8.2) [c]	3.7 (0.8 – 7.8) [c]	0.8 (0.8 – 0.8) [a, b]	<0.001
	IGFBP-3 levels (nmol/L)	22.6 (15.7 – 35.8) [c]	18.4 (2.1 – 34.1) [c]	7.0 (0.7 – 7.0) [a, b]	<0.001
IL-6 (pg/mL)		27 (10 – 106)	20 (10 – 58)	16 (10 – 43)	0.403
Lactate (mmol/L)		1.7 (0.9 – 3.0)	1.6 (0.9 – 5.6)	1.6 (0.8 – 2.0)	0.234
Glucose (mmol/L)		9.1 (4.9 – 13.2) [c]	9.1 (5.8 – 13.5) [c]	6.3 (4.7 – 7.7) [a b]	0.002
Insulin (pmol/L)		41 (14 – 128)	48 (14 – 92)	57 (17 – 329)	0.341
Insulin / glucose (µM/M)		3.9 (1.6 – 11.5) [c]	5.2 (1.5 – 14.2) [c]	9.5 (3.6 – 44.5) [a b]	0.003

Data are expressed as median (range) or numbers (percentage). *P*-values represent Kruskal-Wallis, Pearson χ^2 or Fishers group comparisons, when significant differences were tested between groups: Significantly different compared to infants (a), children (b) and teenagers (c) at $P < 0.05$. † Equivalent dose methylprednisolone (≈ 5 times hydrocortisone dose), ‡ WI score based on maximum intoropic support during surgery and ICU stay, [#] Percentage plasma of total volume added to the circulation during surgery, [##] Total volume per kg body weight added to the circulation during surgery.

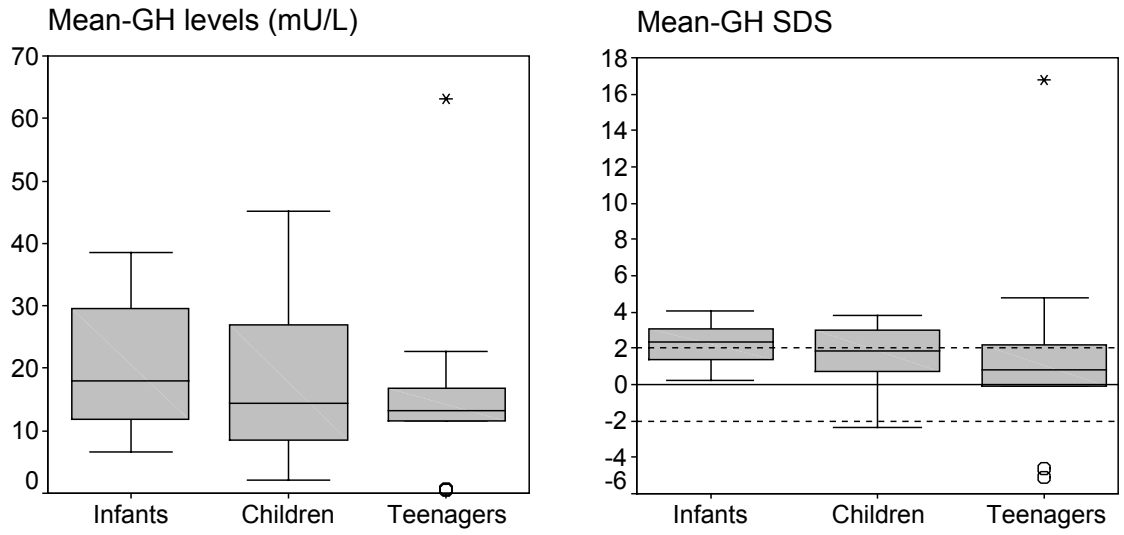

Figure 2. Mean-GH levels and SD-scores in children after cardiac surgery, divided according to age (12).

At the end of surgery, IGF-I and IGFBP-3 SD-scores were lower than reference values in all patients. Surprisingly, IGF-I SD-scores at end of surgery had increased in infants compared to values at start of surgery, but had decreased in teenagers (Figure 1). IGFBP-3 SD-scores had increased in infants and had decreased in both children and teenagers. Multiple regression analysis showed that both IGF-I and IGFBP-3 SD-scores related positively with percentage plasma and initial SD-scores at start of surgery (Table 4), whereas mean-GH levels, IL-6 levels and steroid therapy had no additional influence.

IGFBP-1 levels increased in nearly all patients during surgery (Figure 1). Using multiple regression analysis, we found IGFBP-1 levels at the end of surgery to be related to IL-6 levels and glucocorticoid use and inversely to age (Table 4), whereas IGFBP-1 at the end of surgery was not related to glucose and insulin levels.

Growth factors during the first 24 hours of ICU stay

All patients survived and one child (aged 9.1 years) needed resuscitation after cardiac surgery. The day after cardiac surgery 42 patients were discharged from the ICU to a medium care unit, whereas seven had a longer ICU-stay (range 1.8 – 11.0 days).

At 12h and 24h after surgery, both IGF-I as well as IGFBP-3 SD-scores were significantly lower than reference values (zero SD; $P<0.001$). In contrast to changes during surgery, IGF-I SD-scores decreased in infants during the first 24h of ICU stay. Compared to values at start of surgery, IGF-I SD-scores had decreased in all patients 24h after surgery (Figure 1). IGFBP-3 SD-scores decreased in infants and increased in both children and teenagers during the first 24h of ICU stay. IGFBP-3

SD-scores at 24h were significantly lower compared to values at start of surgery in children and teenagers, but not in infants. IGFBP-1 levels decreased significantly in all patients during the first 24h of ICU stay, resulting in lower levels at 24h compared to pre-surgery levels in infants and children. At 24h after surgery, multiple regression analysis showed that IGFBP-1 was again inversely related to age and insulin levels, whereas IGFBP-1 was not related to IL-6 levels, weight SD-scores, gender or steroid administration during surgery.

Table 4. Multiple regression analyses with depending variables mean-GH level, IGF-I SD-score, IGFBP-3 SD-score, IGFBP-1 level at the end of surgery.

Dependent variable	Independent variable	B	P-value	R^2
Mean-GH level	Age (^{2}Log (years))	-2.479	0.006	0.32
	Dopamine use (Yes=1, No=0)	-7.627	0.038	
	Steroid use (Yes=1, No=0)	-11.082	0.001	
IGF-I SD-score	Percentage plasma [#]	7.218	<0.001	0.41
	IGF-I SD-score at start of surgery	0.445	0.001	
IGFBP-3 SD-score	Percentage plasma [#]	7.218	<0.001	0.55
	IGFBP-3 SD-score at start of surgery		0.031	
IGFBP-1 level	Age (^{2}Log (years))	-0.077	<0.001	0.54
	IL-6 level (^{2}Log (pg/mL))	0.103	0.001	
	Steroid use (Yes=1, No=0)	0.133	0.047	

[#] Percentage plasma of total volume added to the circulation during surgery

Discussion

In this study, we found low SD-scores of IGF-I and IGFBP-3 at start of surgery, which were related to low weight SD-scores and preoperative cyanosis, respectively. At the end of surgery, mean-GH levels were higher than reference values in infants and children and related inversely to age and the use of dopamine and glucocorticoids, but not to IL-6 levels. Glucose levels were significantly higher in patients who received glucocorticoids than in those who did not. At the end of surgery, IGF-I and IGFBP-3 SD-scores were lower than reference values in all age groups, but during surgery values had increased in infants and decreased teenagers. IGF-I and IGFBP-3 SD-scores at end of surgery related to the percentage plasma patients received during surgery and the initial SD-scores at start of surgery, whereas not to IL-6 or GH levels. In contrast, IGFBP-1 levels at the end of surgery related to IL-6 levels, whereas the inverse relation between IGFBP-1 and insulin levels, as found at start of surgery, was lost. Already 24 hours after cardiac surgery, IGF-I, IGFBP-3 and IGFBP-1 levels had returned to values comparable with those at start of surgery.

At start of surgery, we found IGF-I and IGFBP-3 SD-scores to be significantly lower than reference values. Low IGF-I SD-scores related to low weight SD-scores and low IGFBP-3 SD-scores to preoperative cyanosis, suggesting that patients undergoing cardiac surgery have a pre-existing reduction in IGF-I and IGFBP-3 levels, which might be due to an underweight and a compromised clinical status. Diminished levels of IGF-I and IGFBP-3 in combination with underweight is a well known problem in paediatric patients with congenital heart disease (4, 5, 17, 18). We found IGFBP-1 levels to relate inversely with insulin levels and age, which is in accordance with data of healthy non-stressed children (19-21).

At the end of surgery, mean-GH levels were elevated in infants and children. Elevated random GH levels were previously reported after paediatric cardiac surgery, but in these studies no GH profiles were performed (4, 5). We found GH levels to be inversely related to age and dopamine and glucocorticoid administration during cardiac surgery. An interesting finding was the inverse relation between glucocorticoid use and GH levels. Patients in our study who received glucocorticoids, did receive a high dose (30 mg/kg methylprednisolone). This might have influenced GH levels as the acute administration of pharmacological doses may result in a suppression of GH secretion (22). In contrast to data on critically ill children suffering from meningococcal septic shock, we did not find a strong relation between GH levels and IL-6 levels (3). An explanation for this might be the mild elevations in IL-6 levels in patients at the end of cardiac surgery, compared to the extremely elevated IL-6 levels found in children with meningococcal septic shock.

At the end of surgery, we found low IGF-I and IGFBP-3 SD-scores with elevated GH levels, suggesting a GH resistance state. Surprisingly, IGF-I and IGFBP-3 SD-scores increased in infants and decreased in older patients during surgery and related strongly to the levels before surgery and the percentage plasma given during surgery. The fact that infants in our study received a relatively higher percentage of plasma, containing adult levels of growth factors, compared to teenagers, might explain the paradoxical increase in IGF-I and IGFBP-3 in the infants during surgery.

In contrast, IGFBP-1 levels increased during cardiac surgery in nearly all patients. IGFBP-1 levels at the end of surgery related with parameters of disease severity, i.e. IL-6 levels. This is in accordance with data in critically ill children and adults, in whom IGFBP-1 levels increased with increasing disease severity (2, 3, 23, 24). Interestingly, the inverse relation between IGFBP-1 and insulin levels, which was found at start of surgery, was lost at the end of surgery. The manifold metabolic alterations that occur during surgery, for instance the administration of glucocorticoids and the increase in IL-6 levels, might have interfered with the interaction between IGFBP-1 and insulin (2, 24).

During the first 24h after surgery, the changes of IGF-I and IGFBP-3 SD-scores occurred in an opposite direction compared to the changes during cardiac surgery: the levels decreased in younger patients and increased in older patients.

This might indicate that the effect of plasma perfusions was being washed out. Twenty-four hours after surgery, IGF-I and IGFBP-3 had returned to or were lower than the initial values at start of surgery. IGFBP-1 levels decreased in all patients to lower levels than at start of surgery. Furthermore, the inverse relation between IGFBP-1 and insulin levels had returned 24 hours after surgery.

Glucocorticoid administration during cardiac surgery has traditionally been used to protect against the detrimental physiologic alterations induced by exposure of blood to the large areas of synthetic materials from the cardiopulmonary bypass that trigger the production and release of numerous chemotactic and vasoactive substances(25). Most research has focused on the immune modulatory actions of glucocorticoids, whereas only a few have reported on the metabolic side effects, such as hyperglycaemia. Although our study was not designed to investigate the direct effects of glucocorticoid administration on the GH-IGF-I axis and glucose metabolism, we found high doses methylprednisolone (30 mg/kg) to affect GH and IGFBP-1 levels and to result in significantly higher glucose levels at the end of surgery compared to levels in patients who did not receive glucocorticoids, whereas we did not find evidence for the attenuated effect of glucocorticoids on IL-6 levels. As recently has been shown that high blood glucose levels in adult critically ill patients are associated with higher morbidity and mortality and that tight blood glucose control with intensive insulin therapy has benefits in terms of morbidity and mortality (26, 27), the occurrence of hyperglycaemia in this group of children warrants attention. In this perspective, the effects of glucocorticoids (e.g. dose and type) need to be elucidated further (28, 29).

In summary, our study shows that IGF-I and IGFBP-3 SD-scores were already low at start of surgery, which might have been related to underweight and compromised clinical status. At the end of surgery GH levels were elevated but IGF-I and IGFBP-3 SD-scores were decreased. GH levels were inversely related to dopamine and glucocorticoid use, whereas percentage plasma administered influenced IGF-I and IGFBP-3 SD-scores. In contrast, IGFBP-1 levels at the end of surgery related to disease severity, whereas the inverse relation between IGFBP-1 and insulin levels found at start of surgery was lost at the end of surgery. Glucocorticoid administration during surgery was associated with higher glucose levels. Twenty-four hours after surgery, IGF-I and IGFBP-3 SD-scores and IGFBP-1 levels returned to the initial values at start of surgery. Finally, glucocorticoid-induced hyperglycaemia warrants more investigation.

Acknowledgements

We thank Mrs. M. Maliepaard, research nurse, for her technical assistance, B. Dumas, O. Liem, H. Nooteboom and F. van Veghel for helping to collect the GH samples and the nursing staff of the intensive care unit for their support.

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Chapter 8

DISCUSSION

8.1 Introduction

The understanding of the endocrine changes in critically ill children is important as it provides insights in the pathophysiology of the acute stress in children and its differences compared to adults. In addition, it may delineate prognostic factors for survival and may support the rational use of present and future pharmaceutical interventions. Much more than in critically ill adults, the acute phase of critical illness comes into prominence in critically ill children, as they show a very rapid and fierce course of disease, followed by a quick recovery if they survive.

This thesis describes in detail the results of various studies undertaken to evaluate endocrine changes during the acute stress response in critically ill children. The studies were conducted at the pediatric intensive care unit (PICU) of Erasmus MC – Sophia Children's Hospital and the intensive care unit (ICU) of Erasmus MC – Thorax center. The study population consisted of children admitted to the PICU with sepsis or septic shock with purpura and children who underwent open-heart surgery for congenital heart disease. The studies described three hypothalamic-pituitary-end-organ axes:

I. Hypothalamic-pituitary-adrenal (HPA) axis
II. Hypothalamic-pituitary-thyroid (HPT) axis
III. Growth hormone-insulin-like growth factor (GH-IGF-I) axis

In the following section we discuss the main findings of the studies in relation to other published data and elaborate on the implications for clinical practice. We debate the limitations and recommend future research using the knowledge obtained from our studies and literature data.

8.2 Hypothalamic-pituitary-adrenal axis

Adrenal insufficiency in critical illness

Stimulation of the HPA-axis is one of the most important hormonal reactions to critical illness. We found paradoxically lower cortisol levels with higher adrenocorticotropic hormone (ACTH) levels with increasing disease severity in a large group of children with meningococcal sepsis or septic shock on PICU admission (Chapter 2), suggesting a more compromised adrenal function with increasing disease severity. This is in accordance with previous reports (1-3).

To date, criteria to define (relative) adrenal insufficiency in critically ill patients are not generally established and accepted, whereas data in children are very limited as well (4). Some authors have suggested to perform an ACTH-stimulation test to assess the integrity of the HPA-axis in critical illness (5-8), whereas others advocate cortisol levels in a random serum sample (9). Many issues relating to the use of the ACTH-stimulation test in critically ill children still have to be assessed, regarding

dose, cut-off values and timing. Concerning the use of a random cortisol value, many cut-off values for defining adrenal insufficiency have been suggested (9-12). Taking into account several of these cut-off values we found adrenal insufficiency to be present in 10 – 29 % of all children with septic shock and in 13 – 63 % of the nonsurvivors (Table 1).

As cortisol is mainly bound to carrier proteins, the decline in serum cortisol may be the result of lower levels of carrier proteins. We found, however, normal corticosteroid-binding globulin (CBG) in most children with meningococcal disease on PICU-admission (Chapter 2). Our study showed that the low bio-available cortisol levels corresponded with low total cortisol levels, suggesting that bio-available cortisol levels were not more informative about adrenal function than total cortisol levels in this acutely critically ill pediatric population on PICU admission. This is in contrast to studies in critically ill adults reporting higher values of free cortisol in patients due to lowered serum CBG and/or albumin levels, especially during the acute phase of critical illness (13-15).

Table 1. Incidence of adrenal insufficiency in relation with disease severity in our study population, according to various published definitions based on random cortisol assessment only.

Definitions		Incidence in our study population		
Cortisol	Reference	Nonsurvivors (n=8)	Shock-survivors (n=43)	Sepsis survivors (n=9)
< 15 µg/dL (414 nmol/L)	(5)	1 (13 %)	4 (9 %)	0 (0 %)
< 18 µg/dL (496 nmol/L)	(16)	2 (25 %)	4 (9 %)	0 (0 %)
< 20 µg/dL (552 nmol/L)	(7)	3 (38 %)	6 (14 %)	0 (0 %)
< 25 µg/dL (690 nmol/L)	(9)	5 (63 %)	10 (23 %)	1 (11 %)

Factors associated with adrenal insufficiency

In search for factors associated with adrenal insufficiency, we evaluated adrenal enzyme activity of 21-hydroxylase and 11β-hydroxylase, by determining ratios of serum 11-deoxycortisol to 17-hydroxyprogesterone and of cortisol to 11-deoxycortisol. It turned out that decreased cortisol levels were related to decreased activity of 11*β*-hydroxylase, as depicted by decreased cortisol to 11-deoxycortisol ratios, whereas 21-hydroxylase activity was not decreased (Chapter 2) (Figure 1). Furthermore, decreased 11*β*-hydroxylase turned out to be related to etomidate use. This is in accordance with *in vitro* and *in vivo* studies showing that the anesthetic agent etomidate interferes mainly with 11*β*-hydroxylase (17-21). Taking into account clinical and laboratory parameters, we found IL-6 levels and etomidate use to be the major factors related to reduced adrenal function during acute septic shock in children.

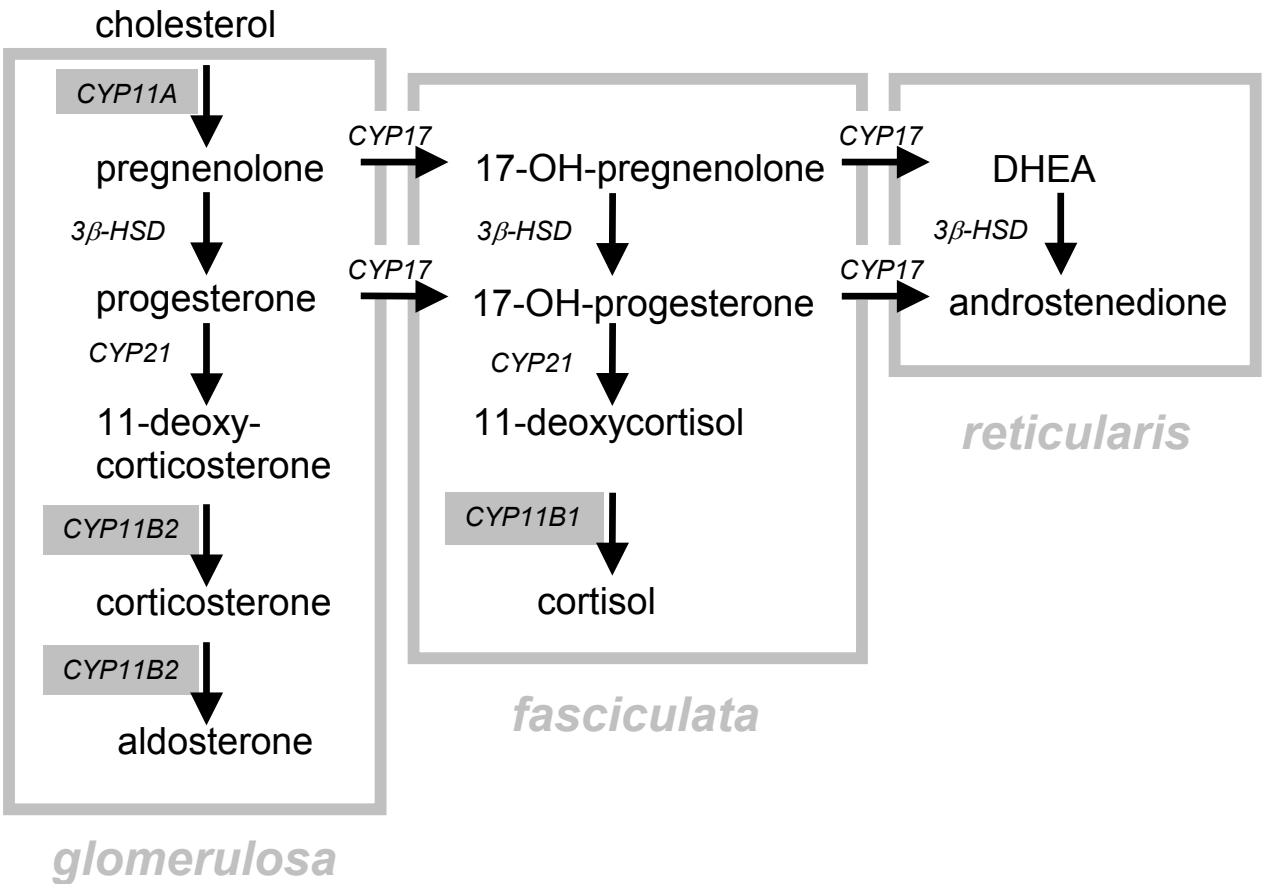

Figure 1. A schematic representation of steroidogenesis in the human adrenal gland and the effect of etomidate. Etomidate inhibits 11β-hydroxylase (CYP11B1), 11β- & 18-hydroxylase (CYP11B2) and cholesterol side-chain cleavage enzyme system (CYP11A) *(shaded enzymes)* with decreasing effectiveness. Decreased CYP11B1 activity will lead to lower levels of cortisol and increased levels of the upstream precursor 11-deoxycortisol. Decreased CYP11B2 will lead to lower aldosterone and higher 11-deoxycorticosterone levels, whereas decreased CYP11A will lead to a generally decreased steroidogenesis. 3β-HSD, 3β-hydroxysteroid-dehydrogenase; CYP21, 21-hydroxylase; CYP17, 17-hydroxylase & 17,20-lyase.

For many years, the long use of etomidate has been withdrawn from long-term sedation regimens due to its reported association with increased mortality (22). One bolus administration of etomidate, however, has remained a first-line anesthetic agent in the setting of rapid sequence intubation, because of its favorable cardiopulmonary profile. It was assumed by many intensive care physicians to give only transient, clinically non-relevant hormonal changes (23-25). Although our study was not designed to examine the direct effects of etomidate for rapid sequence intubation, we found evidence of an impeded adrenal function by etomidate (Chapter 3). Children who received etomidate for rapid sequence intubation showed more signs of adrenal insufficiency, which were specifically related to impaired 11*β*-hydroxylase activity and were associated with decreased glucose levels, compared with children who did not receive etomidate (Table 2). Moreover, of all

children who received etomidate, those who died had received a significantly higher dose of etomidate than those who survived.

Table 2. Incidence of adrenal insufficiency in relation with etomidate use in our study population, according to various published definitions based on random cortisol assessment only.

Definitions		Incidence of adrenal insufficiency		
Cortisol	Reference	Intubated with Etomidate (n=23)	Intubated without Etomidate (n=8)	Not intubated (n=29)
< 15 µg/dL (414 nmol/L)	(5)	4 (17 %)	1 (13 %)	0 (0 %)
< 18 µg/dL (496 nmol/L)	(16)	5 (22 %)	1 (13 %)	0 (0 %)
< 20 µg/dL (552 nmol/L)	(7)	8 (35 %)	1 (13 %)	0 (0 %)
< 25 µg/dL (690 nmol/L)	(9)	14 (61 %)	1 (13 %)	1 (3 %)

Clinical implications

The results of our studies highlight the importance for clinicians to be aware that children with septic shock with purpura may have adrenal insufficiency, especially those who were intubated with etomidate. Although to date there are no strict definitions on adrenal insufficiency assessment in critically ill children, adrenal insufficiency in the case of catecholamine-resistant septic shock may be assumed at a random cortisol level < 18 µg/dL (496 nmol/L) (16). In addition, a cortisol increase ≤ 9 µg/dL (248 nmol/L) after an ACTH-stimulation test may also be suggestive for adrenal insufficiency, although many issues concerning ACTH-stimulation tests in the situation of stress remain to be investigated, such as which dose and which cut-off values should be used and how to interpret the outcome in relation to the initial stress. To date the consensus tells clinicians to assess adrenal function, at least by sampling a baseline cortisol level, in children with catecholamine-resistant septic shock, and always before the initiation of glucocorticoid treatment (16). Awaiting future research, we advocate to simultaneously draw a baseline ACTH level together with the baseline cortisol level (Figure 2).

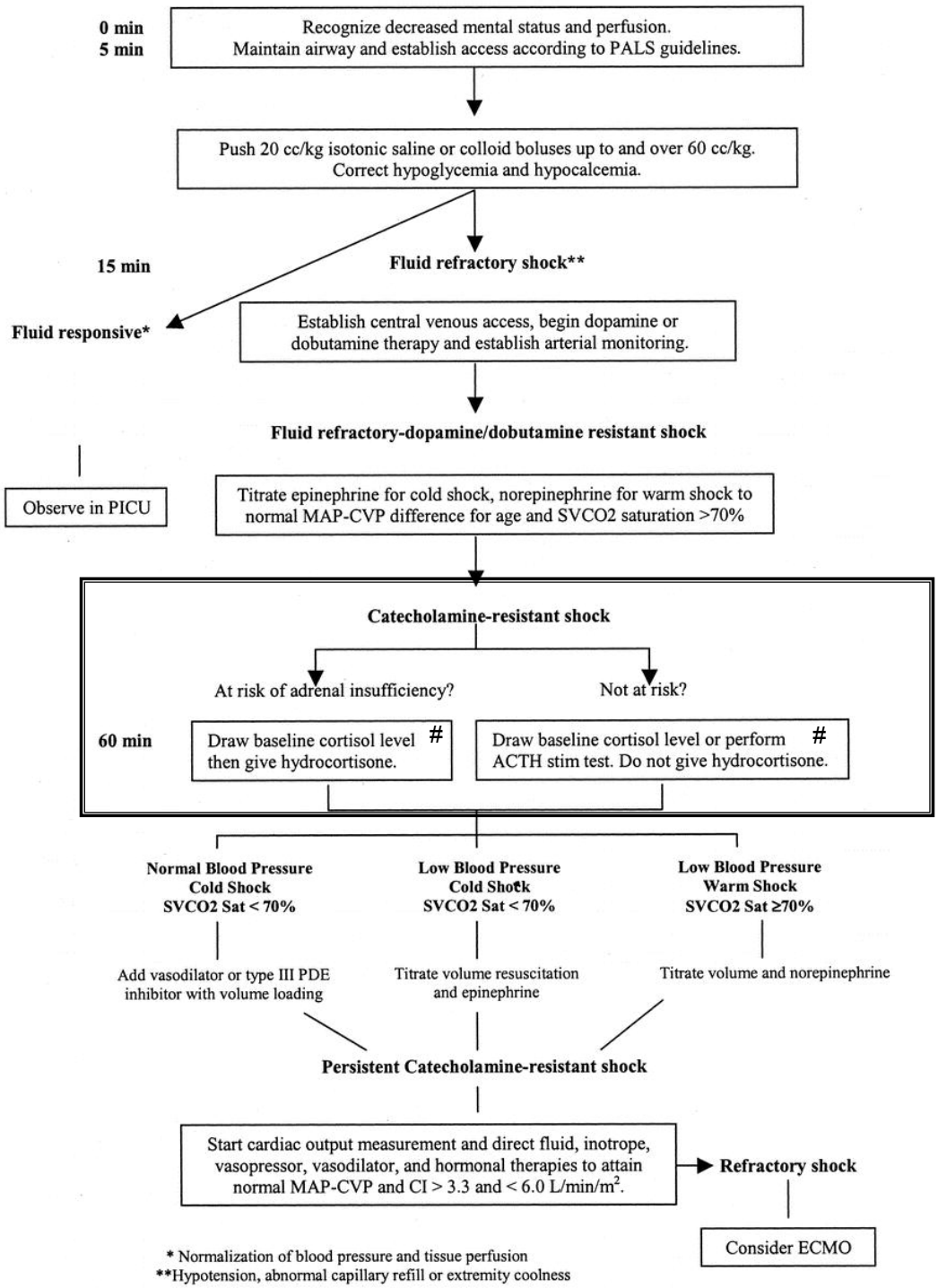

Figure 2. Resuscitation of pediatric septic shock. Adapted from Parker *et al.* (16). (#) We advise to simultaneously draw blood for a baseline ACTH level as well. *PALS*, pediatric advanced life support; *MAP-CVP*, mean arterial pressure–central venous pressure; *ACTH stim*, adrenocorticotropic hormone stimulation; *PDE*, phosphodiesterase; *CI*, cardiac index; *ECMO*, extracorporeal membrane oxygenation.

Hydrocortisone therapy should be reserved for use in children with catecholamine-resistant shock and suspected, e.g. hypoglycemia, or proven adrenal insufficiency and in patients at risk for adrenal insufficiency, such as children who

have previously received steroid therapies for chronic illness and children with pituitary or adrenal abnormalities (16). Considering the dose of hydrocortisone therapy no consensus exists. Dose recommendations vary from 1 – 2 mg/kg for stress coverage to 50 mg/kg for empirical therapy of shock (16). Based on the scarce data in children (26, 27) and studies in adults (28, 29), clinicians should rather use a more 'physiological dose' or 'low-dose' corticosteroid treatment, as high-dose corticoid treatment may also be harmful due to side effects, such as insulin resistance or secondary infections. The duration of steroid therapy should be short, only during the phase of vasopressor support (16). Therapy should be initiated awaiting the results of adrenal function assessment. Finally, based on our results it seems of vital importance to take considerable caution using etomidate during intubation of children with septic shock and to consider combining its administration with glucocorticoids.

8.3 Hypothalamic-pituitary-thyroid axis

Euthyroid sick syndrome in critical illness

We found all children with meningococcal sepsis or septic shock to have signs of euthyroid sick syndrome on PICU admission (Chapter 4 and Chapter 5), as depicted by low TT3 and TT4 levels and high rT3 levels without compensatory elevated TSH levels. This is in accordance with other studies in critically ill children (1, 30-33).

Concerning the relationship between thyroid hormone levels and mortality, however, these studies reported different data. Some showed an association between low levels of TT4 (30) and TT3 (31) and mortality, whereas this relation was not found in other studies (32, 33). We found lower TT4 levels and paradoxically higher TT3/rT3 ratios on PICU admission to be related to mortality, but the predictive value was inferior to that of IL-6 levels (Chapter 4). This indicates that other factors influenced the development of the euthyroid sick syndrome in the early initial phase as well.

In view of the involvement of thyroid hormones in regulating energy expenditure and protein synthesis, low activity of thyroid hormones could be beneficial when exogenous provision of substrate is reduced (34, 35), such as during the initial phase of critical illness (36-38). At this moment it is not yet known if the relatively weak signs of the euthyroid sick syndrome in nonsurvivors might have contributed to the adverse outcome or are due to a lack of time for adjustment to the progressing fulminant disease.

Peripheral thyroid hormone metabolism in critical illness

The peripheral inactivation of thyroid hormones plays an important role in critical illness, especially in the initial phase (38-40). Until recently, the increased rT3 at the expense of TT3 was assumed to be the result of down-regulation of type 1

deiodinase (D1) (39). This view changed since, in addition to D1 down-regulation, type 3 deiodinase (D3) was shown to be induced in critically ill adults (41) (Figure 3). We found lowered TT3/rT3 ratio in all children with meningococcal sepsis, indicating a decreased D1 activity, an increased D3 activity or both (Chapter 4 and Chapter 5). These alterations in peripheral thyroid hormone metabolism related inversely to the duration of disease before admission. Nonsurvivors had paradoxically higher TT3/rT3 ratios, as they experienced a more rapid course of disease than shock-survivors. This suggests that nonsurvivors from meningococcal septic shock lacked the time to develop full-blown euthyroid sick syndrome before PICU admission, although we cannot exclude that nonsurvivors could not sufficiently adapt their HPT-axis to conditions of acute critical illness.

Next to deiodination, sulfation is another pathway of peripheral thyroid hormone metabolism (Figure 3) (40). Interestingly, we found low T4-sulfate (T4S) levels in critically ill children. As shown in Figure 3, T4S is exclusively and rapidly deiodinated by D1. Low T4S levels may indicate high D1 activity, whereas low T3/rT3 ratios suggest low D1 or high D3 activity. Therefore, the combination of low T4S and low T3/rT3 values in our study, suggests that changes in peripheral thyroid hormone metabolism are rather enacted by profound induction of D3 rather than by down-regulation of D1 in early phase of critical illness. Recently published data on T4S levels in critically ill adults, demonstrated T4S levels to be inversely related to hepatic D1 activity and found T4S levels to increase with length of ICU stay (42).

Thyroid hormone binding in critical illness

Because TT4, TT3 and rT3 are mainly bound to carrier proteins, differences in these hormone levels might also be based on varying concentrations of or altered binding to serum carrier proteins, such as thyroxine binding globulin (TBG) and albumin (43). It turned out that reduced TBG levels explained most of the variation in TT4 levels (Chapter 4), whereas albumin did not. In turn, levels of TBG were inversely related to levels of elastase, a serine protease that is released by activated neutrophils and capable of cleaving TBG (44). Taken together, with increasing disease severity both TBG and TT4 levels decreased and elastase levels increased, suggesting that increased turnover of TBG by elastase might have lowered TBG and thus TT4 levels and, as a result, might have exposed tissues to relatively high levels of T4 available for deiodination.

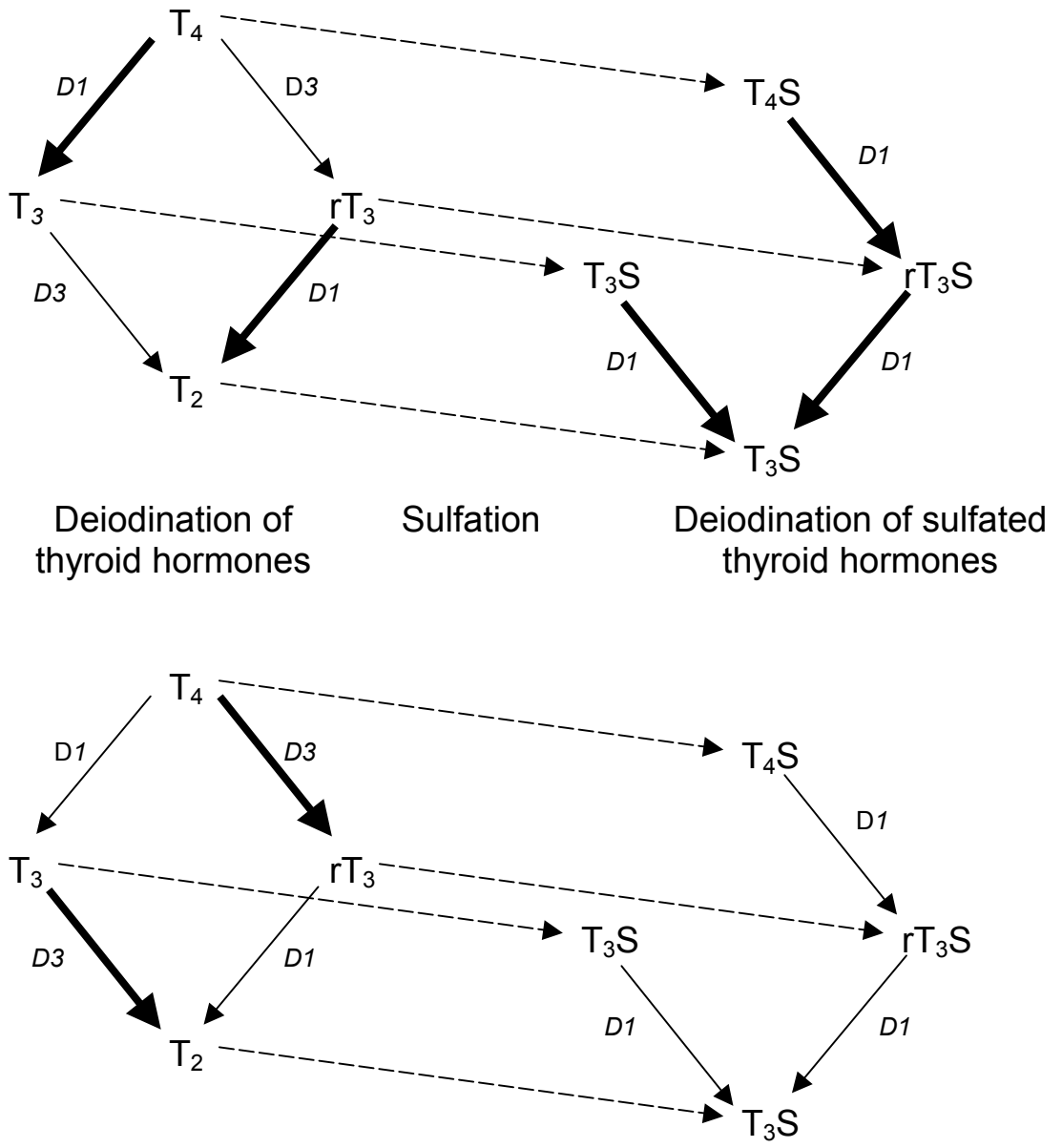

Figure 3. Peripheral thyroid hormone metabolism during normal homeostasis (A) and during critical illness (B). During critical illness deiodination is altered with reduced D1 activity (thin arrow) and increased D3 activity (thick arrow), which both result in increased rT3 levels at expense of TT3 levels (left side). Sulfated thyroid hormones, however, are exclusively deiodinated by D1 (right side) and reduced D1 levels will result in elevated levels of all sulfated thyroid hormones. Sulfation of thyroid hormones is indicated by dotted arrows.

Influence of dopamine in critical illness

The suppressive effect of dopamine on the pituitary gland has previously been described in critically ill adults and children as well as healthy volunteers (45-47). Dopamine directly inhibits anterior pituitary function through inhibitory dopamine receptors, resulting in diminished TSH release (48, 49). In contrast, cessation of

dopamine therapy results in an abrupt rise in TSH levels. Although our study was not designed to investigate the suppressive effects of dopamine, we did find signs of this suppressive effect of dopamine. On PICU admission, dopamine was found to suppress TSH secretion, but not thyroid hormone levels (Chapter 4 and Chapter 5). Only thereafter, during PICU stay, the suppressive effect on thyroid hormone levels came to light (Chapter 5). The relatively short duration of dopamine administration before PICU admission (median, 3.7 h), might explain why the suppressive effect on thyroid hormones was not found on admission.

Time course of euthyroid sick syndrome

Within the group of shock-survivors, the combination of TT4 levels on PICU admission with changes in TT4 and rT3 levels during the first 24 hours of PICU admission and the PRISM score turned out to be prognostic for length of PICU stay (Chapter 5). Although TT3 levels increased from admission onwards in shock-survivors with a short PICU stay, TT3 levels were still decreased at PICU discharge in those who were discharged within 3 days. As thyroid hormones exert anabolic actions (34), this suggests that short stay shock-survivors had still not fully returned to anabolism at PICU discharge.

Clinical implications

The results of our studies show that euthyroid sick syndrome is common in children with meningococcal sepsis on PICU admission. To date, there are no indications that the acute changes in the thyroid axis are harmful and require therapeutic interventions (36, 37, 50, 51), however, no data are currently available to support or refute this hypothesis (52). The setting in which thyroid hormone administration in the acute phase of critical illness has been studied, is short-term T3-administration in adults and children undergoing elective cardiac surgery (53-55). Although these studies show an improved post-operative cardiac function, they do not show an improved outcome. One must be careful extrapolating these data to other clinical settings of euthyroid sick syndrome, such as meningococcal septic shock. To date, we do not think that there is an indication for thyroid hormone therapy in the acute phase of meningococcal disease, whereas an indication in pediatric cardiac surgery should await the results of large controlled trials (56).

Concerning the use of dopamine in critical illness, short-term administration might be useful in the acute management of circulatory failure (57), but as also stated by others, prolonged administration should best be avoided (58, 59), as it induces or aggravates central hypothyroidism and deteriorates catabolism and cellular immune dysfunction. Consequently, alternative inotropics, which do not exert a pituitary suppressive effects, might be used.

Concerning the use of thyroid hormones levels for prediction of outcome, results from our studies showed that thyroid hormone levels on admission were not superior over IL-6 in predicting mortality, as higher disease severity was not reflected

in more signs of euthyroid sick syndrome. On the other hand, a combination of thyroid hormone values on PICU admission and changes over the first 24 hours of PICU admission were predictive for length of PICU stay (Table 3) and might, thus, be a useful tool to identify those children at risk for a long stay at the PICU.

Table 3. Prediction model for duration of PICU stay in shock-survivors (R^2 = 0.64).

PICU-stay (^{10}log (days)) =	0.631
	– 0.004336 * TT4 $_{\text{on admission}}$ (nmol/L)
	– 0.007902 * change $_{(24h - \text{admission})}$ in TT4 (nmol/L)
	+ 0.753 * change $_{(24h - \text{admission})}$ in rT3 (nmol/L)
	+ 0.413 * PRISM score $_{(\text{over first 6h})}$

8.4 Growth hormone-insulin-like growth factor-I axis in meningococcal disease

GH resistance in critical illness

We found in the majority of acutely ill children with meningococcal disease (Chapter 6) normal to high GH levels in combination with low levels of total IGF-I, total IGFBP-3 and ALS on PICU admission, suggesting a GH resistance state. This is in accordance with data in critically ill adults and children (60-64). In order to evaluate whether the high levels of total GH in children with meningococcal disease were also bioactive, we determined bioactive GH levels as well. It turned out that total GH levels related very strongly with bioactive GH levels, indicating that the high circulating GH levels were bioactive. As GH resistance may be associated with reduced GH-receptor function and serum GHBP levels are assumed to reflect the extracellular part of the GH-receptor (GHR) (65), we determined GHBP levels. To our surprise, GHBP levels were within the normal range in most children and positively related with IGF-I, IGFBP-3 and ALS levels, suggesting that the extracellular part of GHR might be still functioning (Figure 4). This does not exclude a disturbance in the transmembrane or intracellular part of the GHR or the post-GHR signaling pathway, which cannot be investigated in serum samples. One of the possibilities might be that the GH-receptor signaling was blocked by an intracellular negative feedback loop induced by the suppressors of cytokine signaling, the SOCSs. SOCS proteins are rapidly induced upon exposure to cytokines, GH, growth factors and fasting, and play an important role in the cellular response to GH (66-68). Other levels of the post-GHR-signaling pathway might also be involved, such as Src homology 2-containing tyrosine phosphatase (SHP-2) or signal transducer and activators of transcription (STAT) (66, 69). In critically ill adult patients, 3 days after surgery and in protracted critical illness, decreased GHBP levels accompanied the GH resistance state, suggesting interference at the level of the extracellular part of

the GHR in the chronic phase of critical illness, by either by decreased expression or increased cleavage (70, 71). In children, however, the combination of normal to elevated GH, low IGF-I, normal GHBP, elevated cytokine levels and a fasting condition in the early phase of critical illness is more suggestive for a disturbed post-GHR signaling rather than a disturbance at the level of the extracellular part of the GHR.

Factors related to GH levels

In our study in children with meningococcal sepsis, GH levels varied between normal to extremely high. In search for factors related to circulating GH levels in the initial phase of critical illness, we found elevated IL-6 levels to be one of the major factors related to elevated GH levels, besides age and dopamine use, which were both inversely related to the GH levels. Interestingly, we found GH levels to be inversely related to cortisol levels. Both GH and cortisol exert direct substrate-mobilizing actions. As cortisol levels decreased with increasing disease severity, the extremely elevated GH levels in these children might have been an ultimate attempt of the body to provide essential substrates for survival in these children. Nevertheless, it might be that other factors affect both cortisol and GH levels.

Factors related to IGFBP-1 levels

In our study, IGFBP-1 levels correlated strongly with parameters of disease severity and were extremely high in nonsurvivors (Chapter 6). In critically ill adults, IGFBP-1 levels have also been reported to increase with increasing disease severity and to be predictive for mortality (72, 73). In contrast to normal conditions (74) we did not find a relation between IGFBP-1 and insulin levels. The multitude of metabolic alterations that occur during critical illness might have caused this. IGFBP-1 has been suggested to fulfill a counter-regulatory role by binding free IGF-I in order to block the insulin like activity of free IGF-I, thereby preventing hypoglycemia, for which the brain is especially vulnerable (60). We found indeed evidence of the inverse relation between IGFBP-1 and free IGF-I levels. In our study, nonsurvivors had extremely high IGFBP-1 levels and the lowest free IGF-I levels. As a consequence, glucose uptake into cells might have been further decreased in the already existing situation of minimal nutritional supply due to fasting. Together with the low levels of alternative metabolic substrates, such as NEFAs (Chapter 6), many cells might have been deprived from energy in a situation of very high metabolic demands.

Clinical implications

In the initial phase of critical illness, the GH-IGF-I axis shows major disturbances. To date, it is unknown whether these alterations, such as high GH levels, are harmful, adaptive or just a side effect of many other factors that occur during the acute phase of stress. Future research is needed to clarify this.

8.5 Growth hormone-insulin-like growth factor-I axis before and after cardiac surgery

IGF-I and IGFBP-3 levels at start of surgery

We evaluated the GH-IGF-I axis in children before and after cardiac surgery and assessed the influence of age, disease severity and medical interventions. At start of surgery, we found levels of total IGF-I and IGFBP-3 to be significantly lower than reference values and to relate to lower weight and preoperative cyanosis. The combination of low levels of IGF-I and IGFBP-3 and underweight is a well-known problem in children with congenital heart disease (75, 76) and has also been found in other studies on pediatric cardiac surgery (62, 63).

GH profiles at end of surgery

At the end of surgery, mean-GH levels were higher than reference values in infants and children but normal in teenagers. Previous studies reported low random GH levels and low urinary GH levels in pediatric cardiac surgery (62, 63). We found GH levels to be inversely related to age and the use of dopamine and glucocorticoids during cardiac surgery, but not to IL-6 levels. An explanation for the latter might be that IL-6 levels were only mildly elevated. In addition, two-thirds of the children received high dose methylprednisolone, which might have inhibited the generation of IL-6.

IGF-I and IGFBP-3 levels at end of surgery and 24 hours thereafter

Despite elevated GH levels, we found low total IGF-I and IGFBP-3 SD-scores at the end of surgery. Surprisingly, total IGF-I and IGFBP-3 SD-scores had increased in infants and decreased in older patients during surgery. Compared to teenagers, infants received a relatively more human donor plasma, as depicted in higher percentage plasma of total volume, which contained thus higher concentrations of growth factors. This might well explain the increase in total IGF-I and IGFBP-3 SD-scores in infants. At the end of surgery, total IGF-I and IGFBP-3 SD-scores related strongly to the levels before surgery and the percentage plasma of total volume given during surgery, whereas not to GH or IL-6 levels. During the first 24h after surgery, the levels in total IGF-I and IGFBP-3 SD-scores returned to values at the start of surgery.

IGFBP-1 levels before and after surgery

As in normal conditions, in which IGFBP-1 is GH-independently regulated and rapidly influenced by metabolic factors, primarily by insulin (74, 77-79), we found IGFBP-1 levels to be inversely related to insulin levels at the start of surgery. However, this relation had disappeared at the end of surgery. This is in accordance with data in critically ill children and adults, in whom IGFBP-1 levels increase with

increasing disease severity (60, 61, 73, 80). The multitude of metabolic alterations that occurred during open-heart surgery, for instance the use of glucocorticoids and the increase in IL-6 levels, might have had more influence on IGFBP-1 transcription than the suppressive effect of insulin. Interestingly, 24 hours after surgery, IGFBP-1 levels had declined even below levels at start of surgery and related again inversely to insulin levels.

Clinical implications

In our study low total IGF-I levels were related to a underweight. Although this is a well-known problem in children with congenital heart disease (62, 63, 75, 76), these data suggest that nutritional support in this group of children still warrants more attention. Furthermore, one must take account of the amount of plasma used during surgery when interpreting serum levels of IGF-I and IGFBP-3 after surgery, as human plasma products contains growth factors.

Although our study was not designed to investigate the direct effects of glucocorticoid administration on the GH-IGF-I axis and glucose metabolism, we found that high doses of methylprednisolone (30 mg/kg) affected GH and IGFBP-1 levels and resulted in much higher glucose levels at the end of surgery compared levels in those who did not receive glucocorticoids. Glucocorticoid administration during cardiac surgery has traditionally been used to protect against the detrimental physiologic alterations induced by exposure of blood to the large areas of synthetic materials from the cardiopulmonary bypass that trigger the production and release of numerous chemotactic and vasoactive substances (81), but the metabolic effects have been underexposed. As it has been shown recently that tight blood glucose control with intensive insulin therapy has beneficial effects on morbidity and mortality in adult critically ill patients (82, 83), the effect of hyperglycemia on outcome in this group of children should be further evaluated. In this perspective, it may be discussed whether lower doses of glucocorticoids should be used (84, 85).

8.6 Synthesis of the growth hormone-insulin-like growth factor-I axis

We found in both study populations, children with meningococcal sepsis and children after cardiac surgery, sustained to enhanced GH levels, but low levels of IGF-1 and IGFBP-3 (Table 4). The patient groups differed, however, in several aspects, such as IL-6 levels and glucocorticoid use. IL-6 levels were much lower in children after cardiac surgery than in children with meningococcal sepsis, in whom IL-6 levels were extremely elevated. The lower IL-6 levels in children after cardiac surgery might have been due to lower disease severity and inflammation, but could also be due to the glucocorticoid therapy, which might have tempered cytokine generation (86). Children with cardiac surgery received more often glucocorticoid therapy and also in

much higher doses than children with meningococcal sepsis, whereas some children with septic shock experienced adrenal insufficiency. In addition, children with congenital heart disease suffered from preexisting underweight, whereas children with meningococcal sepsis did not (unpublished observations).

High GH levels related to high IL-6 levels in critically ill children with sepsis, but not in children after open-heart surgery, whereas in both groups GH levels turned out to be inversely related to dopamine use. Furthermore, GH levels related inversely to cortisol levels in critically ill children with sepsis and inversely to high dose glucocorticoid treatment in children after open-heart surgery. This indicates that the GH secretion in critically ill children was stimulated by high IL-6 levels and inhibited by dopamine use and high cortisol levels. As in normal conditions, younger children in both populations had higher GH levels than older children.

Total IGF-I levels were lower than reference values in both populations, at PICU admission and after cardiac surgery. Total IGF-I levels related, however, inversely to IL-6 levels in children with sepsis, but to initial IGF-I values at the start of surgery in children with congenital heart disease. The latter values in turn were related to underweight. Both inflammatory cytokines and nutritional deficiency, have been suggested as causes of the low IGF-I levels in the initial phase of critical illness (60).

As in normal conditions (74), IGFBP-1 levels related inversely to insulin levels before cardiac surgery, whereas this relation disappeared after open-heart surgery and did also not exist in septic children. The multitude of metabolic alterations that occurred during sepsis and open-heart surgery might have caused this. In both populations, IGFBP-1 levels related positively to IL-6 levels, whereas IGFBP-1 related also to glucocorticoid use in children after cardiac surgery.

In conclusion, our data emphasize that endocrine changes in the initial phase of critical illness show similarities, some of which were also related to disease specific factors. Therefore, one may not unreservedly extrapolate these endocrine changes from one disease state to the other.

Table 4. GH-IGF-I axis and clinical parameters at PICU admission in children with meningococcal sepsis or after open-heart surgery

	Meningococcal disease			Open-heart surgery
	Nonsurvivors (n=4)	Shock-survivors (n=29)	Sepsis-survivors (n=6)	(n=49)
Mean-GH (mU/L)	131 (48 – 148)	13 (6 – 19)	16 (11 – 32)	15 (11 - 26)
SDS	5.7 (3.2 to 6.0)	1.5 (–0.2 to 2.5)	1.9 (0.1 to 3.0)	2.1 (0.6 - 3.0)
Total IGF-I (nmol/L)	1.1 (0.4 – 2.9)	5.8 (4.6 – 8.3)	5.8 (2.6 – 23.7)	5.9 (4.4 - 7.2)
SDS	-3.9 (-5.9 to -1.9)	-2.2 (-2.8 to -1.2)	-1.4 (-3.8 to -0.6)	-1.3 (-2.2 to -0.4)
IGFBP-3 (nmol/L)	11 (9 – 12)	20 (16 – 28)	26 (15 – 59)	31 (28 – 36)
SDS	-7.5 (-8.7 to -6.7)	-4.9 (-5.6 to -3.2)	-3.2 (-6.6 to -1.8)	-2.7 (-3.4 to –2.0)
IGFBP-1 (nmol/L)	33.4 (10.2 - 47.6)	5.1 (3.2 – 8.1)	2.3 (0.7 – 6.1)	15.7 (9.7 – 22.1)
Lactate (mmol/L)	5.5 (4.8 – 9.6)	3.9 (2.5 – 5.2)	2.1 (1.5 – 2.6)	1.6 (1.3 – 2.4)
IL-6 (pg/mL)	1571923 (347548 – 2690384)	63233 (6406 – 150240)	366 (129 – 6853)	21 (10 – 39)
Dopamine therapy (%)	0 (0)	10 (34)	0 (0)	22 (45)
Glucocorticoids therapy (%)	1 (25)	4 (14)	0 (0)	32 (65)
Glucose (mmol/L)	4.6 (1.8 – 10.1)	6.5 (5.5 – 10.1)	8.5 (7.6 – 11.4)	8.6 (6.7 – 10.4)
Insulin (pmol/L)	29 (29 – 336)	104 (40 – 242)	81.6 (52 – 203)	52 (27 – 73)
Insulin/Glucose (µM/M)	15 (6 –34)	15 (7 – 32)	10 (5 –19)	6 (4 – 8)

Data are expressed as median (25 to 75 percentile) or numbers (percentage).

8.7 Future perspectives

More studies in different populations of critically ill children

The endocrine changes in acutely critically ill children with meningococcal sepsis or after open-heart surgery showed differences and might therefore not unreservedly be extrapolated to critically ill children due to other diseases or with co-morbidity. Although limited data have recently been published, future research should explore the endocrine changes in different groups of critically ill children, due to for instance trauma, burns, respiratory distress and severe neurological status, and in children with co-morbidity (87, 88).

Alternative tools for endocrine hypothalamic-pituitary-end-organ function assessment

Future research should take effort in obtaining tissue samples from critically ill patients, preferably serially sampled and combined with serum samples, to assess hormone tissue levels and receptor density and/or function. For example, the intracellular activity of cortisol is subject to the cortisol-cortisone conversion under the influence of 11β-hydroxysteroid dehydrogenases (11β-HSD) and to glucocorticoid receptor expression. It will, however, be difficult to obtain tissue samples of vital organs, such as liver or muscle, from critically ill children. Not only because of the increased risk of bleeding due to coagulation disturbances in children with meningococcal septic shock, but also because many parents will refuse this kind of invasive assessment to be used in their critically ill child. Peripheral blood mononuclear cells (PBMCs) are relatively easy to obtain, via blood sampling, but as gene expression varies among tissues, PBMCs may not always reflect gene expression of other tissues. Another possibility to obtain tissue samples more easily are post mortem biopsies, as shown in studies on critically ill adults (41, 42, 89-91). Post mortem biopsies may however not reflect the acute phase of critical illness. In addition, many parents refuse post mortem investigations because of various personal reasons. Finally, although results from animal studies may not unreservedly be extrapolated to humans, these studies remain important in case of critical illness.

Assessment of the influence of genetic factors

Considerable work has already been done on exploring the influence of genetic factors on the host response to meningococcal sepsis (92, 93). The studies presented in this thesis showed hormonal responses in relation to disease severity of critical illness in children and found differences between survivors and nonsurvivors, some of which were related to medication use. It remains however to be explored whether genetic factors might play a role in the hormonal response to stress. Nonsurvivors might not have had the genetic constitution to adapt adequately to the

extreme demands of severe stress. Interesting genes for investigation of polymorphism might be the glucocorticoid receptor (94), GH-receptor, which belongs to the cytokine receptor super family, genes of the post GHR signaling pathway, some of which are shared by the post insulin receptor pathway (83), such as suppressor of cytokine signaling (SOCS) (66, 95, 96) and genes of the thyroid hormone pathway (97, 98).

Hormonal intervention studies

The studies described in this thesis had an observational nature and were therefore not designed to evaluate the effects of different hormonal interventions on the outcome of critically ill children. Although several hormonal interventions have been studies in critically ill adults (28, 82, 99-108), of which most were conducted in protracted critical illness, only a few hormonal interventions have been tested in children (55, 109-114). Given the rare occurrence of protracted critical illness in children and differences between the endocrine and metabolic adaptations between critically ill adults and children, the findings from studies in critically ill adults may not unreservedly be extrapolated to critically ill children. Future hormonal intervention studies in pediatric critical illness, will need large numbers of children and therefore a multi-center design may be indispensable. Future studies are needed to clarify for example specific situations in which corticosteroid replacement is beneficial and to determine the optimal dose and duration of replacement.

Concluding remarks

The results of our studies emphasize the importance of endocrine evaluation of critically ill children. Future research should further explore the endocrine adaptations to stress in order to clarify the endocrine changes in other clinical conditions with the ultimate aim to evaluate if influencing the various hormonal pathways would reduce mortality and morbidity of life-threatening diseases in children.

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Chapter 9

SUMMARY AND CONCLUSIONS

The understanding of the endocrine changes in critically ill children is important, as it provides insights in the pathophysiology of the acute stress in children and its differences compared with adults. Furthermore, it delineates prognostic factors for survival and supports the rational use of present and future pharmaceutical interventions. Much more than in critically ill adults, the acute phase of critical illness comes into prominence in critically ill children, as they show a very rapid and fierce course of disease, followed by a quick recovery if they survive. This chapter presents a comprehensive summary of the results of various studies undertaken to evaluate endocrine changes seen during the acute stress response in critically ill children suffering from sepsis or septic shock with purpura (**Chapters 2 to 6**) or undergoing open-heart surgery (**Chapter 7**). These studies evaluated three hypothalamic-pituitary-end-organ axes:

I. Hypothalamic-pituitary-adrenal axis (**Chapter 2** and **Chapter 3**)
II. Hypothalamic-pituitary-thyroid axis (**Chapter 4** and **Chapter 5**)
III. Growth hormone / insulin-like growth factor axis (**Chapter 6** and **Chapter 7**)

Chapter 1 provides a general overview of these hormonal axes and the current knowledge on the changes during the acute phase of critical illness in children and adults.

Chapter 2 describes several aspects of adrenocortical function in relation with disease severity in children with meningococcal sepsis or septic shock on PICU admission. The most severely ill children had more signs of adrenal insufficiency, as depicted by lower median cortisol/ACTH ratios. In contrast with data in critically ill adults, bio-available cortisol levels were not more informative on adrenal function than total cortisol levels. Decreased adrenal function was strongly inversely related to IL-6 levels and at least partly to a decreased 11β-hydroxylase activity, but not to a decreased 21-hydroxylase activity. In addition to IL-6 levels, one single bolus of etomidate during intubation was related to a decreased adrenal function and 11β-hydroxylase activity.

Chapter 3 describes retrospectively the influence of one single bolus of etomidate used for intubation on adrenal function in children with meningococcal sepsis on PICU admission, 12 and 24 hours thereafter. Children who received etomidate had significantly more signs of impaired adrenocortical function, such as lower cortisol, higher ACTH and 11-deoxycortisol levels, than those who did not receive etomidate, independently of intubation. The median dose of the etomidate bolus was significantly higher in children who died compared with those who survived. All, except one, children who died had received etomidate. Within 24 hours cortisol/ACTH ratios increased significantly in children who had received etomidate, resulting in cortisol/ACTH ratios 24 hours after admission which were comparable to

those in the children who had not received etomidate. Our data imply that even one single bolus of etomidate negatively influences adrenal function and thereby might increase risk of death. Therefore, considerable caution should accompany the administration of etomidate in children with septic shock. Future research should elucidate the potential role for concomitant steroid replacement when etomidate remains in use.

Chapter 4 describes several aspects of the thyroid function in relation with disease severity in children with meningococcal sepsis or septic shock on PICU admission. The study shows that all critically ill children with sepsis or septic shock had signs of euthyroid sick syndrome on PICU admission, as depicted by a decreased TT3/rT3 ratio. More signs of euthyroid sick syndrome did not inevitably reflect higher disease severity at the time of PICU admission, indicating that other factors influenced the development of euthyroid sick syndrome in the early initial phase as well. Alterations in peripheral deiodination were related to duration of illness and seemed enacted by a profound induction of type 3 deiodinase rather than down regulation of type 1 deiodinase, suggesting that children who died from meningococcal septic shock lacked the time to develop full-blown euthyroid sick syndrome before PICU admission. Low TT4 levels were related to increased cleavage of TBG by elastase. Dopamine-treated children showed only a reduction of TSH levels and no difference in other thyroid hormone levels compared to the non-dopamine-treated children at the time of PICU admission. Values of TT3/rT3 and TT4 were predictive for mortality, but not more informative than IL-6 levels.

Chapter 5 evaluates thyroid function in relation to length of PICU stay in children who survived septic shock with purpura during the first 48 hours of PICU stay. All children surviving from meningococcal septic shock showed features of the euthyroid sick syndrome. Changes in thyroid function on admission were influenced by the duration of illness prior to admission. Changes in thyroid hormone levels over the first 24h of admission, especially the TT3 drop and the rT3 increment were prognostic for length of PICU stay, in addition to TT4 levels on admission and PRISM score. Thyroid hormone concentrations at PICU discharge were still not normalized in short stay shock-survivors, suggesting that those children had still not fully returned to anabolism at PICU discharge. Dopamine was found to have an influence on the course of thyroid levels.

Chapter 6 describes several aspects of the GH-IGF-I axis in relation with disease severity in children with meningococcal sepsis or septic shock on PICU admission. The majority of children with meningococcal sepsis had normal or elevated GH levels with decreased total IGF-I levels, suggesting a GH resistance state on PICU admission. This was most striking in the most severely ill children. The bioactivity of serum GH paralleled total GH levels. Surprisingly, GHBP levels were within the

normal range and related positively to total IGF-I, IGFBP-3 and ALS levels, suggesting reduced post-GHR signaling rather than decreased GHR function. IGFBP-1 levels increased with increasing disease severity and correlated inversely with free IGF-I levels, emphasizing its counter-regulatory role in critical illness.

Chapter 7 describes the GH-IGF-I axis in children before and after open-cardiac surgery and determines influencing factors. At start of surgery, IGF-I and IGFBP-3 SD-scores were already low, which might have been related to underweight and compromised clinical status. At the end of surgery GH levels were elevated but IGF-I and IGFBP-3 SD-scores were decreased. GH levels were inversely related to dopamine and glucocorticoid use, whereas the plasma administered influenced IGF-I and IGFBP-3 SD-scores. IGFBP-1 levels at the end of surgery related to disease severity, whereas the inverse relation between IGFBP-1 and insulin levels found at start of surgery was lost at the end of surgery. Glucocorticoid administration during surgery was associated with higher glucose levels. Twenty-four hours after surgery, IGF-I and IGFBP-3 SD-scores and IGFBP-1 levels returned to the initial values at start of surgery.

In **Chapter 8** we describe the results of our studies in the context of the literature and elaborate on the implications for clinical practice. We discuss the limitations and recommend future research.

Chapter 10

SAMENVATTING EN CONCLUSIES

Het hormonale stelsel speelt een belangrijke regulerende rol in stofwisseling van mens en dier, zowel in gezondheid als in ziekte. Hormonen zijn in het lichaam gevormde chemische stoffen die via de bloedstroom bepaalde organen tot werkzaamheid aanzet. Dit proefschrift beschrijft de hormonale veranderingen die optreden tijdens de acute fase van levensbedreigde ziekte in kinderen, de “acute stress reactie”; in het bijzonder van kinderen opgenomen op de pediatrische intensive care vanwege bloedvergiftiging (sepsis en septische shock) met de meningokok bacterie (*Neisseria meningitidis*) (Hoofdstukken 2 tot en met 6) of na een open-hartoperatie (Hoofdstuk 7). Met dit onderzoek beoogden we de kennis en het inzicht in de hormonale regelmechanismen van de acute stress reactie bij kinderen met acuut levensbedreigende aandoeningen te verbreden, het therapeutisch handelen in relatie hiermee te evalueren en indien mogelijk aan te passen. Tevens beoogden we hormonale parameters te vinden om een prognose te kunnen stellen voor de duur van de ziekte en overleving.

In dit hoofdstuk geven we een beknopte samenvatting van de resultaten van onze onderzoeken naar de hormonale aspecten van de acute stress reactie tijdens ernstig zieke. Drie hormonale assen worden beschreven:

I. Hypothalamus-hypofyse-bijnier as (**Hoofdstukken 2 en 3**)
II. Hypothalamus-hypofyse-schildklier as (**Hoofdstukken 4 en 5**)
III. Groeihormoon-insulin-like growth factor as (**Hoofdstukken 6 en 7**)

Hoofdstuk 1 geeft een introductie van de drie hormonale assen en de kennis omtrent veranderingen van deze drie hormonale assen tijdens de acute stress reactie in kinderen en volwassenen.

Hypothalamus-hypofyse-bijnier as

Het stresshormoon cortisol speelt een belangrijke rol in het instandhouden van de bloeddruk, het vrij maken van energie en het voorkomen een te sterke, waardoor schadelijke, afweerreactie. Cortisol wordt geproduceerd in het schors van de bijnieren, onder invloed van verschillende enzymen. De activiteit van deze enzymen is indirect te meten door verhoudingen te berekenen van de tussenvormen van cortisol in het bloed. Het aanzetten van de productie van cortisol in de bijnier staat onder invloed van het adrenocorticotroop hormoon (ACTH) dat in de hersenen wordt geproduceerd. Cortisol remt weer de productie van het stimulerende hormoon ACTH, zodat niet te veel cortisol wordt geproduceerd, dit mechanisme heet “negatieve terugkoppeling”. In het bloed wordt cortisol gebonden aan bindingseiwitten, waardoor minder dan 10% vrij in het bloed circuleert. Het vrije cortisol is biologisch actief. Als te weinig cortisol wordt geproduceerd tijdens levensbedreigende ziekte (bijnierinsufficiëntie) heeft dit tot gevolg dat de bloeddruk

te laag wordt en niet meer goed reageert op bloeddruk verhogende medicatie, de glucose productie te kort schiet en het afweersysteem niet adequaat genoeg reageert.

Hoofdstuk 2 beschrijft verschillende aspecten van de bijnierschorsfunctie in relatie tot de ernst van ziekte bij kinderen met meningokokken sepsis bij opname op de pediatrische intensive care. Met toenemende ernst van ziekte werden meer tekenen van bijnierinsufficiëntie gevonden, zich uitend in lagere cortisol spiegels en hogere ACTH spiegels. In tegenstelling tot ernstig zieke volwassen patiënten, bleken in deze ernstig zieke kinderen de niet-gebonden cortisol spiegels goed overeen te komen met de totale cortisol spiegels en waren de vrije cortisol spiegels dus niet informatiever over de bijnier functie dan de totale cortisol spiegels. Verminderde bijnierfunctie bleek voornamelijk gerelateerd aan interleukine (IL-6), een parameter voor ernst van ziekte, en aan een verminderde activiteit van het bijnierenzym 11β-hydroxylase. Daarentegen bleek de activiteit van het bijnierenzym 21-hydroxylase niet verlaagd. Naast de ernst van ziekte bleek het eenmalig gebruik van het narcosemiddel etomidaat bij intubatie gerelateerd te zijn aan verminderde 11β-hydroxylase activiteit en verminderde bijnierschorsfunctie.

Hoofdstuk 3 beschrijft de studie naar de invloed van het eenmalig gebruik van etomidaat op de bijnierfunctie van kinderen met meningokokken sepsis bij opname, en 12 en 24 uur daarna. Kinderen die etomidaat hadden gekregen bij intubatie toonden significant meer tekenen van verminderde bijnierschorsfunctie dan kinderen die geen etomidaat hadden gekregen bij intubatie. Bij deze groep kinderen werden lagere spiegels van cortisol en hogere spiegels van ACTH en 11-deoxycortisol (een voorloper van cortisol) gevonden. Van alle kinderen die etomidaat hadden gekregen, was de gemiddelde dosis etomdiaat significant hoger in kinderen die overleden vergeleken met de kinderen die overleefden. Vierentwintig uur na opname was de cortisol/ACTH ratio significant gestegen bij kinderen die etomidaat hadden ontvangen, wat resulteerde in vergelijkbare cortisol/ACTH ratio's 24 na opname vergeleken met kinderen die geen etomidaat hadden ontvangen. De data lieten zien dat zelfs een enkele dosis etomidaat de bijnierschors negatief beïnvloedt en daarmee een extra risicofactor is voor overlijden. Derhalve dient het gebruik van etomidaat bij kinderen met septische shock ter discussie te staan. Het tegelijkertijd geven van een dosis glucocorticoïden dient sterk overwogen te worden.

Hypothalamus-hypofyse-schildklier as

Onder invloed van het thyroïd stimulerend hormoon (TSH) uit de hersenen, produceert de schildklier het hormoon thyroxine (T4). In de lever, nieren en spieren van gezonde mensen zetten enzymen, die deiodinasen heten, T4 om in het actieve

schildklierhormoon T3. Dit heet ook wel het perifeer schildkliermetabolisme, waarbij type 1 deiodinase een belangrijke rol speelt. Het schildklierhormoon T3 heeft een voornamelijk anabole invloed op de stofwisseling, met andere woorden een stimulerende werking op groei en ontwikkeling. De regulatie van de hypothalamus-hypofyse-schildklier as gaat via een negatieve terugkoppeling: als er voldoende T4 en T3 is wordt de afgifte van TSH geremd. Tevens worden ook T4 en T3 gebonden in het bloed aan bindingseiwitten, met name T4-bindend eiwit (TBG), en is het effect afhankelijk van het ongebonden, biologisch actieve hormoon.

Tijdens ziekte wordt het T4 omgezet in het inactieve reverse T3 (rT3) en wordt het actieve T3 versneld afgebroken, zonder dat het TSH stijgt wat normaliter zou gebeuren door de negatieve terugkoppeling. Deze veranderingen worden samen het “euthyroid sick syndrome” genoemd en men veronderstelt dat dit een adaptatie van het lichaam is om energie te sparen en eiwit opbouw voor groei uit te stellen ten gunste van overlevingsstrategieën.

Hoofdstuk 4 beschrijft de evaluatie van de schildklierfunctie in relatie tot ernst van ziekte in kinderen met meningokokken sepsis of septische shock bij opname. Alle kinderen hadden tekenen van “euthyroid sick syndrome” bij opname, zich uitend in een verlaagde T3/rT3 ratio zonder verhoogde TSH spiegels. Bij opname was echter een toename van ziekte ernst niet gerelateerd aan meer tekenen van “euthyroid sick syndrome”. Dit geeft aan dat bij opname ook andere factoren dan ernst van ziekte invloed hadden op het ontwikkelen van het “euthyroid sick syndrome”. De duur van ziekte voor opname bleek gerelateerd te zijn aan de schildklierwaarden. Hoe korter ziek hoe minder tekenen van het “euthyroid sick syndrome”. De veranderingen in het perifere schildkliermetabolisme bleken meer gerelateerd aan de inductie van het enzym type 3 deiodinase dan aan de vermindering van het enzym type 1 deioninase. Daarnaast waren lage T4 spiegels in het bloed gerelateerd aan een verhoogde afbraak van TBG, het belangrijkste bindingseiwit van T4, door elastase. Het bloeddrukverhogende middel dopamine bleek alleen een associatie te hebben met lagere spiegels van TSH, echter niet op de spiegels van andere schildklierhormonen. Tenslotte bleken hogere T3/rT3 ratio’s en lagere T4 spiegels een voorspellende waarde te hebben voor mortaliteit. Hun predictieve waarde was echter niet belangrijker dan die van verhoogde IL-6 spiegels, een bekende parameter voor ernst van ziekte.

Hoofdstuk 5 beschrijft verschillende aspecten van de schildklierfunctie in relatie tot ernst van ziekte in kinderen die de meningokokken septische shock overleefden, tijdens de eerste 48 uur na opname. Alle kinderen hadden tekenen van “euthyroid sick syndrome” bij opname. De T3/rT3 ratio, een maat voor perifeer schildkliermetabolisme, was omgekeerd gerelateerd aan de duur van ziekte voor opname op de pediatrische intensive care. Veranderingen in de schildklierhormoon spiegels gedurende de eerst 24 uur na opname, vooral de daling in T3 en de stijging

in rT3, waren voorspellend voor de opnameduur op de intensive care. Daarnaast waren lagere T4 spiegels bij opname en de hogere ziektescore “PRISM score” voorspellend voor een langere opnameduur. Bij ontslag van de intensive care waren de schildklierhormoon spiegels nog niet normaal hetgeen suggereert dat er nog geen volledig herstel is van de anabole functies.

Groeihormoon-insulin-like growth factor as

Groeihormoon (GH) heeft een anabole invloed op de stofwisseling, zowel direct als indirect via insulin-like growth factor-I (IGF-I). GH wordt geproduceerd in de hersenen en komt voor in diverse vormen met bijbehorende verschillende biologische activiteiten. GH bindt aan een GH-receptor, die bestaat uit drie delen: het extracellulair, transmembraneus en intracellulair. Activatie van de GH-receptor, door middel van dimerisatie, resulteert met name in de lever in de productie van groeifactoren zoals IGF-I en bindende eiwitten zoals insulin-like growth factor binding protein (IGFBP-3) en acid-labile subunit (ALS). Als het extracellulaire deel van de GH-receptor wordt gekliefd komt het in het bloed als het GH bindende eiwit “GHBP”. De regulatie van de GH-IGF-I as loopt ook via een negatieve terugkoppeling: IGF-I remt de afgifte van GH. IGF-I wordt in het bloed gebonden aan bindingseiwitten, met name IGFBP-3. De activiteit van IGF-I wordt bepaald door het ongebonden, biologisch actieve deel (vrij IGF-I).

Tijdens ziekte worden lage spiegels van IGF-I en IGFBP-3 gevonden, terwijl de spiegels van GH normaal blijven of verhoogd zijn. Deze veranderingen worden samen ook wel “GH resistentie” genoemd.

Hoofdstuk 6 beschrijft de evaluatie van de GH-IGF-I as in relatie tot ernst van ziekte in kinderen met meningokokken sepsis of septische shock bij opname op de pediatrische intensive care. Het merendeel van de kinderen had normale of verhoogde spiegels van GH met verlaagde spiegels van IGF-I, hetgeen past bij een beeld van GH resistentie. De GH resistentie was het meest uitgesproken bij de meest zieke kinderen. De spiegels van het biologisch actieve GH was vergelijkbaar met de spiegels van het immunoreactieve, totale GH in het bloed. Tegen de verwachtingen in werden normale spiegels van het GHBP gemeten, die positief correleerde met de spiegels van totaal IGF-I, IGFBP-3 en ALS. Deze bevindingen suggereren dat de GH resistentie veeleer veroorzaakt wordt door verminderde functie van het traject na de GH-receptor, de “post-GH-receptor signaaltransductie”, dan door een verminderde expressie van het extracellulaire deel van de GH-receptor. Spiegels van het IGF bindingseiwit IGFBP-1 stegen met toenemende ernst van ziekte en correleerde omgekeerd met de ongebonden (vrij) IGF-I spiegels.

Hoofdstuk 7 beschrijft de studie naar de GH-IGF-I as in kinderen vóór en na open-hartoperatie en de evaluatie van de verschillende factoren die van invloed zijn op de GH-IGF-I as. Aan het begin van de operatie waren de standaard deviatie (SD) scores van IGF-I and IGFBP-3 spiegels al verlaagd vergeleken met gezonde kinderen van dezelfde leeftijd. Tevens bleek dat lagere SD scores voor IGF-I en IGFBP-3 gerelateerd waren aan lage SD scores voor gewicht en een verminderde klinische conditie van de kinderen. Aan het einde van de operatie werden hoge GH spiegels gevonden en verlaagde IGF-I and IGFBP-3 SD scores. De GH spiegels waren omgekeerd gerelateerd aan de toediening van de medicamenten dopamine en glucocorticoïden, terwijl het gebruik van donorbloedproducten de SD scores van IGF-I en IGFBP-3 positief beïnvloedde. IGFBP-1 spiegels aan het einde van de open-hartoperatie waren gerelateerd aan de ernst van ziekte en niet meer aan de insuline spiegels, zoals aan het begin van de open-hartoperatie. Het gebruik van glucocorticoïden tijdens open-hartoperatie was gerelateerd aan hoge glucose spiegels. Vierentwintig uur na de open-hartoperatie, waren IGF-I, IGFBP-3 en IGFBP-1 spiegels weer teruggekeerd naar het niveau van aan het begin van de operatie.

Hoofdstuk 8 bespreekt de resultaten van de diverse studies in relatie met de literatuur, bediscussieerd de klinische implicaties, bespreekt de beperkingen en geeft suggesties voor toekomstig onderzoek.

DANKWOORD

Dit proefschrift beschrijft studies die onder bijzondere omstandigheden gedaan werden en kwam tot stand met de hulp van velen, die ik hierbij wil bedanken.

Allereerst wil ik alle kinderen en ouders bedanken. Jullie vormden tenslotte de basis van dit proefschrift. Ondanks de acute stress waarin jullie verkeerden, deden jullie belangeloos mee. Dankzij jullie medewerking zijn we weer een stap verder gekomen in de zorg voor acuut ernstig zieke kinderen. Dank jullie wel!

Mijn promotor, Prof.dr. A.C.S. Hokken-Koelega, beste Anita, dank voor de kans die je me gegeven hebt met dit project. Jouw enthousiasme voor de acute stress respons heb je al in het eerste telefoongesprek op mij overgedragen en het heeft mij sindsdien niet meer losgelaten. Ik bewonder je doorzettingsvermogen en gedrevenheid. Ondanks je volle agenda, maakte je altijd tijd vrij. Dank je wel!

Mijn copromotor, Dr. K.F.M. Joosten, beste Koen, dank je voor je uitstekende begeleiding, opbouwende adviezen en goede samenwerking. Ondanks dat ik geen voetbal fan ben, vormden we een goed team. Dank je wel!

Prof.dr. T.J. Visser en Prof.dr. F.H. de Jong, beste Theo en beste Frank, dank jullie voor het meedenken en de adviezen bij de artikelen; en samen met Prof.dr. D. Tibboel de snelle beoordeling van mijn manuscript.

Prof.dr. S.L.S. Drop, beste Sten, dank dat ik onderzoek heb mogen doen bij de subadeling Endocrinologie. Ook wil ik je bedanken voor je bereidheid plaats te nemen in de grote commissie.

Prof.dr. A.J.J.C. Bogers, dank u voor de mogelijkheid die u mij gaf onderzoek te doen op het Thorax centrum, uw kritische blik en uw bereidheid plaats te nemen in de grote commissie.

Dr. J.A. Hazelzet, beste Jan, dank je voor de samenwerking, kritische blik en je bereidheid plaats te nemen in de grote commissie. Je stond altijd paraat en was voor mij een belangrijk aanspreekpunt op de ICP.

Prof.dr. A.B.J. Groeneveld wil ik bedanken voor zijn bereidheid plaats te nemen in de grote commissie.

Alle artsen en verpleegkundigen van de ICP en de IC van het Thoraxcentrum: dank jullie voor jullie medewerking op die hele drukke momenten.

Dr. W.C.J. Hop, beste Wim, dank je voor al je adviezen. Je bent voor mij een stimulans geweest om de kneepjes van de statistiek te leren.

Dr. J. van Doorn, beste Jaap, dank je voor je kritische blik en de ervaring die ik bij je in Utrecht heb mogen opdoen. Werken in een laboratorium vergt ontzettende precisie en leert je dat laboratorium uitslagen meer zijn dan alleen maar getallen. Ook wil ik alle laboranten uit Utrecht danken voor hun hulp en de gezelligheid.

PhD L. Fryklund, dear Linda, thank you for stimulating advises and the continuous interest in this study. I thank Pfizer for their financial support.

Marianne Maliepaard, dank je voor alle research verpleegkundige hulp. Je was een steunpilaar voor de studie en een plezier om mee samen te werken. Veel succes in de psychologie. Ook Beatrix wil ik danken voor haar hulp in de beginfase.

Mijn collega's van de subafdeling endocrinologie: dank jullie voor de fijne jaren. Fellow-kamer, Endo-kamer, Madrid, Ljubljana en Bazel, huwlijken, BBQ, diners, shoppen en endo-... Met name wil ik Ellen, Marije, Dederieke, Annabelle, Daniëlle, Robert en Venje bedanken voor de weekenden die jullie voor mijn klaar stonden. Zonder jullie had ik dit onderzoek niet volgehouden! Venje we zijn binnenkort allebei klaar! Ellen en Marije, collega's, vriendinnen en nu mijn paranimfen!

Clementien, het was fijn om met jou samen te werken aan onze gemeenschappelijke studiepopulatie. De samenwerking verliep soepel en is mede dankzij je positieve instelling een succes geworden. Ook je collega´s, Edwin, Pieter, Gwenda, Marieke (E), Debby, Sander en Vishal, dank ik voor hun hulp tijdens de weekenden.

Bertine, Olivia, Heike en Fleur, dank jullie voor de hulp bij de groeihormooncurven en het schrijven.

Marloes, Leon, Jaqueline, Carine en alle collega (ex)onderzoekers: dank jullie voor de koffie, lunches, borrels en sinte-kerstdiners.

Caroline, Justine, Anne en Jolanda, lieve vriendinnen, binnenkort ben ik er weer.

Lieve Berry en Esther, mijn ouders, dank jullie voor jullie onvoorwaardelijke liefde, steun en geloof in mij. Lieve Berry en Marianne, dank jullie voor de hulp bij de acute stress om dit boekje op tijd bij de drukker te krijgen. Lieve Gijs, mijn broer en zakenwaarnemer, lieve Stefanie en kleine Thibault dank jullie voor alle steun. Merci.

Lieve Luc, Gerda, Eva,Tamino en Ramy, mijn Belgische familie, dank jullie voor jullie warmte.

Lieve Ramses, dank voor de tekeningen, je geduld, je humor en nog veel meer ...

CURRICULUM VITAE

Marieke den Brinker was born on April 13th, 1972 in Amsterdam. She passed her secondary school exam (VWO) at the Hervormd Lyceum Zuid in Amsterdam in 1990. In 1992, she passed her Propedeuse Economics at the University of Amsterdam. In 1992 and 1993 she traveled South-east Asia and Australia with a friend. In 1993 she started her medical training at the University of Antwerp, Belgium and passed her first year successfully. During the year she studied in Antwerp, she met her boyfriend, Ramses de Pauw. From 1994 onwards she continued her medical training at the University of Amsterdam. During her medical training she participated in several scientific research projects. She participated in the Amsterdam HIV Cohort at the International AIDS Therapy Evaluation Center (IATEC, head: Prof.dr. J.M Lange) in Amsterdam, which resulted in her first article on liver enzyme elevations during highly active anti-retroviral therapy published in 2000, and in a project on nausea during reading with magnifiers at the scientific institute for low vision and (re)habilitation (SILVUR) in Amsterdam (head: Dr. B.P.L.M. den Brinker). Besides this she taught physiology to medical students at the University of Amsterdam. After obtaining her medical degree in 2001 she started as a PhD student at the Department of Pediatric Endocrinology and the Department of Pediatric Intensive Care of the Erasmus MC – Sophia Children's Hospital in Rotterdam (supervised by Prof.dr. A.C.S. Hokken-Koelega), working on the research presented in this thesis. As a part of her PhD training she worked for two months on the laboratory of the Department of Pediatrics of the Wilhelmina Children's Hospital – University Medical Center Utrecht. In November 2005, she has started her clinical pediatric residency in training (GSO) at the University of Gent in Gent, Belgium (head: Prof.dr. D. Matthys) for which she is posted the first year in the Erasmus MC – Sophia Children's Hospital in Rotterdam.

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