The Endocrine and Metabolic Stress Responses in Critical Illness in Childhood

by

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Abstract

Critical illness in childhood is of immense clinical significance, since 1 : 1000 paediatric hospital admissions requires intensive care for a mean duration of 4 days with a mortality rate of 6.4%. The stress of critical illness induces a coordinated response in multiple organ systems, including the neuro-endocrine, metabolic and immune systems, in order to enhance the probability of survival. The aims of the present study were to describe the magnitude and nature of the metabolic and endocrine response (MER) over the first few days of critical illness in children, to relate it to illness severity, patient age and diagnosis. Interactions between different hormones and metabolites were examined with particular emphasis on the GH axis, lipid metabolism and adrenocortical responses.

60 children admitted to PICU were studied (31 with meningococcal sepsis). Blood levels of lactate, pyruvate, acetoacetate, β-hydroxybutyrate, free fatty acids, glucagon, insulin, GH, IGF-1, IGFBP-3, IGFBP-1, T_4, TSH, cortisol, aldosterone, renin and catecholamines were measured by microassays.

Collectively, all children showed evidence of endocrine stress, however there appeared to be little correlation between the MER at presentation and classical measurements of critical illness severity (PROM). Plasma lactate levels, however, correlated better with many of the metabolites and hormones. The MER may describe aspects of illness severity not addressed by the current scoring systems. Significant differences were observed between the different diagnostic categories and the MER at presentation. Plasma aldosterone levels were significantly lower in the patients with meningococcal disease. These were dissociated from appropriately elevated plasma renin levels. Hyperreninaemic hypoaldosteronism has not been reported previously in critically ill children. Plasma cortisol levels were elevated in the meningococcal sepsis category, they fell faster than in other critically ill children. Plasma cortisol levels were lower in the meningococcal patients with higher inotrope requirement. Plasma levels of aldosterone correlated with elements of the IGF-1 axis, again a feature not previously described. The retrospective assessment of initial design of the study and areas of future research are also addressed.

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List of abbreviations

ACTH	Adrenocorticotrophic hormone
ALS	acid labile subunit
ARDS	adult respiratory distress syndrome
AVP	Arginine vasopressin
CNS	central nervous system
CRF	corticotrophin-releasing factor
CV	coefficient of variation
DA	dopamine
DHEAS	dihydroepiandrosterone sulphate
GH	growth hormone
GMSPS	Glasgow Meningococcal Septicaemia Prognostic Score
5-HT	5 hydroxytryptamine
HPA	Hypothalamo-Pituitary-Adrenal Axis
IGF-1	insulin growth factor 1
IGFBP-1	insulin growth factor binding protein 1
IGFBP-3	insulin growth factor binding protein 3
IL –10	interleukin 10
NA	noradrenaline
NEFA	non-esterified free fatty acids
PGE_2	prostaglandin E_2
PICU	paediatric intensive care unit
PRISM	Paediatric Risk of Mortality score
PROM	Predicted Risk of Mortality
T_3	3,3',5-triiodothyronine
T_4	Thyroxine
TBG	Thyroid binding globulin
TNFα	Tumour necrosis factor-α
TRH	Thyroid releasing hormone
TSH	Thyroid stimulating hormone

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The Stress Response to Critical Illness in Childhood

Chapter 1 - The endocrine and metabolic effects of critical Illness in children.

1.1 Introduction

1.1.1 The clinical and socio-economic aspects of critical illness in childhood

Critical illness in childhood is of immense clinical and socio-economic significance in paediatric medicine since as much as 1 : 1000 admitted to hospital requires intensive care for a mean duration of 4 days. The mortality rate is 6.4%, and whilst little is known of the cumulative morbidity, the total financial cost amounts to more than £50 million each year in the U.K.(7). The basic understanding of the stress response to critical illness is of crucial importance in order to improve the management of children in intensive care and thus reduce the morbidity and mortality rates of critically ill children.

1.1.2 Stress, stress response and critical illness

Stress, a response to aversive stimuli, is a concept that is difficult to define because its interpretation tends to vary according to the various disciplines. The phenomenon of the stress-response to physical insult has long been recognised. John Hunter in 1749 in his "Treatise on Blood, Inflammation and Gunshot wounds" wrote: "...there is a circumstance attending accidental injury which does not belong to disease, namely that the injury done, has ... a tendency to produce both the disposition and the means of cure"(8).

Hans Selye, a pioneer in addressing general principles of physiology and pathophysiology in the exploration of stress, defined stress as "the non-specific response of the body to a demand" (9). He emphasized the role of

an integrated response of multisystems rather than isolated reflexes. Although virtually all organs are affected by exposure to a hostile environment, the neuroendocrine, cardiovascular, immune, and gastrointestinal systems are first to experience functional changes. These result in a series of coordinated responses organised to enhance the probability of survival. These coordinated responses are referred to as the "stress response". The integration of such orchestrated neuroendocrine – immune systems responses to critical illness, which alter the internal milieu are depicted in Fig. 1.1.

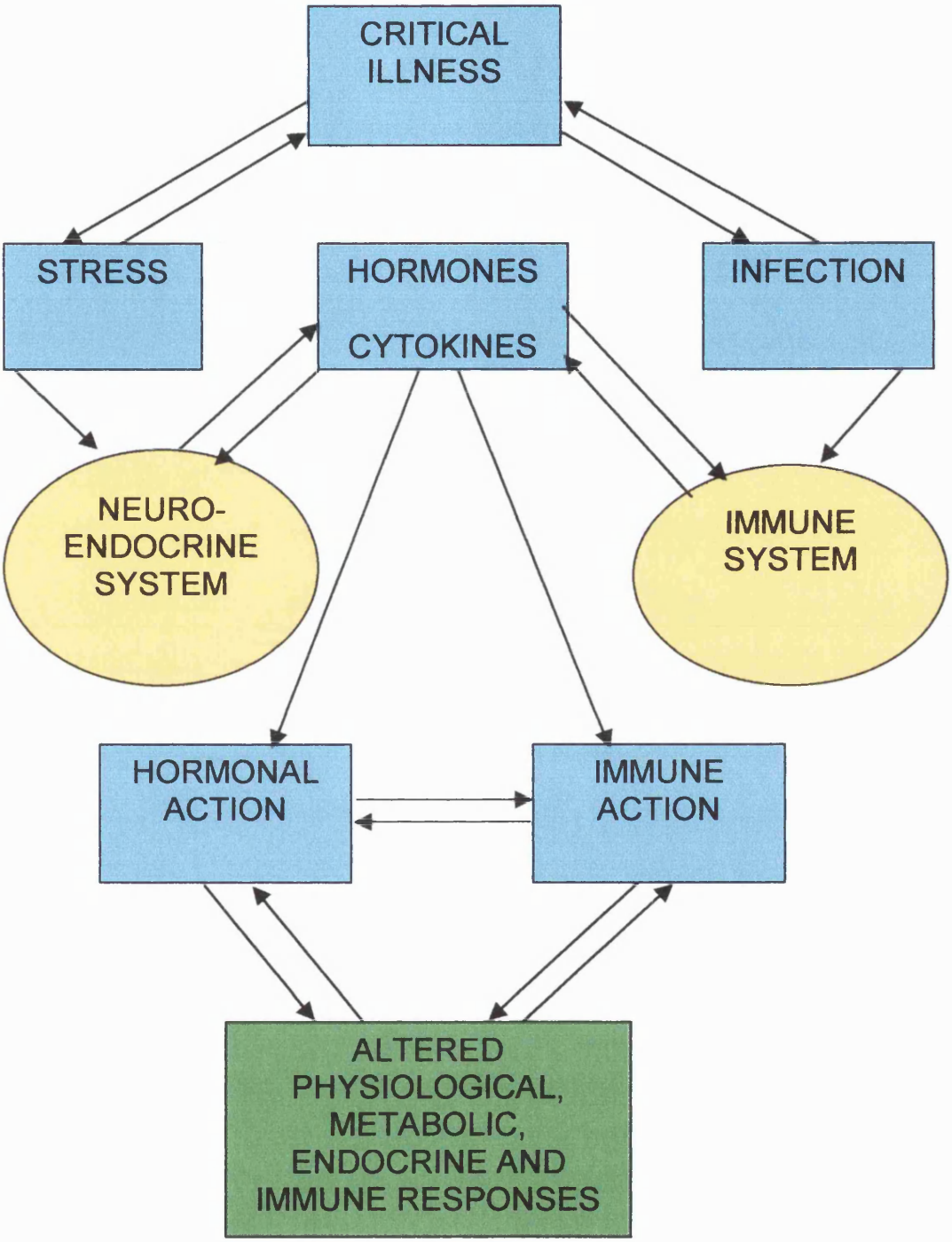


Fig. 1.1 The stress of critical illness and its effect on the neuroendocrine-immune systems can result in altered physiological, metabolic and immune responses.

1.1.3 Stress Response in Critical Illness – Beneficial or Harmful?

It seems likely that the acute phase of the stress response has evolved, subject to evolutionary pressures, to optimise survival in non-critical illness or injury. Profound metabolic and endocrine changes have been described in critically ill adults as part of the stress response. Modern intensive care is a new phenomenon, allowing patients to survive who would not have done so previously (2). Both the acute phase and chronic phase stress response in critical illness may represent human physiology reacting to circumstances which it has not evolved to deal with (10) and in that, this adaptive and beneficial response may turn into a deleterious one, due to its magnitude, duration and timing, complicating clinical management and increasing both patient morbidity and mortality.

Factors which adversely affect outcome include the age of the patient (11-13), the most vulnerable being the young and the elderly, the severity of the illness (14), the duration of the illness – especially prolonged critical illness (2), pre-existing conditions, such as catabolic diseases including chronic infections and cancer (15), and the use of some drugs, for example, dopamine (16), that are often used in the management of critically ill patients.

1.2 The stress response

The physiology or pathophysiology of the stress response needs first to be described, as the endocrine and metabolic changes may have prognostic significance, whether or not they have a causal role in outcome or are just markers for disease severity.
It is also necessary to understand the interactions of the different mediators of the stress response in order to design interventions to improve patient outcome.

The stress response may be divided into the afferent response to insult, which is until now the least well characterised, its central coordination and the efferent response. Afferent is taken to mean conducting inward to a part or organ, whilst efferent signifies a signal that is carried, conveyed away from the centre.

1.2.1 The afferent response to stress

The afferent limb of the stress response receives stimulae which include nociceptive afferents from the damaged areas, baro and volume receptor inputs responding to hypotension and hypovolaemia, stimulae from osmo, gluco, and hormone receptors responding to changes in the composition of blood (17). Pro inflammatory cytokines, particularly TNF-α, IL-1 and IL-6, which may be produced locally by macrophages and leucocytes are also thought to play a role in the afferent limb.
Neuronal afferent fibres play a pivotal role in the development of the stress response. Hume and Egdahl in 1959 demonstrated that division of afferent fibres suppressed the development of the adrenocortical response to hind-limb injury in rats (18). Blockage of afferent input by epidural anaesthesia was found to diminish the hormonal response to stress in adults (19;20).
Most sensory inputs pass through either the reticular activating system or the thalamus to the amygdala and sensory cortex, which then communicate

with the hippocampus. Brain circuits therefore play a central role in the coordination of the stress response.

1.2.2 The central coordination of the response to stress

Proof of central nervous system involvement in the stress response was provided by the work of Claude Bernard, who in 1849 (21) demonstrated that puncturing the 4th ventricle in dogs precipitated glycosuria. However, removal of the brain above the hypothalamus did not alter the ability of dogs to respond to operative stress, as measured by elevations in adrenocortical secretions (18). In humans however, input from higher centres may modulate the efferent arm of the stress response. Centrally acting high dose morphine anaesthesia has been shown to block cortisol and growth hormone response to surgical stress in humans (22).
Although the stimulae which activate the stress response are variable, the resultant stimulation of the endocrine and metabolic systems to the CNS response have been considered to represent a final common pathway to stress. Fig 1.2 depicts the brain circuits participating in the regulation of the neuroendocrine stress response.

Multiple brain structures are involved in the organization of responses to aversive or stressful stimulae. Among them are the hypothalamus, septo-hippocampal system, amygdala, cingulated and prefrontal cortices and parts of the brainstem (23). The release of corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) from the hypothalamus are considered to be an integral part of the final common pathway in the stress response. However it has become increasingly apparent that different types of stress are characterised by altered contributions of CRF and AVP and probably other factors, depending on the aetiology of the stressor (24). Therefore, in contrast to a non-specific response to stress, there exist specific biological pathways which are specific for different types of stressor.

Glucocorticoids play a key regulatory role in the neuroendocrine control of the hypothalamo-pituitary-adrenocortical axis and on the termination of the stress response by exerting negative feedback at the levels of the hypothalamus and pituitary (25), whilst activation of mineralocorticoid receptors in the hippocampus decrease the HPA axis activity (26). This results in a highly complex central coordinating system, which may be modified, attenuated or altered at many different levels, depending on the nature of the stressors, the ensuing pathophysiological responses, the genetic and physical status of the individual.

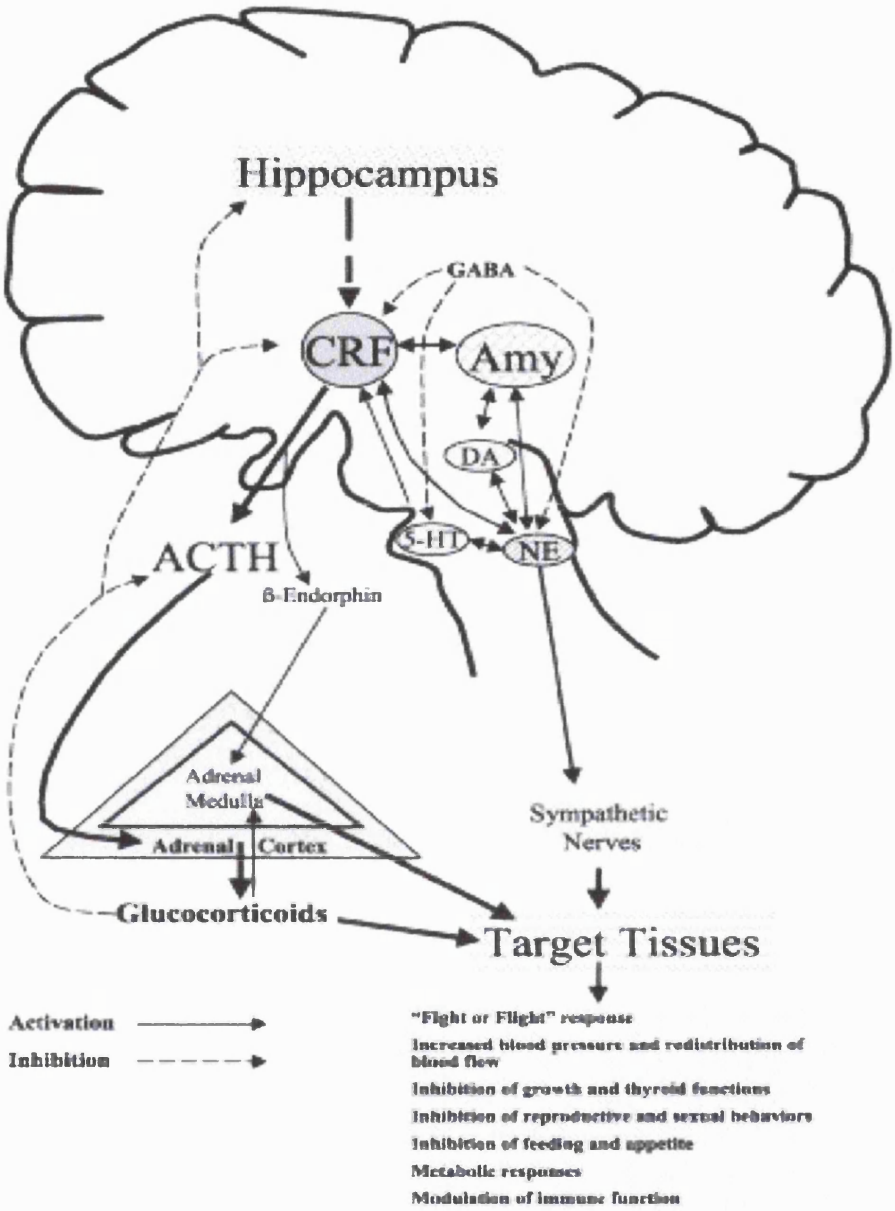


Fig. 1. 2 Brain circuits participating in the regulation of the neuroendocrine stress response (adapted from (1)).

CRF=corticotrophin-releasing factor in the hypothalamic paraventricular nucleus; 5-HT=serotonin in the dorsal raphe nucleus; NE=noradrenaline in the locus coeruleus; DA=dopamine in the mesolimbic system; Amy=amygdala; GABA=gamma-amino-butyric acid.

1.2.3 The efferent response to stress

Efferent pathways include both the sympathetic and parasympathetic nervous systems, which act directly on blood vessels redistributing blood flow and promote hormone release e.g. adrenaline from the adrenal medulla (27). Other efferent mediators include hormones and cytokines. The picture is however more complex than previously perceived.

The neuroendocrine response to stress is almost instantaneous, with increased catecholamine release from the sympathetic nervous system and adrenal medulla. This is followed by the release of CRF and vasopressin from the parvicellular neurons, release of oxytocin from the neural lobe of the pituitary and 5-10 seconds later by the secretion of pituitary ACTH (28). The release of anterior pituitary hormones prolactin and GH are increased as is renin release from the kidney (28). The glucocorticoid response follows approximately 30 minutes to an hour post onset of stressor (28;29).

Van den Berghe has subdivided the endocrine efferent stress response into 3 phases during the course of critical illness in adults; the acute phase, the chronic phase and the recovery phase as illustrated in Fig 1.3. In the acute phase, which encompasses the first hours to a few days after the onset of critical illness, the function of the anterior pituitary is essentially maintained or even amplified, whilst the anabolic target organ hormones are inactivated. Both cortisol and ACTH are elevated in the acute phase (2).

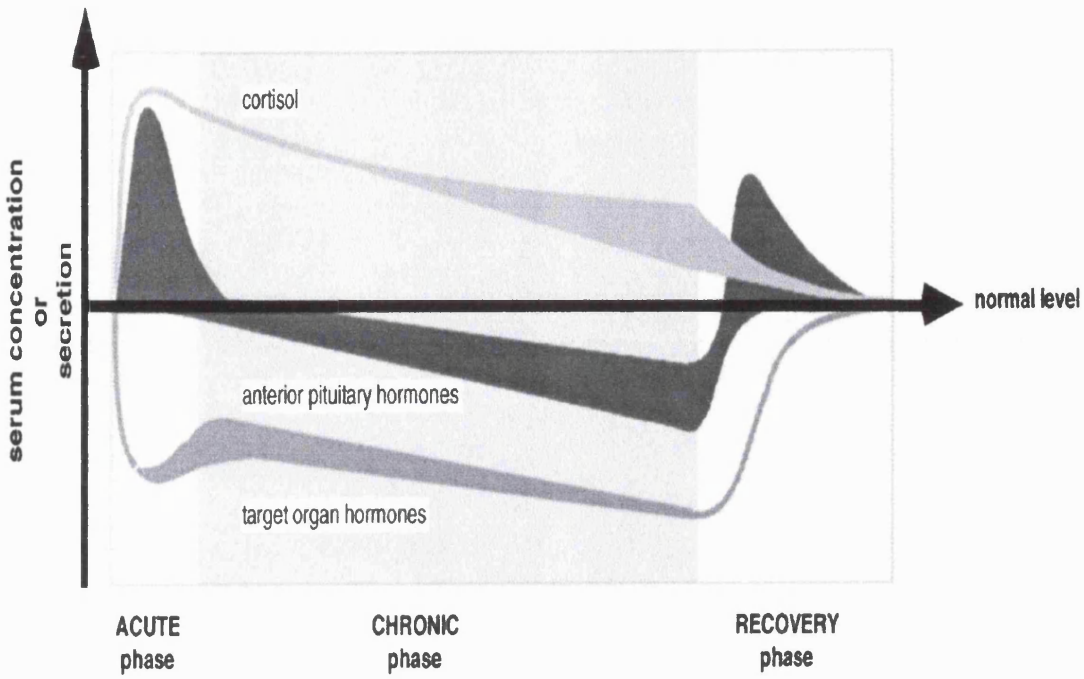


Fig 1.3 Simplified concept of the acute, chronic and recovery phases of critical illness, illustrating the pituitary dependant changes (adapted from (2)).

The acute changes are triggered by the release of the "stress" or "catabolic" hormones (30), with the emergence of insulin resistance (31), together with low IGF-1 and T3 levels (32-35). The net metabolic effect is to cause hyperglycaemia due to increased hepatic glucose output and reduced clearance because of insulin resistance (36). Fat becomes the major fuel with intense lipolysis and increased oxidation of fatty acids (37). There is accelerated protein breakdown and sodium and water retention (38-40).

Prolonged illness causes a wasting syndrome, where despite adequate nutrition, catabolism is not reversed.

In the chronic phase in adults, reduced secretion of anterior pituitary hormones occurs with reduced activity of target tissues. Cortisol secretion is a notable exception, increased secretion being maintained through a peripheral drive (2).

Of note is that critically ill adult non-survivors have increased circulating levels of immunosuppressive factors i.e. Interleukin–10 (IL-10) and prostaglandin E_2 (PGE_2). There is evidence of depressed cell-mediated immunity with lower total lymphocytes, decreased T helper and natural killer cells within 6 days of major injury (3;41) (Fig.1.4). These changes coincide with the chronic prolonged phase of critical illness.

These changes are reversed during the third phase, namely the recovery phase, during which time the sensitivity of the anterior pituitary is restored to normal feedback control (2).

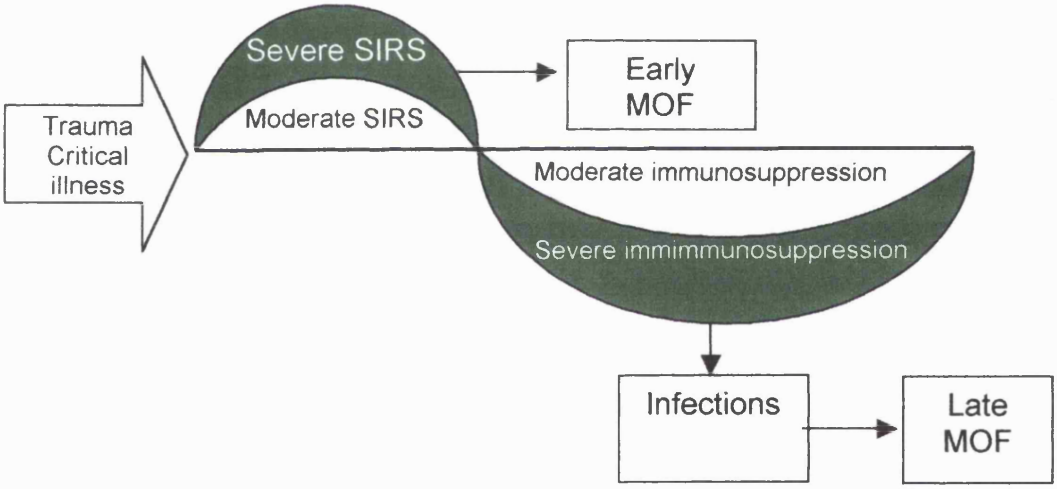


Figure 1.4 Inflammatory response to major trauma (adapted from (3)). SIRS= systemic inflammatory reponse syndrome, MOF= multiorgan failure.

1.3 Changes in metabolic and endocrine axes in stress

1.3.1 Carbohydrate metabolism

Patients with critical illness show high raised glucose turnover, as demonstrated using stable isotope techniques (42). Increased hepatic glucose output occurs due to a combination of glycogenolysis and increased gluconeogenesis. Peripherally there is increased lactate production but also an increase in glucose uptake, occurring independently of insulin (43) and increased utilization. Plasma insulin levels tend to rise in response to illness or injury (31;44), but are themselves ineffective in lowering the elevated blood glucose concentration, as the critically ill patients acquire insulin resistance. The insulin to glucose ratio over this time-frame becomes inappropriately high (45). Plasma insulin levels gradually rise over the first few days after insult, in part reflecting the deregulation of adrenergic inhibition of insulin secretion. An exaggerated insulin response has been documented to glucose infusion (46). Elevated levels of blood glucose should lower basal hepatic glucose output through a negative feedback mechanism. However, this does not occur in critical illness. Clinically, exogenous infusion of dextrose/glucose may cause a rise in plasma glucose in critically ill individuals, due to the degree of hepatic insulin resistance despite maximal endogenous insulin release (47). This is supported by the data from Wolfe 1985, who demonstrated only partial suppression of hepatic glucose output on exogenous glucose infusion in cases of surgical trauma or infection (48).

Peripheral glucose uptake is also increased, reflecting the increased glucose turnover, however this is not dependent on insulin (43). Non-insulin mediated glucose uptake occurs down concentration gradients with the aid of glucose transporters eg. GLUT-1. This uptake is probably influenced by cytokines, where TNF-α has been shown to increase in vivo glucose utilization in macrophage rich tissues (49).

Table 1.1 gives a summary of the changes in carbohydrate metabolism during critical illness.

Table 1.1 Summary of the changes in carbohydrate metabolism during critical illness

Gluconeogenesis[1]	↑
Lactate production	↑
Glycogen synthesis	↓
Peripheral glucose uptake	↑
Peripheral glucose utilization	↑
Glucose intolerance	↑
Insulin resistance	↑

[1]In severe sepsis, initial hyperglycaemia during which gluconeogenesis is increased is often followed by suppressed gluconeogenesis and hypoglycaemia

1.3.1.1 Lactate

Blood lactate levels tend to rise in critical illness, unless hepatic clearance and recycling overcomes increased peripheral delivery. Increased lactate production, as demonstrated by kinetic studies (47), is thought to occur due to poor tissue perfusion and an increase in anaerobic glycolysis. Tissue hypoxaemia may also clinically be reflected in patients exhibiting metabolic acidosis or low pO_2. However, blood lactate/pyruvate ratios tend to remain unaltered in critical illness (50), so this must reflect rising glycolytic activity. Fig 1.5 shows lactate cycling within the Cori cycle.

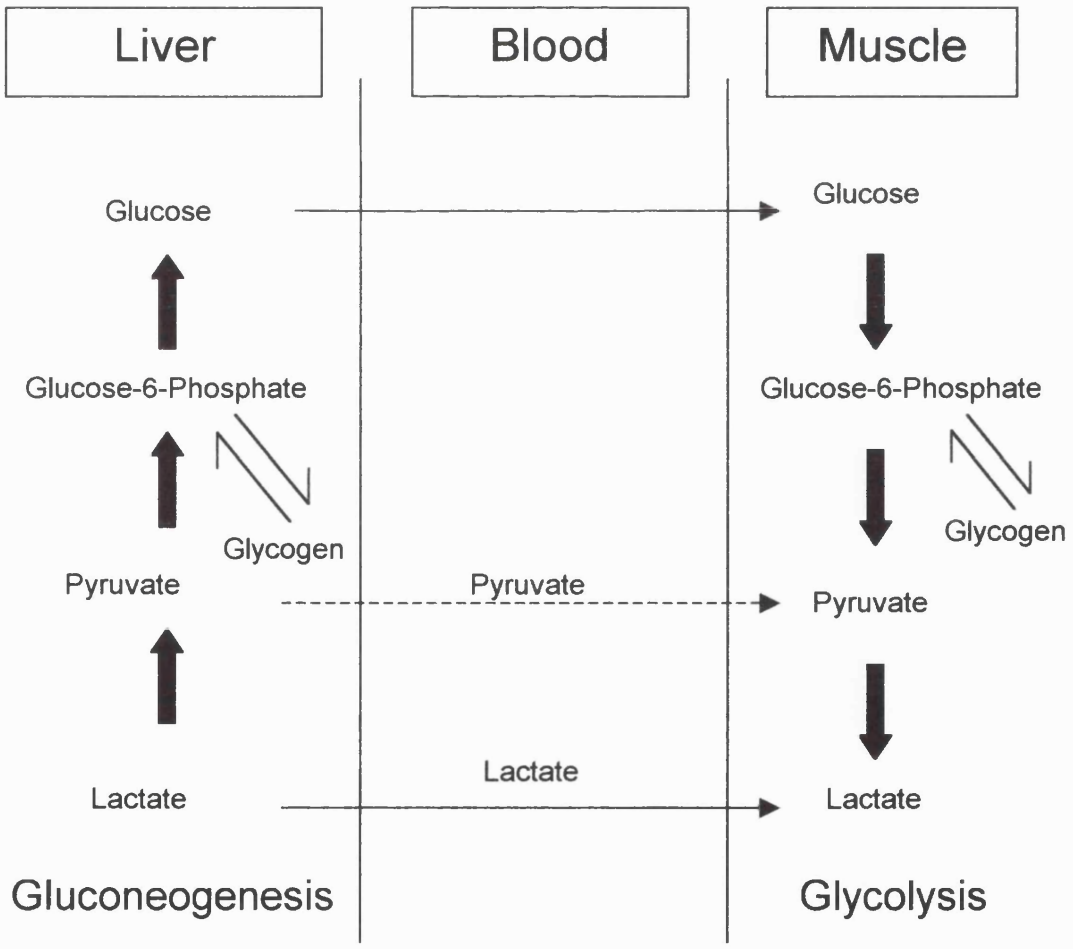


Figure 1.5 The Cori cycle.

1.3.2 Fat metabolism

An overview of lipid metabolism in the whole body is depicted in Fig 1.6. The net output of non-esterified free fatty acids (NEFA) from adipose tissue represents the balance between lipolysis or breakdown of triacylglycerols and the esterification of NEFA. Lipolysis, as measured by the rate of appearance of glycerol in the plasma, is markedly increased following trauma (37). There appears to be discrepancy in findings since several studies on burns and trauma cases have reported relatively normal or mild elevation in plasma NEFA concentration. However, increased rates of NEFA/triglyceride cycling have been shown to account for this (51;52). Both NEFA and glycerol levels do not correlate with injury severity, although higher levels are observed in patients with moderate, as opposed to minor injuries.

The main stimulus to hormone sensitive lipase are the catecholamines. Insulin in contrast is the most important inhibitor (53). In vitro studies have suggested that both glucagon and ACTH may also stimulate lipolysis to a certain degree (53), although this has not been substantiated in vivo.

As lipolysis increases glycerol levels rise. Glycerol clearance depends on its conversion to glucose within the liver and in healthy subjects, it usually constitutes 2% of the substrate for glucose formation. In septic patients this may rise to as much as 20% (54).

NEFA are converted into acetyl CoA in hepatic mitochondria, from which acetoacetate and other ketone bodies are formed and released into the circulation. Ketone bodies become an important fuel for the CNS during fasting (55).

There is significant correlation between plasma ketone body and NEFA concentrations (56).

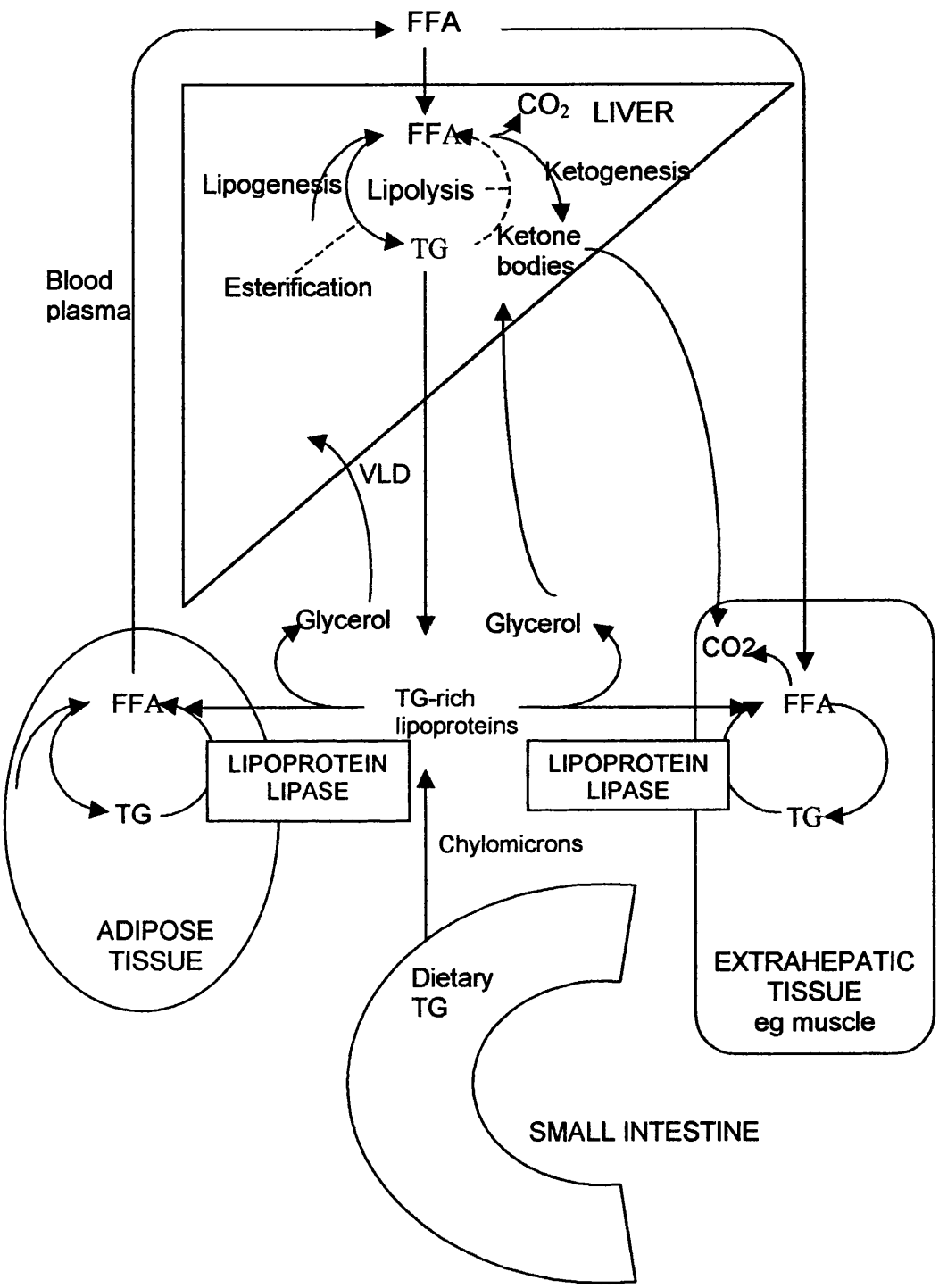
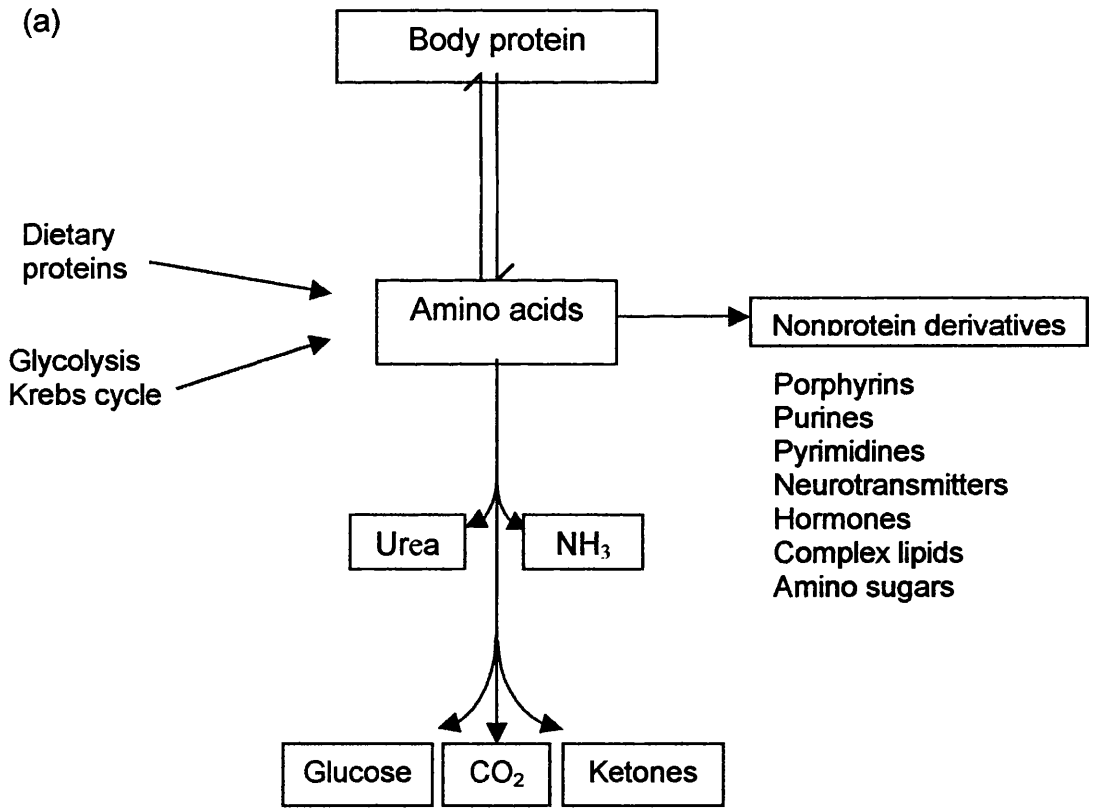


Figure 1.6 Overview of lipid metabolism in the whole body. (VLDL= very low density lipoproteins; FFA= free fatty acids; TG= triacylglycerol.)

1.3.3 Protein Metabolism

In a healthy human adult, normal protein turnover amounts to 1-2% of total body protein per day. This protein turnover results predominantly from degradation of muscle proteins to amino acids. However, approximately 75-80% of the released amino acids are re-utilized for new protein synthesis. The remainder are metabolised into nitrogenous waste and glucose, ketones and/or carbon dioxide (Fig 1.7).

Figure 1.7 (a) Overall sources and utilisation of amino acids.



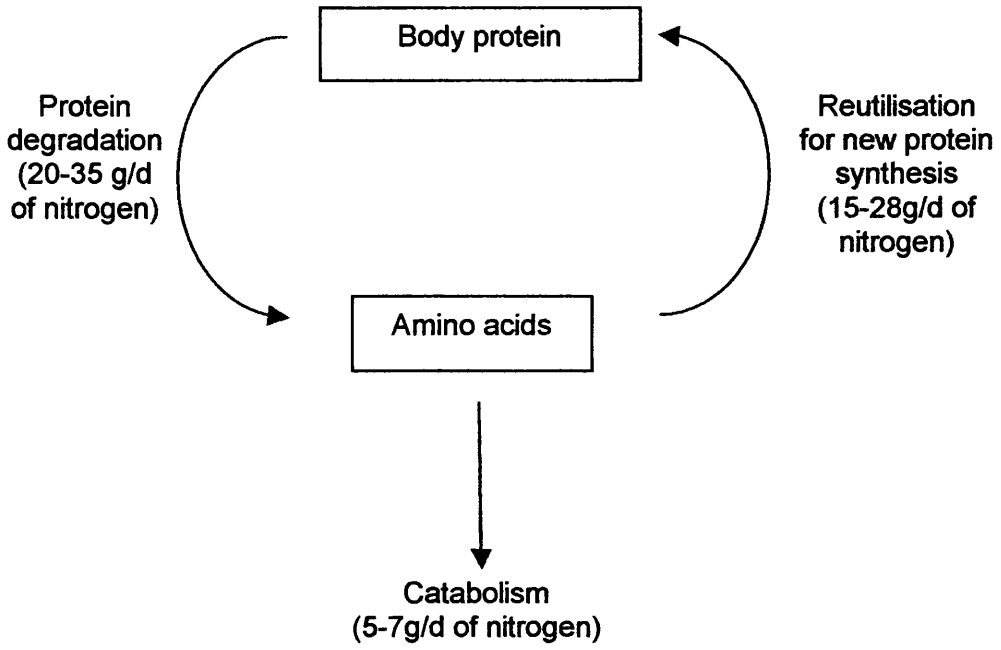
In the acute phase post physical insult a large increase in urinary nitrogen excretion has been observed (17;57), reflecting the increased mobilization, release and breakdown of amino acids from skeletal muscle to meet increased demands for fuel and wound repair. Protein turnover studies have reflected increases in both protein synthesis and breakdown with the net balance in favour of protein degradation (38-40).

Hormonal mediators of catabolism include cortisol, glucagon and the catecholamines (36). Insulin fails to exert its expected anabolic effect on protein turnover. Frayn demonstrated a striking positive relationship between insulin concentration and nitrogen excretion at various times after injury (17). Purified human IL-1 has been shown to have a direct proteolysis stimulating effect on muscle in vitro (58) and TNF-α has been implicated in the pathogenesis of cachexia, promoting catabolism of visceral tissue and skeletal muscle (59). Direct attempts to investigate the role of IL-1 in man on protein metabolism, by stimulating its production by means of etiocholanolone injection have shown little effect (60). However, on combination with couterregulatory hormone infusion, many of the responses to injury were simulated (17;61). Both morbidity and mortality in critically ill children and adults have been related to the severity of the stress response and the magnitude of loss of body protein (62).

In prolonged critical illness a non-specific wasting syndrome has been described, where despite feeding protein continues to be lost from vital organs and tissues, reflecting a continuing increase in protein degradation and a decrease in synthesis (2) leading to a severe loss of body mass. Clinically, this results in muscle atrophy, leading to increasing weakness and prolonging ventilator dependence (63). Intestinal mucosal atrophy occurs, and along with disturbed gastrointestinal motility prolongs the need for parenteral nutrition. Delay in tissue repair and immune dysfunction jeopardise the healing process and contribute to continuing dependency on intensive care support (64).

Figure 1.7 (b) Quantitative relationships for protein and amino acid turnover.

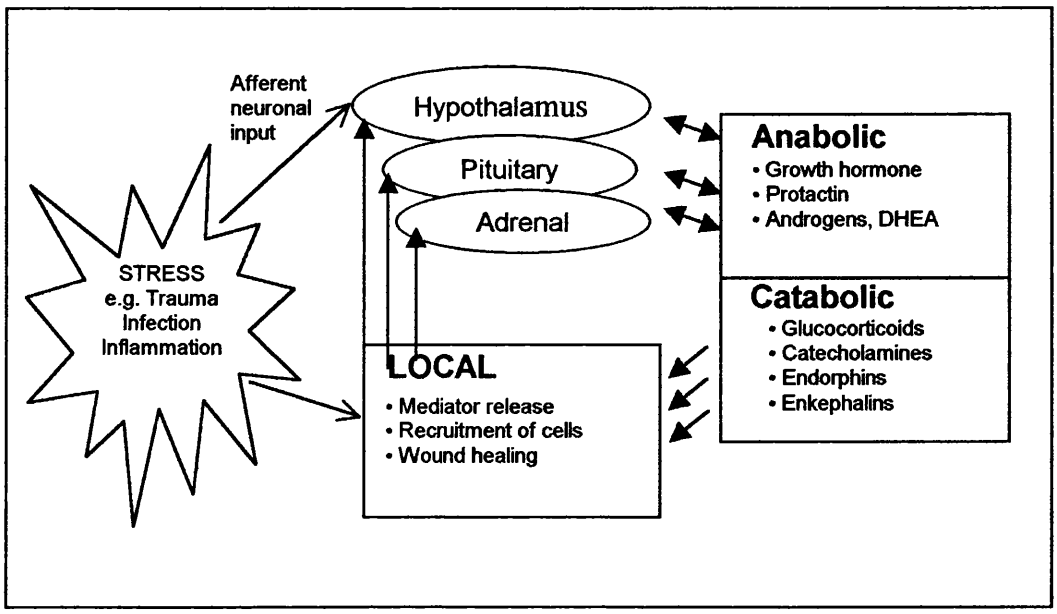
(b)



1.3.4 Mediators of Metabolic Change in Critical Illness

Both acute and chronic phases of critical illness are typically associated with a metabolic shift towards catabolism. The hallmarks of catabolic reactions involved in the stress response to critical illness include protein loss and negative nitrogen balance, increased glycogenolysis and gluconeogenesis (elevated blood glucose, lactate and alanine levels) and accelerated lipolysis (elevated free fatty acids). Catabolic changes in the energy balance after body insult and stress are the result of a cascade of different reactions. During the course of critical illness, the production and output of catabolic mediators predominate for various lengths of time. Cortisol is a key catabolic factor and has general effects on tissue metabolism. Along with cortisol, catecholamines are decisive catabolic players and are produced as a result of the stimulation of the adrenal medulla and from the activated sympatheic nervous system. In concert with cortisol (and glucagon) catecholamines stimulate hepatic glycogenolysis and gluconeogenesis, as well as lipolysis. Sequelae of the whole body to stress are depicted in Fig 1.8 a&b and illustrate the integrated actions of hormones as mediators of metabolic changes in the major organs.

Fig 1.8 a) Sequelae of the whole body to stress.



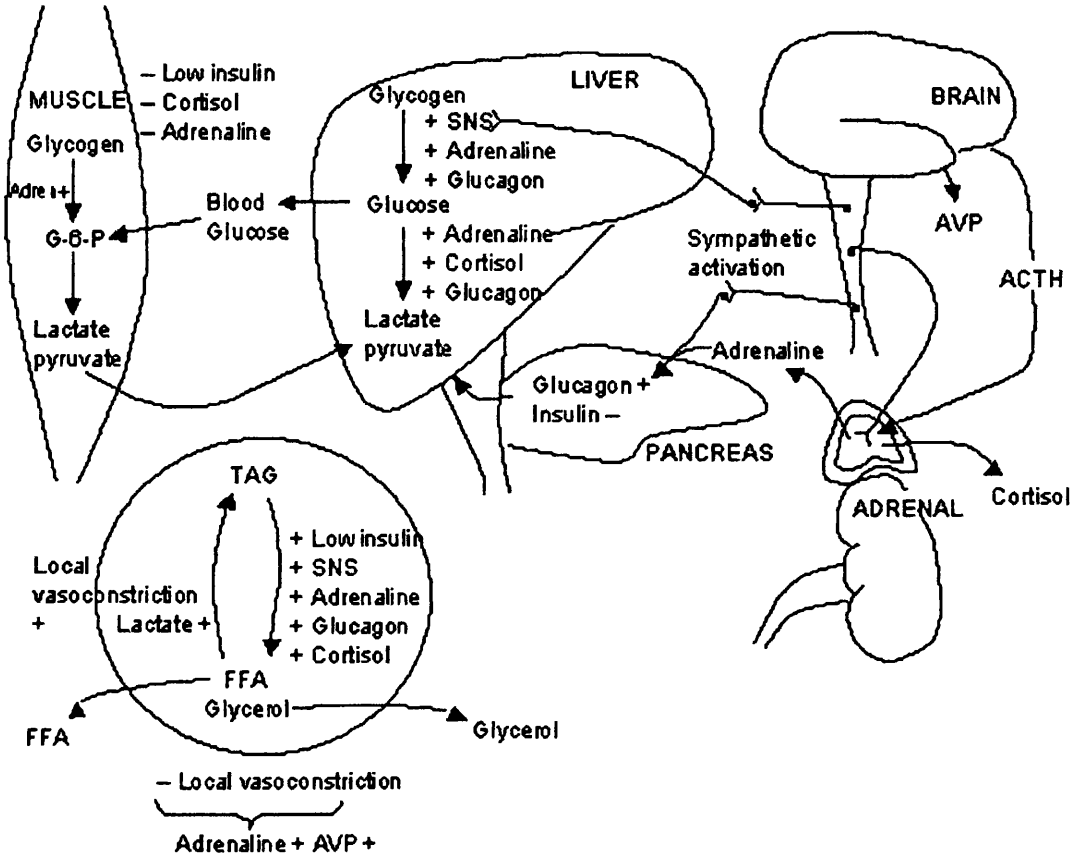


Fig. 1.8 b) Central nervous system control of metabolism in response to stress. Primary events ate the activation of the sympathetic nervous system and release of adrenaline from the adrenal medulla. The pituitary releases AVP and ACTH. Sympahoadrenal activation acts on the pancreas, promoting glucagon and inhibiting insulin release. ACTH promotes cortisol release. As a result, liver and muscle glycogenolysis and hepatic gluconeogenesis are stimulated, resulting in increased glucose release. Glucose uptake into the muscle not increased due to the action of cortisol, adrenaline and low insulin. Lipolysis is stimulated in adipose tissue as insulin release is relatively low. FFA (free fatty acid) release however is limited to a degree due to local vasoconstriction (adrenaline and AVP). Elevated tissue lactate will also promote reesterification.

The relative contribution from the sympathetic nervous system activity and circulating adrenaline may be difficult to separate and assess, the overall result being a composite pool of catecholamines acting directly on tissues involved, through indirect actions on other hormones and by altering local blood flow.

Catecholamine levels are known to rise rapidly and reach maximum levels shortly after the insult, be it trauma (65) or myocardial infarction (66) and return to the normal range within the first 24 hours (17;27), maximum levels correlating with insult severity (14). Moreover, plasma catecholamine levels achieved are above threshold for inducing metabolic changes (67). These include hepatic glycogenolysis and gluconeogenesis, stimulation of lipolysis and indirectly diminishing peripheral glucose uptake (36). However, more importantly, both circulating adrenaline and sympathetic activity inhibit insulin release and stimulate glucagon release through a β-effect by action on the pancreas (68).

Glucagon levels are also known to rise rapidly within 12 hours of insult e.g. trauma, surgery (14), thereafter returning to normal. Its main effects are on the liver, stimulating gluconeogenesis, ketogenesis (69) and promoting amino acid uptake.

Experimental evidence documenting the importance of glucagon in stressed humans was provided by Jahoor, where somatostatin was infused into patients with burn injury, thereby blocking their endogenous insulin, glucagon and growth hormone release (70). The insulin was then replaced by infusion. These manipulations revealed that hepatic glucose production rates fell in the patients with burn injury compared with that in the healthy controls.

It must be emphasisied, however, that the counterregulatory hormones tend to act synergistically, and experimental models show that combined infusion of glucagon, cortisol and adrenaline for 72 hours in normal subjects produce a greater rise in blood glucose, elevation in basal metabolic rate and negative nitrogen balance as compared to small or negligible effects when the hormones are infused in isolation (71).

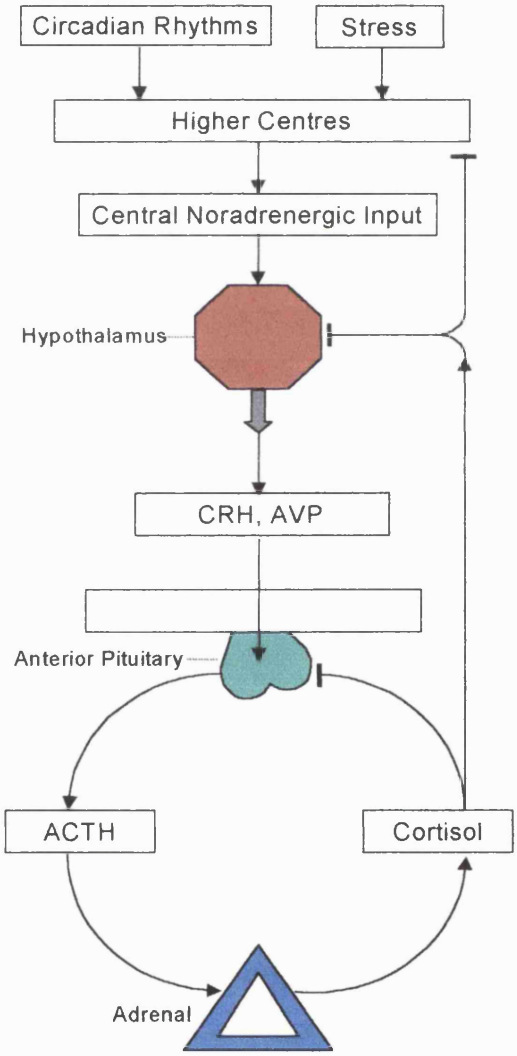
However, in the clinical scenario, counterregulatory hormones often begin to fall before the metabolic changes have peaked (72), which suggests that other pathways may exist which contribute to the metabolic response to acute insult.

Other mediators of metabolic changes in stressed individuals include cytokines and bacterial endotoxin, which may in itself exert a direct effect or indirectly alter counterregulatory hormone and cytokine release and influence organ perfusion. The degree of the contribution to, the metabolic and endocrine stress response requires further elucidation.

1.3.5 The Hypothalamo-Pituitary-Adrenal (HPA) Axis

The schematic representation of the neuroendocrine regulation of the HPA axis is given in Fig 1.9.

Figure 1.9 Neuroendocrine regulation of the hypothalamic-pituitary-adrenal (HPA) axis in humans. (CRH=corticotrophin releasing hormone, AVP= arginine vasopressin, ACTH=adrenocorticoprophic hormone).



A functioning hypothalamo-pituitary-adrenal axis is essential for survival. The absence of an appropriate rise in cortisol on stress, results in rapid decompensation and carries a high mortality (73).

Acute stress evokes major activation of the HPA axis, with elevations in both plasma ACTH and cortisol, which in turn is driven by hypothalamic secretagogues CRF and vasopressin. These are further influenced by cytokines and other secretagogues (74;75). Catecholamines are also implicated in the HPA stimulation (76).

Plasma levels of cortisol have been found to correlate with severity of insult, where magnitude of cortisol rise relates to the extent of myocardial infarction, as defined by cardiac enzyme changes (77) and the duration of elevation of cortisol relating to severity of the surgery (78). A positive patient outcome is related to a lower basal plasma cortisol and a fully preserved adrenal reserve on administration of ACTH (79). The normal circaidian pattern to cortisol secretion is disturbed, being either lost (80), or reset (81).

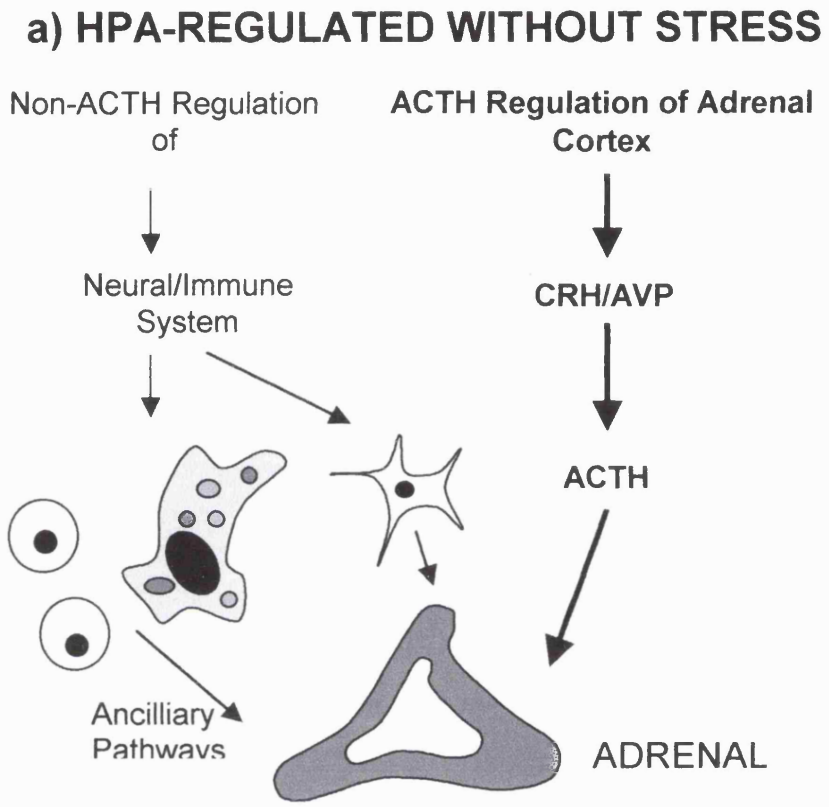
Hypercortisolism of the acutely stressed individual influences metabolism, stimulating lipolysis, protein catabolism and facilitating gluconeogenesis, liberating substrate for energy metabolism. Hypercortisolism also diminishes the activated immune response, thus preventing 'overshoot' in the initial phases of the stress response. Conversely, cytokines can influence peripherally the response to glucocorticoids by upregulating the reductase activity of the steroid shuttle (82).

A biphasic pattern is displayed by the HPA axis if critical illness becomes prolonged, where ACTH levels fall, but cortisol levels usually remain elevated, indicating an alternative pathway of activation, possibly involving endothelin (80;83) (Fig. 1.10).

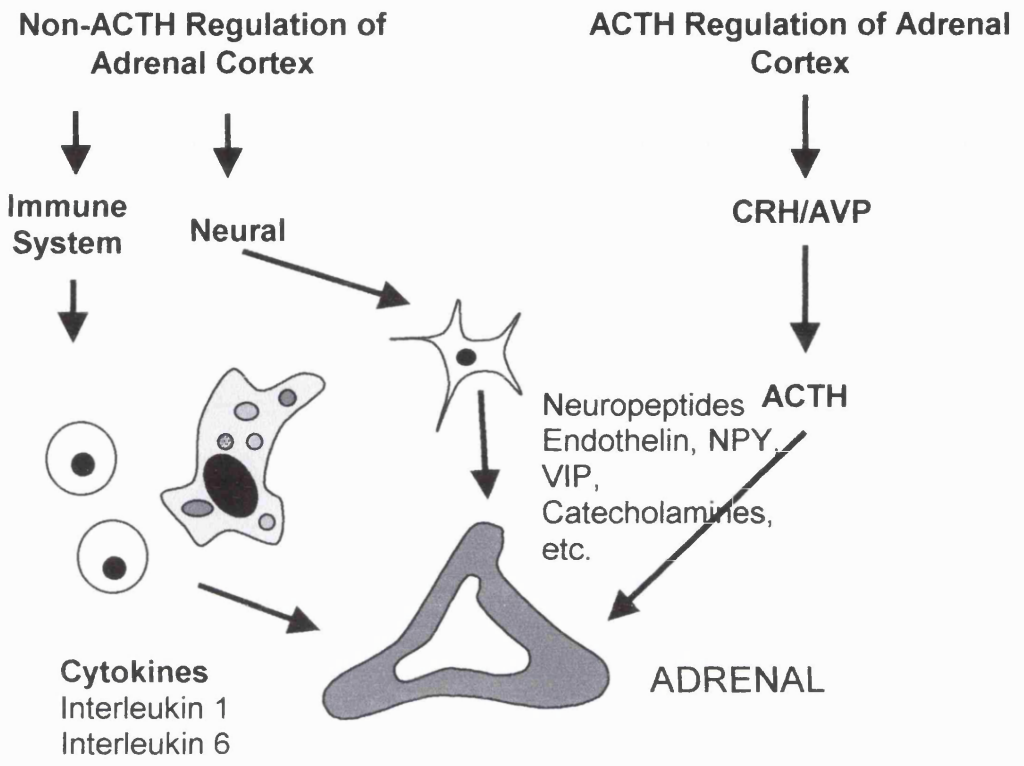
Fig 1.10 a) HPA regulation without stress.

b) HPA regulation in stress and critical illness.

(adapted from (4)).



b) HPA-REGULATION IN STRESS AND CRITICAL ILLNESS.



In the acute phase of the stress response the rise in plasma aldosterone is thought to be due to the activation of the renin-angiotensin system (23). In prolonged illness, however, aldosterone levels fall, despite the persistence of elevated renin (84). Levels of dihydroepiandrosterone sulphate also fall. It has been suggested that intraadrenal pregnenalone is diverted away from mineralocorticoid and androgen synthesis in order to preserve glucocorticoid output (84;85).

The subject of cortisol and aldosterone biosynthesis, secretion and metabolic effects have been described in detail in the introduction to chapter 8.

1.3.6 The Growth Hormone (GH) / IGF Axis

Fig 1.11 depicts the feedback system regulating GH release in the hypothalamus, pituitary and peripheral organs.

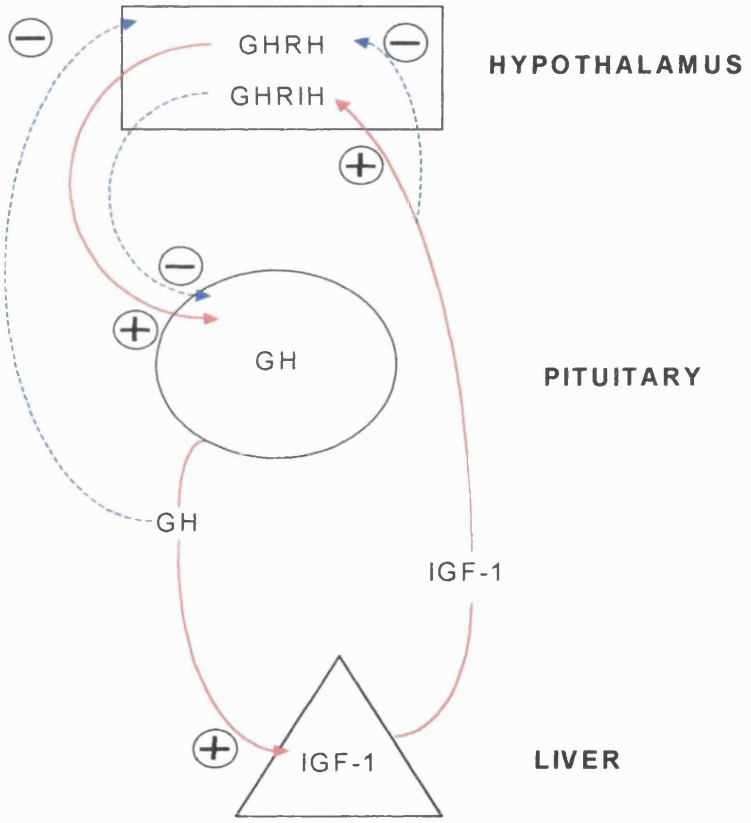
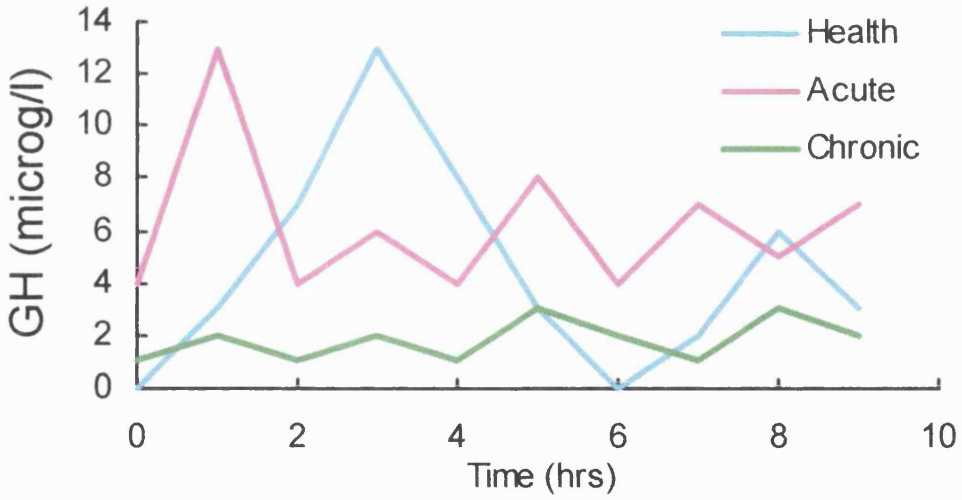


Figure 1.11 Feedback system regulating growth hormone release. (GH= growth hormone, IGF-1= insulin growth factor-1, GHRH= growth hormone hormone releasing hormone, GHRIH= Growth hormone releasing inhibitory hormone).

GH secretion in health is pulsatile, with the frequency and amplitude changing with age (24). The concentrations at the peaks determine growth velocity in children, the concentrations at the troughs being important for its metabolic actions (86). Thus, dynamic measurements are necessary to interpret changes in GH secretion. The question of GH pulsatility in critical illness has been addressed by the studies by De Zegher and Van de Berghe where they describe a reduced pulse amplitude but elevated interpulse levels of GH during the acute phase of critical illness in adults (5) (Fig. 1.12).

Fig. 1.12 GH pulsatility in health, the acute phase of critical illness and the chronic phase of critical illness in adults (adapted from (5)).



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In the first few days of illness, despite an increase in the GH interpulse level, there is a decrease in plasma IGF-1 (87) and IGFBP-3 concentration. Maiter et al have shown that pulsatile GH delivery has a greater effect on IGF-1 gene expression than continuous infusion (88), which may in part explain the low plasma IGF-1 levels found in critical illness.

Increased activity of the specific IGFBP-3 protease alters the balance of growth peptides, resulting in increased dissociation of IGF-1 from the ternary complex and shortening the IGF-1 plasma half-life (33). The profile is, therefore, one of acquired GH resistance.

The indirect anabolic actions of GH are reduced, while raised basal plasma GH concentrations promote increased lipolysis and insulin antagonism in order to provide metabolic fuels at the expense of muscle protein loss (89). In the chronic phase pulsatile GH secretion is low and this correlates positively with reduced activity of target tissues (90).

Drugs given in ITU also affect GH secretion. Thus, dopamine suppresses GH pulsatility still further (16), which may aggravate catabolism.

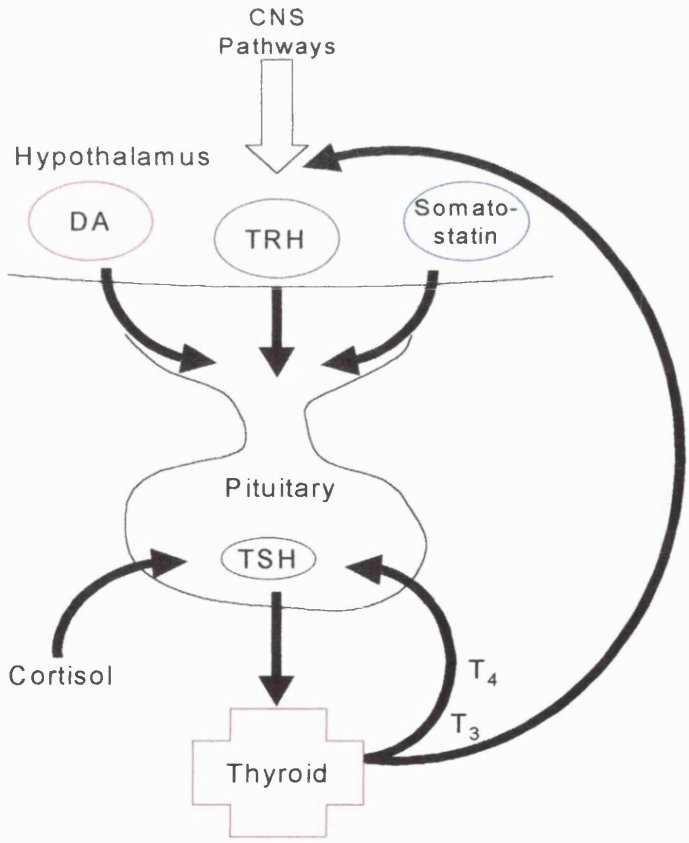
In paediatric intensive care dopamine infusion aggrevates partial hypopituitarism, in neonates suppressing prolactin, GH and TRH secretion, but in children suppressing only prolactin and TRH (91). This is an age related effect of stress.

In prolonged illness the reduced GH pulsatility positively correlates with low circulating levels of IGF-1, and IGFBP-3. However, on giving the GH secretagogues such as GHRH to critically ill adults restores GH pulsatility, and increases IGF-1, IGFBP-3 (90;92), albeit for the short duration of the infusion. This suggests that it is the hypothalamic control of the pituitary which is depressed.

1.3.7 Thyroid Axis

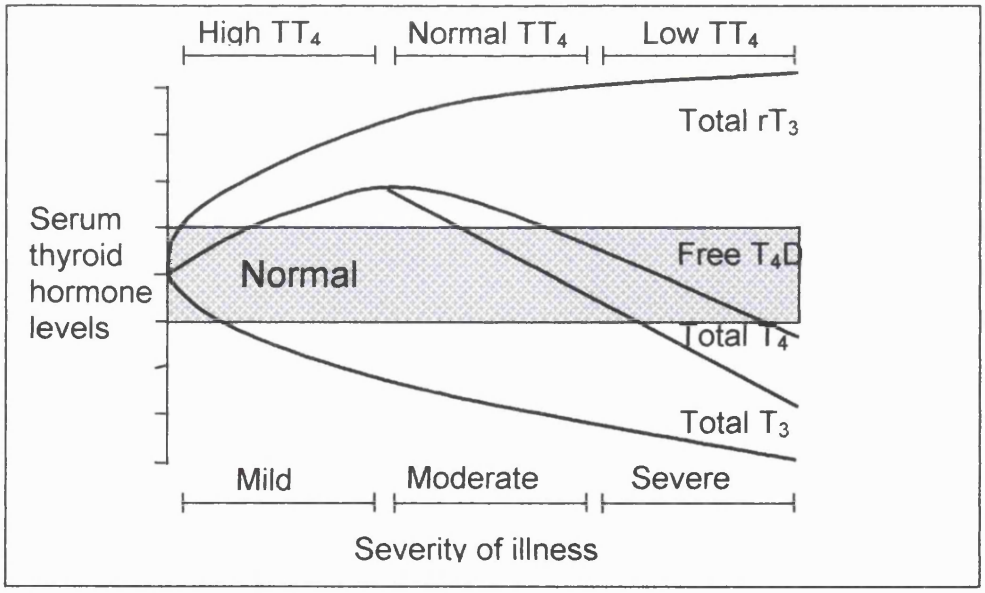
Feedback systems tend to control and regulate the hormonal axes. A pictorial representation of the feedback regulation and interactions of the major humoral mediators of TSH secretion is given in Fig 1.13.

Figure 1.13 Interactions of the major humoral mediators of thyroid-stimulating hormone (TSH) secretion. CNS= central nervous system, DA= dopamine, TRH= thyroid releasing hormone, TSH= thyroid stimulating hormone).



The "sick euthyroid syndrome" is a well described phenomenon, where during non-thyroidal illness, but also caloric deprivation, serum concentrations of thyroid hormones are often abnormal. The most consistent change being low serum 3,3',5-triiodothyronine (T_3) level that progressively decreases with increasing severity of disease (Fig 1.7). Low T_4 levels are related to high mortality (93). Other changes include alterations in serum binding and in thyroid hormone kinetics. The sequelae of changes in serum thyroid hormone levels in relation to the severity of illness is depicted in Fig 1.14.

Fig. 1.14 Schematic representation of the continuum of changes in serum thyroid hormone levels in patients with non-thyroidal disorders relative to the severity of illness (from (6)).



In the acute phase following insult or injury, target organ hormones are low. The decline in plasma T_4 and T_3 levels becomes apparent within 6-12 hours of injury and continue to fall, reaching minimal levels within 4 days (94).
Thyroidal T_3 production is normal, yet extrathyroidal generation of T_3 from T_4 is decreased, both due to decreased T_4 availability for conversion and decreased conversion per se. In a group of critically ill patients, Kaptein et al described that the transfer of serum T_4 to tissues was inhibited by 50% and plasma T_3 production rate was decreased by 75% (95). Serum rT_3 levels rise due to decreased hepatic clearance, although the production rate (extrathyroidal) does not change in critical illness (96). Responsiveness to T_3 may also be modulated, since in animal models, hepatic nuclear T_3 receptors appear to decrease in number and in occupancy (11;97).

Lower binding of T_4 to thyroid binding globulin (TBG) has been documented in non-thyroidal illness (98). This is thought to be due to the rise in desialylated TBG, which has a lower affinity for T_4 (99). Its presence has been documented in non- thyroidal illness, as it migrates slowly on electrophoretic gel (100). No evidence for the presence of inhibitors to TBG binding T_4 has been found in non-thyroidal illness (99).
The absence of elevation in TSH in response to low circulating T_3 levels suggests that there exists an altered feedback setting at the hypothalamopituitary level (101). Reduced TRH gene expression and enhanced nuclear T_3 receptor occupancy within the thyrotrophs has been documented in the rat model (102).
In prolonged critical illness pulsatile TSH release is decreased and T_3 levels remain low. Dopamine infusion, used clinically in the ICU setting, may further suppress TSH release from the pituitary (103). A rising plasma TSH level is a marker of recovery (90).

1.3.8 Immune Axis

It is now well documented that the neuroendocrine network strongly interacts with the immune system. Neuroendocrinoimmunology is a fast growing field of research, which appears increasingly relevant in the stress response. It is known that the immune system produces peptide hormones and expresses peptide receptors that are classically described in the hypothalamo-pituitary axis (Table 1.2).

Table 1.2 Immune cells synthesise and secrete pituitary hormones and neurotransmitters in stress and inflammation

Cell type	Hormone, neurotransmitter
T-lymphocytes, thymocytes	ACTH, endorphins, enkephalins, LH, TSH, growth hormone (and IGF-1), prolactin, somatostatin
B-lymphocytes	ACTH, endorphins, FSH, growth hormone (and IGF-1)
Monocytes, macrophages	ACTH, endorphins, growth hormone (and IGF-1)

ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone; IGF-1,

Conversely, the neuroendocrine system also produces cytokines and expresses cytokine receptors. Fig 1.15 illustrates the putative mechanisms by which proinflammatory cytokines released from activated immune/ inflammatory cells stimulate the HPA axis.

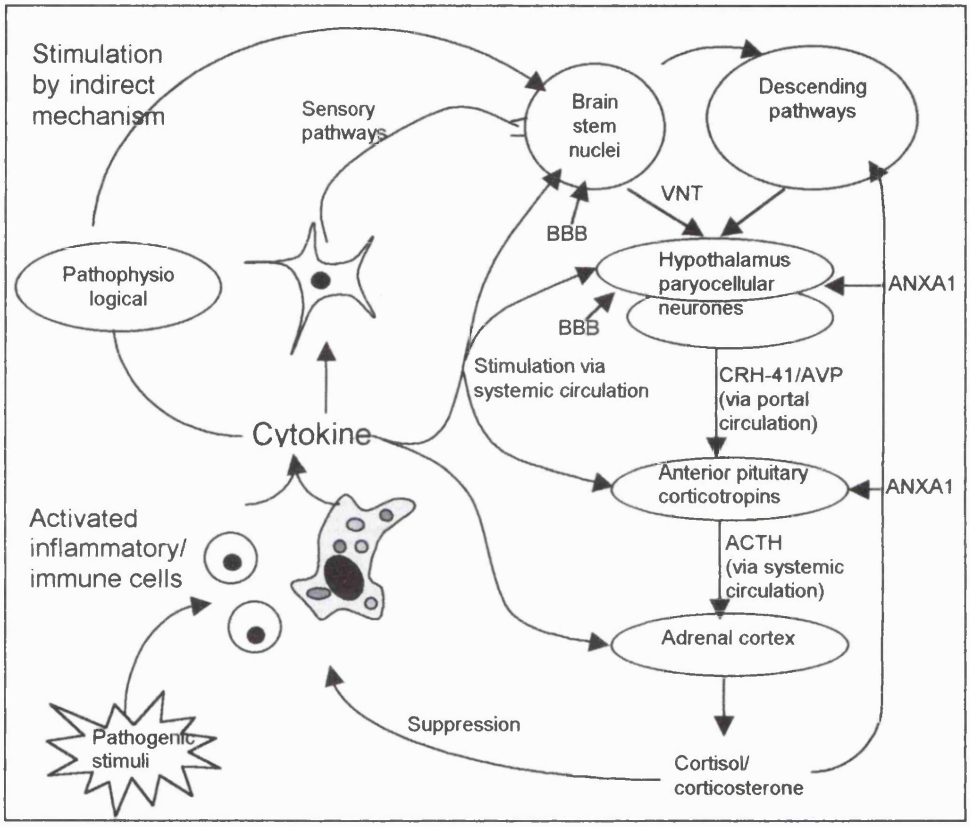


Fig 1.15 The putative mechanisms by which proinflammatory cytokines released from activated immune/ inflammatory cells stimulate the HPA axis. (ANXA1= annexin1; VNT= ventral noradrenergic tract).

Polypeptide proinflammatory cytokines such as TNF-α play a dual role as primary mediators of infection, injury and inflammation and play a role in tissue homeostasis and host defence. Their function depends on their concentrations, duration of cell exposure and the presence of other mediators in the cellular environment. Activation of the immune axis can

occur in the efferent, central and afferent limbs of the stress response. An immune challenge that threatens the stability of the internal milieu can be regarded as a stressor. Thus cell products from the activated immune system, predominantly the cytokines TNF-α, IL-1, IL-6 stimulate CRF secretion and hence activate both the HPA axis and the sympathetic nervous system in inflammatory stress (104), the products of which may then selectively suppress cellular immunity and favour humoral immune responses.

Elevated cytokine levels of TNF-α, IL-1, IL-6, IL-8 found in human sepsis correlate with severity and patient outcome (105). Infusion of recombinant TNF-α into humans results in the systemic inflammatory response syndrome with fever, haemodynamic abnormalities, leucopenia, elevated liver enzymes and coagulopathy (106-109) and may lead to end-organ dysfunction. TNF-α release induced after endotoxin infusion (110), causes a septic-like state after infusion, can activate production of other mediators and is elevated in patients with clinical sepsis. It should therefore be considered a central mediator of sepsis.

However, therapies designed to modulate or interrupt the cytokine cascade have proved largely unsuccessful and have shown little impact on patient survival e.g. infusion of anti-TNF-α monoclonal antibody (111;112). It therefore appears that the stress immune responses are still poorly understood, since attempts at modulation of the proinflammatory immune response to stress are largely ineffective in improving morbidity and mortality.

Prolonged critical illness is associated with anergy or immune suppression where patients display an increased susceptibility to infection. The corresponding animal models with burn injury, the suppressed cellular immunity is associated with decreased production of IFN-γ and IL-12 and increased production of IL-10 ie .Th2 shift (113). It appears that stress-immune interactions are highly complex and still poorly understood.

1.4 Stress response in Childhood

In contrast to the enormous literature on the stress response in adults, little information exists on stress responses in children (114). Only recently better micro-analytical tests have been developed to allow the study of complex changes taking place in children (11). This has opened the potential for building a 'comprehensive map' of the endocrine, metabolic and immune changes that occur in a broad range of illnesses in the neonate to the adolescent. Although the principles of the stress response are similar in both adult and paediatric populations, there do exist important differences. The stress response in childhood varies with patient age, disease severity and disease entity and also exhibits genetic polymorphism.

1.4.1 The effect of age

The human body is capable of responding to painful or harmful stimulae from as early as of 16 – 20 weeks gestation, by mounting an endocrine response to invasive procedures (115) or hypoxia (116), through to old age. However, the patterns of response, both qualitatively and quantitatively vary with age of subject. An investigation carried out by Anand et al showed that there were significant differences in the stress responses to elective surgery between the preterm and term neonate, the infant and child compared to the adult (117). There was little change in the total plasma concentration of gluconeogenic amino acids (alanine, glutamine, glycine, valine, proline and lysine) in full term babies, whereas preterm infants had a short lived fall (14). Similar differences in the total gluconeogenic substances were also observed in older children undergoing elective surgery. There was a very different pattern of change, with an initial increase in concentrations, followed by a fall lasting at least 48 hours after surgery (16).

In adults, a marked stress response causes hypermetabolism, increased oxygen consumption, raised temperature, increased protein turnover, increased cardiac output, increased suseptability to infection (118). Infants,

in contrast to adults, do not increase their whole body protein turnover and metabolic rates after major operations. It has been speculated that the lack of catabolic response in this age group is due to diversion of protein synthesis products from growth to tissue repair (119). Indeed evidence of slower growth velocity has been documented post major burns in children (120). Different age groups, therefore, have different patterns of metabolic response to stress.

1.4.2 Severity of illness

Severity of the illness has been considered to determine the magnitude of the stress response. The pattern of hormonal changes has been shown to be related to the severity of surgery (using the surgical severity score) in neonates; the greater the severity of surgery, the greater the endocrine response (14).

Medical illness in infants also is shown to produce a clear correlation between the rise in plasma cortisol level and the severity of illness. The plasma cortisol levels on admission in infants with acute bronchiolitis when plotted against the illness severity scores appear to be equivalent to those documented in open heart surgery (121).

Thus, the severity of illness appears to be the key driver for the magnitude of the stress response. Other evidence includes correlation between burns surface area and blood hormone concentration in children (122) and correlation between initial hormone concentration and Glasgow coma score in head injured children (123).

However, in the PICU setting, the scenario is apparently more complex, since mortality prediction models currently in use eg. PROM (Predicted Risk of Mortality) and GMSPS (Glasgow Meningococcal Septicaemia Prognostic Score) have not been correlated to indices of metabolic and endocrine stress. This may, however, reflect to the fact that metabolic and endocrine responses to critical illness in childhood may describe some aspects of patient illness severity not addressed by PROM or GMSPS scoring systems,

which are in themselves not accurate enough to base decisions on individual patient management.

1.4.3 Disease entity

Important differences between severe infection and other causes of critical illness have begun to emerge. Wilmore et al observed that alanine infusion increased hepatic glucose output in patients with burn injury, but had no effect on those with super-added sepsis (124). Factors modulating this response, whether endotoxin or cytokines, remain to be elucidated. However, the relevance of this observation to the clinical situation in humans is that the cases of hypoglycaemia in overwhelming sepsis have been described and unsurprisingly indicate a poor prognosis (125). Significant differences in the stress responses of different diagnostic categories of critically ill children have been described on admission to PICU even when the effects of age and disease severity are taken into account (126). Further studies are therefore needed to determine the mechanisms and pathophysiological functional effects of these differences.

1.4.4 Genetic polymorphism

Biological variation has been considered as a plausible explanation why some patients develop serious post-insult complications and others do not. Van Dissel et al, 1998 (127) reported that a predominantly anti-inflammatory cytokine profile ie. low TNF-α/high IL-10 ratio was associated with fatal outcome in febrile patients. Westendorp at al, 1997 (128) described that certain cytokine secreting phenotypes ie. Low TNF/high IL-10 may contribute to fatal meningococcal sepsis.

Critical illness outcome may be causally related to the inflammatory response severity. A recent study has shown that angiotensin –converting enzyme (ACE) insertion (I) / deletion (D) polymorphism is related to the

severity of meningococcal disease in children. ACE DD genotype is associated with increased illness severity (129).
Inherited traits towards high and low levels of HLA-DR expression are further evidence of a genetic component in the immune response to injury (130) where monocyte human leukocyte antigen-DR (HLA-DR) expression correlates closely with clinical outcome in severely injured patients at high risk for infection.

1.5 Catabolism in critically ill children

The importance of the stress response relates to the fact that critical illness leads to severe loss of body mass. Children may be more vulnerable than adults since their resting energy expenditure rates and protein requirements are higher. Moreover, the glucose requirement is also higher due to limited glycogen stores and the demand for glucose in the child's large brain (10). If catabolism is detrimental to adults, then intuitively, it must be worse for volatile young children. Hence it is pertinent to attempt to modulate or decrease catabolism in the PICU setting.

1.6 Modulation of the stress response

In order to reduce catabolism in the critically ill, provision of adequate calories, protein and essential nutrients has been instituted, which forms part of the clinical management of patients. However current forms of nutritional support, although able to reduce catabolism, are inadequate to induce net protein anabolism or positive nitrogen balance (63). Losses of 4-8g nitrogen/day (25-50g protein/day) in adults have been reported despite aggressive feeding in severely catabolic patients and the weight gain associated with the provision of specialised nutrition often represents a gain in total body fat and extracellular fluid. Hyperalimentation has been associated with complications in the PICU setting (131), including hyperglycaemia, electrolyte disturbances, abnormal liver function tests, azotemia and respiratory insufficiency due to excess CO_2 production (132).

Current administration guidelines emphasize that overfeeding may be of detriment to the critically ill patient.

The finding that preterm and term neonates mount substantial stress responses to surgery under anaesthesia with nitrous oxide and curare and that prevention of this response by fentanyl or halothane anaesthesia may be associated with an improved postoperative outcome has led to the use of potent anaesthesia and analgesia for surgical procedures in neonates (133;134).

Other strategies for attenuating protein catabolic loss employ the administration of growth factors and anabolic hormones. The aim of reducing the time period of convalescence and the cost of treatment prompted trails using GH, due to its protein sparing influence and promotion of fat utilisation. Other anabolic agents have included insulin and IGF-1.
GH treatment in adults after major surgery (135), trauma (25) and sepsis (136) causes elevated IGF-1 levels and a positive nitrogen balance. Some studies also demonstrate net increase in protein synthesis, but only for the duration of treatment. These studies, however, do not document the clinical outcome measures such as length of hospital stay, survival and infection rates which are important in the assessment of the clinical benefit of this treatment. Controversy still remains as to whether GH treatment improves inspiratory muscle power, showing benefit in adult patients with chronic obstructive pulmonary disease (137), but little benefit on the period of weaning from mechanical ventilation in critically ill adults (138).

In critically ill children, on the other hand, very little information is available regarding the benefits of GH therapy.
Herndon, et al. (139), reported on their experience with GH therapy in 150 children with severe burns. They had shorter donor-site healing times and hospital stay which reduced the overall treatment costs. There was no increase in mortality in the treated group. The main side effect was hyperglycaemia in some patients, insulin added to the regimen appeared to

confer further anabolic effect. There have been no studies on the use of GH in other forms of critical illness in children.

Recently, two large, independent, prospective, multicentre, double-blind, randomised, placebo-controlled trails (involving over 500 critically ill adults) have been carried out to evaluate the beneficial effects of GH therapy and improvement in patient outcome (140). The GH doses used were large, approximately 10 to 20 times higher than those usually given as replacement therapy in adults with GH deficiency. The rationale for the high dose was based on the evidence of GH resistance in critically ill patients and the information of dose levels used in previous studies.

Of major concern regarding these studies is that the mortality rate of patients receiving GH therapy was twice that of the placebo groups. There was also a rise in morbidity. Speculations have been made to explain these detrimental effects.

It has been proposed that exogenous GH might lead to excessive lipolysis, reduction in splanchnic blood flow, glucose deprivation of glucose dependant tissues, limitation of glutamine mobilization with subsequent acidosis, oxidative stress and gastrointestinal toxicity, and altered or exaggerated immune responses (2), which in turn might increase the incidence of multiorgan failure, septic shock and uncontrolled infection as causes of death in the GH treated group (140).

Another approach to stress response modulation has included the use of hypophyseal secretagogues in restoring pituitary responsiveness in the prolonged phase of critical illness (141). In this investigation selective pituitary-endocrine axes were reactivated, for the duration of the intervention, with preserved peripheral responsiveness, so that the presence of feedback inhibition protected from dose related side effects. However, it still remains to be evaluated whether these interventions would produce metabolic benefit and improve clinical outcome.

The hormonal modulation of the stress response has nevertheless proved beneficial in the latest large study involving 1548 critically ill adult surgical patients showing both a decrease in mortality and morbidity. Since

hyperglycaemia and insulin resistance are classical features of the stress response, intensive insulin therapy was instituted to maintain blood glucose levels between 4.4 and 6.1 mmol/l. Mortality was effectively halved, and the effect was most pronounced in long-stay ITU patients.This was mainly due to a reduction in multi-organ failure with a proven septic focus. Intensive insulin therapy reduced overall in-patient mortality by 34%, bloodstream infections by 46%, the median number of red cell transfusions by 50%,critical illness polyneuropathy by 44% and patients receiving intensive therapy were less likely to require prolonged mechanical ventilation and intensive care (142).

Modulation of the stress response in critical illness has proven to be a complex field, in part by the heterogeneity of the underlying pathologies found in patients receiving intensive care, the difficulty in pinpointing the exact time of onset of medical illness (unlike elective surgical cases) and the numbers that need to be studied to achieve sufficient power to recognise significant effects. These problems will be more profound in the paediatric population where the stress response varies with the age of the patient.

1.7 Conclusions

The metabolic and endocrine responses to acute stress are highly complex, and are both qualitatively and quantitatively different in critically ill children compared with critically ill adults. In critical illness human physiology is reacting to circumstances which it has not evolved to deal with and thus may be inappropriate and deleterious to survival (10). In the adult, the acute and prolonged critical illness result in different neuroendocrine paradigms. Despite the evidence that critical illness is of major importance in paediatric practice, both in clinical and economic terms, little attention has been given

to the metabolic, endocrine and immune changes in this population and is, as a consequence poorly understood. Previous studies in children have concentrated on single diagnostic categories, single acute insults, the initial phase post injury and defined age brackets. Critical illness with its multiple diagnostic categories, multiple insults, providing an acute on chronic picture, and larger age group (3 months to 16 years) therefore provides a considerable challenge.

Attempts at modulating the stress response have resulted in some improvements in patient outcome, however, the use of GH in critically ill adults has resulted in a considerable increase in mortality rate. The reasons for which still to need be elucidated.

It is apparent that a comprehensive understanding of the neuroendocrinoimmunological interactions in the stress response of critical illness is required before further pharmacological interventions are considered. The pathophysiology at the biochemical and endocrine level during the acute and prolonged phases of critical illness in childhood needs proper definition and extensive investigation and provides areas for future research.

1.8 Aims of the study

Since little is known of the pathophysiology of critical illness in children, the aim of the study was

- to describe the magnitude and nature of the metabolic and endocrine response to critical illness in childhood,
- to correlate this with patient severity and age,
- to describe whether patterns of metabolic and endocrine response are diagnosis specific both on admission to PICU and in the first few days post onset of critical illness.

Chapter 2 - Laboratory Methodologies.

2.1 Blood sample collection and storage

2.5 ml of blood was collected from an indwelling peripheral arterial catheter in patients weighing more than 10kg, and 2ml from patients weighing less than 10kg into a heparinized tube, which had been stored on ice before and after collection. 250μl aliquots were then immediately transferred to into preprepared and cooled tubes containing 500μl of 0.7M perchlorate to deproteinize the sample and prevent enzymatic destruction and interconversion of intermediary metabolites. These samples were further diluted as required with distilled water just prior to laboratory analysis.

Since glucagon is known to be rapidly degraded in plasma, it was necessary to process and freeze the samples within minutes of collection, so samples were processed rapidly, taken on ice, immediately centrifuged at 12000 rpm for 5 minutes at 4°C, the supernatant being separated into aliquots and frozen at -70°C. The plasma samples were batched and analysed, for the measurement of growth hormone, cortisol, aldosterone, IGF-1, IGFBP-1, IGFBP-3, glucagon, insulin, TSH, T4 and non-esterified free fatty acids (NEFA) and beta-hydroxybutyrate concentrations. The supernatant of the perchlorate- treated samples were used for lactate, pyruvate and acetoacetate determinations.

The principal methods of analysis for the measurement of levels of circulating intermediary metabolites and hormones were enzymatic assays, radioimmunoassays, immunoradiometric assays and ELISA, and these will be described in detail in the following sections.

2.2 Measurement of circulating intermediary metabolite concentrations

Concentrations of intermediary metabolites lactate, pyruvate and acetoacetate were measured on the Cobas Bio centrifugal analyser. The incubation temperatures for pyruvate and acetoacetate assays were 25°C and for lactate 37°C.

The levels of NEFA and β-hydroxybutyrate were measured using the Cobas Mira Plus analyser.

2.2.1 Preparation of standards

Stock solution of each standard were made up in distilled water from dried salts, as follows.

- L-Lactate : 50mmol/L 0.120g/25mls
- Pyruvate (sodium salt) : 10 mmol/L 0.0275g/25mls
- Acetoacetate (lithium salt) : 20mmol/L 0.054g/25mls

Standard Curve preparation.

200μl aliquots of each standard solution were made up to 10mls with 0.46M perchloric acid (PCA). The mixed standard and 0.46M PCA were further mixed as follows to produce seven serial dilutions for each intermediary metabolite assayed.

Table 2.1 Preparation of standards.

Standard no	Volume of mixed standard	Volume of 0.46M PCA(μL)
STD 1	12.5	987.5
STD 2	62.5	937.5
STD 3	125	875
STD 4	250	750
STD 5	500	500
STD 6	750	250
STD 7	1000	0

2.2.2 Lactate

Principle of the method

Lactate was measured using lactate dehydrogenase (LDH), which converts lactate to pyruvate with concommitant oxidation, the co factor NAD is reduced to NADH.

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

The associated increase in fluorescence emission of light at 450nm, caused by NADH excited by light at 340nm is directly correlated with lactate concentration.

Reagents used were from the Sigma Lactate kit No 826-UV. Working solutions were prepared as follows:

1. 10mg of NAD, 2ml of glycine buffer at pH 9.2 (containing 0.6mol/L glycine),
2. 4ml of deionized water
3. 0.1ml of LDH solution.

Standards 1,3,4,5,6,7 were used at concentrations 0.075, 0.75, 1.5, 3.0, 4.5, 6.0 mmol/L. Standard curve for lactate is given in appendix 1.

The assay range was 0.075-6.0 mmol/L whole blood. For higher readings, a 1 : 2 dilution was made and the assay repeated.

2.2.3 Pyruvate

Principle of the method.

Pyruvate is measured using the reaction catalysed by lactate dehydrogenase (LDH), which converts pyruvate to lactate and produces NAD from NADH.

NADH when excited by light at 340nm, emits light at 450nm by fluorescence. The associated decrease in fluorescence emission of light at 450nm caused by a decrease in NADH during the reaction is directly correlated with the pyruvate concentration, as equimolar concentrations of pyruvate and NADH are utilized to produce lactate.

Reagents used were as follows:- (from Pyruvate kit Cat No 726 from Sigma Diagnostics Ltd.)

1. Stock NADH - 1mg NADH reduced form, disodium salt, tetrahydrate and 2 mls of Trizma base solution (containing Tris(hydroxymethyl) aminomethamine, 1.5mol/L and sodium azide 0.05% as preservative)
2. Working NADH - 80µl of stock NADH and 6 mls of base solution
3. Working LDH - 50µl of kit LDH and 1ml of 3.2M ammonium sulphate.

Standards 2,3,4 were used at concentrations 75, 150, 300 µmol/L. Standard curve for pyruvate is given in appendix 1.

The assay range was 30-300 µmol/L whole blood.
For higher concentrations a 1 : 2 dilution was done and the assay repeated.

2.2.4 Acetoacetate

Principle of method.

Acetoacetate is measured using the reaction catalysed by β-hydroxybutyrate dehydrogenase (β-HBDH), which utilizes NADH to convert acetoacetate to β-hydroxybutyrate and NAD.

$$\text{Acetoacetate + NADH} \xrightarrow{\beta\text{-HBDH}} \beta\text{-hydroxybutyrate + NAD}$$

Hence a decrease in the NADH concentration is monitored, where NADH, when excited by light at 340nm, emits light at 450nm by fluorescence, which directly correlates with acetoacetate concentration.

Reagents used were as follows :-

1. 0.5M phosphate buffer pH 7.4. This was prepared by mixing 20.8g KH_2PO_4 with 86.5g $K_2HPO_43H_2O$ and making up to 1L of distilled water.
2. Stock NADH - 1mg of NADH and 1.33mls of 0.5M phosphate buffer.
3. Working NADH - 250μl of stock NADH and 10mls of phosphate buffer.
4. Working HBDH - (from Boeringer Mannheim Cat No 127841) 400μl enzyme initially diluted with 400μl distilled water.

Standards 2,3,4 were used at concentrations 150, 300, 600 μmol/L. Standard curve for acetoacetate is given in appendix 1. The assay range was 30-600 μmol/L whole blood. For higher levels, a 1 : 2 dilution of the sample was done and the assay repeated.

2.2.5 β-Hydroxybutyrate

Principle of Method.

The method used to measure the level of β-hydroxybutyrate in plasma is based on its conversion to acetoacetate by the enzyme 3-hydroxybutyrate dehydrogenase. In this reaction the cofactor NAD is reduced to NADH. The assocaited change in absorbance is directly correlated to the D-3-hydroxybutyrate concentration.

β-hydroxybutyrate+ NAD $\xrightarrow{\text{3-hydroxybutyrate dehydrogenase}}$ Acetoacetate + H + NADH

Reagents used were from the Randox "RANBUT" kit Cat No J89210 which contained the following:-

1. Buffer at pH 8.5 (consisting of Tris buffer 100mmol/L, EDTA 2mmol/L and oxalic acid 20mmol/L)
2. NAD and 3-hydroxybutyrate dehydrogenase. (These were diluted in 10 mls of buffer).
3. Standards of D-3-hydroxybutyrate at 1 and 4mmol/L. The 4mmol/l standard was then further diluted with distilled water to give a 2mmol/l standard. The standard curve is shown in appendix 1.

The assay range was 0.1 to 2.5 mmol/l.

Any samples giving readings above 2.5mmol/L were rerun after a 1 in 3 dilution with 0.9% saline.

2.2.6 Nonesterified free fatty acids (NEFA)

Principle of method.

The plasma concentration of NEFA was measured, using a procedure which employs three sequential enzymatic reactions carried out by acyl-CoA synthase (ACS), acyl-CoA oxidase (ACOD) and a peroxidase, under appropriate enzymatic reaction conditions (the principle of which is shown in equations i-iii). Firstly, the cofactor CoA in the presence of ATP, Mg^{2+} ions and NEFA is converted to Acyl-CoA by ACS. Then the Acyl-CoA in the presence of O_2 produces H_2O_2 by the reaction with ACOD. The resultant H_2O_2 is then utilized by a peroxidase for the oxidative condensation of a chromophor, 3-methyl-N-ethyl-N-(β-hydroxyethy)-aniline (MEHA), to produce a purple dye that can be measured by absorbance at 550nm wavelength. Therefore the amount of NEFA in the sample was determined from the optical density measured at 550nm.
The presence of ascorbic acid as an antioxidant in the plasma or biological sample can be a major interference in the above mentioned NEFA assay. However, this is normally prevented by the addition of ascorbate oxidase to the reaction mixture at the onset of the assay.

i) NEFA RCOOH + ATP + CoA $\xrightarrow{\text{ACS}}$ Acyl-CoA + AMP + Ppi

ii) Acyl-CoA + O_2 $\xrightarrow{\text{ACOD}}$ 2,3-trans-Enoyl-CoA + H_2O_2

iii) H_2O_2 + MEHA $\xrightarrow{\text{peroxidase}}$ purple dye

Reagents used were from the NEFA/C kit (Code 994-75409) Wako Chemical GmbH. The standard solution was 1.0mmol/L oleic acid. Standard curve is shown in appendix 1. The range of the assay was up to 2.5mmol/l. Samples giving readings above 2.5mmol/L ie. above the assays limit of linearity were rerun after a 1 : 2 dilution with 0.9% saline.

2.2.7 Inter and Intra- assay coefficient of variation (CV)

Precision values for each of the metabolites for levels at low, medium and high concentrations are given below.

The intra-assay variation was calculated from the mean result of five batches, each containing five replicates at each level. The inter-assay variation was calculated from all the results obtained from five batches of five replicates at each level.

The laboratory had previously found a high intra- and inter-assay CV for pyruvate and acetoacetate at lower concentrations when the assay was run at 37°C. Hence, assays of these metabolites were carried out at 25°C, which gives a better linearity with time and it also improves the sensitivity of the assay. The intra and inter-assay CV values are given in table 2.2.

Analyte	MEAN CONC.	INTRA-ASSAY CV%	INTER-ASSAY CV%
Lactate (mmol/L)	0.20 1.06 4.27	5.5 1.8 2.1	7.5 5.1 3.4
Pyruvate (μmol/L)	40 128 303	6.7 4.7 1.6	8.7 5.8 1.8
Acetoacetate (μmol/L)	40 209 551	6.7 2.7 1.8	7.9 6.8 7.5
β-hydroxybutyrate (mmol/L)	0.12 0.45 1.09	4.0 1.7 0.9	5.8 2.4 2.5
NEFA (mmol/l)	1.40	Not routinely conducted by laboratory	1.01

Table 2.2

The intra- and inter assay CV values for metabolites.

2.3 Measurement of plasma hormone concentrations

2.3.1 Glucagon

Glucagon was measured by the double antibody technique and sequential radioimmunoassay using Diagnostic Products Corporation kit no. KGND1.

Protocol of the method.

After thawing, 200 μl of each sample was incubated with 100 μl anti-glucagon antibody for 24 hours. A further 100 μl of ^{125}I-labelled glucagon was added to compete with the glucagon in the sample for antibody with a further 24 hour incubation. Separation of the bound from free glucagon was achieved by use of 1ml of a precipitating solution consisting of goat anti-rabbit gamma globulin and dilute polyethelene glycol in saline. After centrifugation, the precipitate containing the antibody bound fraction was counted for radioactivity (on a gamma counter) and compared with the standard calibration curve.

The calibration curve was constructed from kit provided samples and their serial dilutions from zero calibrator to correct for non-specific binding through 25,50,100,500 and 1000 pg/ml.

The sensitivity of the assay was 13pg/ml. The intra-assay coefficient of variation (CV) was 15.7% at 35 pg/ml, 4.4% at 151pg/ml and 4.1% at 564 pg/ml. These results were calculated from 20 pairs of tubes in a single assay. The inter-assay coefficient of variation (CV) was 15.7% at 37pg/ml, 6.5% at 159pg/ml and 5.7% at 534pg/ml.

2.3.2 Insulin

Insulin was measured by a solid phase ^{125}I radioimmunoassay using the Coat-A-Count Diagnostics Product Corporation kit no. TKIN1.

Protocol of the method.

This solid phase radioimmunoassay has insulin-specific antibody immobilized to the wall of polypropylene tubes in which ^{125}I-labelled insulin competes for a fixed time with insulin in the patient sample for sites on the insulin-specific antibody. The competition is terminated by decanting the supernatant from the tubes since the antibody is tube-bound. Counting each tube in the gamma counter for 1 minute yielded a number, which was converted by way of a calibration curve to the concentration of insulin present in each patient sample.

The calibration curve was constructed from kit provided samples and their serial dilutions from zero calibrator through 5, 15, 50, 100, 200, 400 μIU/ml of insulin. 200 μl of each specimen, the calibration specimens and tubes for non-specific binding and total counts had 1ml of the ^{125}I Insulin added to each. Incubation was carried out for 3 hours at 37°C.

The detection limit for the assay was 1.3 μIU/mL .

The intra-assay CV was 10.1% at 16 μIU/mL, 8.0% at 35 μIU/mL, 5.0% at 76μIU/mL and 4.5% at 95μIU/mL. These values are based on 20 degrees of freedom. The inter-assay CV was 10.0% at 16μIU/mL, 7.1 at 35 μIU/mL, 7.2% at 76μIU/mL and 4.9% at 95μIU/mL for 20 different assays.

2.3.3 Growth hormone

An immunoradiometric assay was used for GH from NETRIA (North Thames Region Immunoassay Unit).

Protocol of the Method.

A sheep anti-GH antiserum and a mouse monoclonal anti-GH antibody were used for the immunoradiometric assay (IRMA) for human GH. Samples containing human GH (hGH) were simultaneously reacted with the solid phase bound sheep anti-GH antiserum directed toward a unique site on the hGH molecule and a ^{125}I-labelled mouse monoclonal antibody directed against a distinctly different antigenic site on the same hGH molecule. The labelled antibody was prepared by radioiodination of a purified monoclonal antibody using Chloramine T and was subsequently purified on Sephacryl S-300. Following the formation of the solid phase-hGH-^{125}I monoclonal antibody sandwich, all reagents were added simultaneously and the reaction was allowed to occur at room temperature overnight with samples placed on a rotatory mixer. Separation was achieved by centrifugation and two washing steps were used to minimise the non-specific binding. The radioactivity bound to the solid phase was measured with a gamma counter for 5 minutes and it was directly proportional to the concentration of hGH present in the test sample.

The reagents used for the initial incubation in each tube were as follows:-

1. 100μl of sample or standard
2. 300μl of assay buffer (made up from 10ml of stock phosphate buffer 0.5M pH 7.4, 1ml of 10% sodium azide, 1g of Bovine serum albumin, 5ml 10% Tween 20 to 100 ml distilled water)
3. 50 μl labelled antibody (100,000 counts per 60 secs)
4. 50 μl solid Phase First Antibody (used as supplied, but kept mixing during assay, using a magnetic stirrer).

The standards used were prepared in GH-free animal serum being reconstitited with 2ml distilled water. The standards used were as follows:-

0.5mU/L, 1.0mU/L, 5.0mU/L, 10.0mU/L, 50.0mU/l and 100.0mU/L. The assay range was 0.5-100.0mU/L.
The wash buffer was made up from 100ml stock 0.5M Phosphate buffer, 50ml 10% Tween 20 to 1 L with distilled water.
The sensitivity of this assay was 0.2mU/L. The intra-assay CV was 2.7% at 0.8mU/L, 2.4% at 4.5mU/L and 2.6 at 86.5 mU/L with n=10. The inter-assay CV was 3.3% at 7.7 mU/L, 5.2% at 21.7 mU/L and 5.5% at 45.8 mU/L with n=10.

2.3.4 IGF-1

The IGF-1 assay used was an in house radioimmunoassay with acid-ethanol extraction.

Protocol of Method.

The acid-ethanol extraction procedure was used to remove the IGF-1 from its binding proteins which would otherwise interfere with the assay. A 50μl of standard or sample serum was added to 200μl of acid-ethanol (made up from 437ml ethyl alcohol, 14.5ml concentrated hydrochloric acid and 48ml of water), mixed and then spun at 3000rpm for 20 min at 4°C. The temperature is important for good extraction. Then a 125 μl of supernatant was removed and added to 50μl of 0.855M Tris base (hydroxymethyl methylamine) to neutralise the acidity. This was then re-centrifuged and the supernatant was used for further analysis.
The standard curve was constructed from dilutions of human serum standard giving the following concentrations 5, 15, 30, 180, 550ng/ml.
The samples (25 μl), after acid-ethanol extraction, were diluted with assay buffer (4.14g of monobasic phosphate, 3.72g of EDTA, 0.5ml Tween,10ml of 2% sodium azide, made up to 800ml with distilled water and pH made up to 7.5 with sodium hydroxide), giving a final dilution of 1 in 25.

Assay procedure.

A 50μl of sample, standard or quality control, was added to 50μl of ^{125}I-IGF-1 label added and 150μl of antibody (polyclonal antiserum R557A rabbit antihuman raised against highly purified IGF-1). Blank, non-specific binding and total count tubes were processed as well. These samples were incubated over 16 hours at 4°C, then mixed with 300μl of PEG-mix (125ml 40% polyethylene glycol, 50ml of 0.2M tris-HCL and 25ml distilled water with 0.3g bovine gamma-globulin), incubated for 1 hour, and centrifuged 300rpm for 20-30 min at 4°C. The supernatant was discarded and the precipitate was counted for radioactivity in the gamma counter.

The working range of the assay was 5 - 600ng/ml.

The sensitivity of the assay as 13ng/ml. The intra-assay CV was 9.0% at 45 ng/ml, 6.5% at 243ng/ml and 4.7% at 698 ng/ml. The inter-assay CV was 10.5% at 75ng/ml, 10.1% at 196ng/ml and 5.1% at 698mg/ml.

2.3.5 IGFBP-1

Total IGFBP-1 was measured by a coated tube immunoradiometric assay kit from Diagnostic Systems Laboratories no. DSL-7800.

Protocol of the Method.

As with the GH assay, this procedure employs a 2-site immunoradiometric assay (IRMA), a non-competitive assay in which the IGFBP-1 is sandwiched between two antibodies, firstly, the solid phase, immobilized to the walls of the tube and secondly to radiolabelled antibody for detection. The unbound material was removed by decanting and washing tubes.

The standards used were 0.5, 2.0, 10.0, 40.0, 80.0 and 160.0ng/ml of IGFBP-1 in non-human serum with azide as preservative.

25μl of standards, controls and specimens were used in the coated tubes along with 200μl of assay buffer. This was incubated at 25°C for 4 hours on a shaker at 180rpm, after which the tubes were decanted and washed twice

with 2ml of deionized water. 200μl of the second antibody ^{125}I-labelled anti IGFBP-1 was added to each tube and incubated in similar conditions for 18-22 hours. The tubes were then decanted, taking care to blot any droplets adhering to the rim and then washed 3 times with deionized water and decanted as above. The tubes were then counted for radioactivity in a gamma counter for 1 minute. Total count tubes were plain to which the ^{125}I-labelled anti-IGFBP-3 was added.

The sensitivity of the assay was 0.33ng/ml. The assay range was 0.8 - 140ng/ml. The intra-assay CV was 5.2% at 5.2ng/mL, 4.6 at 50.2ng/mLand 2.7 at 144.6ng/mL. The inter-assay CV was 3.5% at 5.1ng/mL, 6.0 at 47.0ng/mL and 3.6 at 142.0ng/mL.

2.3.6 IGFBP-3

Active IGFBP-3 was measured by Enzyme-Linked Immunosorbent Assay (ELISA) kit from Diagnostic Systems Laboratories no. DSL-10-6600.

Protocol of the Method.

The ELISA procedure for measuring IGFBP-3 is an enzymatically amplified two-step sandwich type immunoassay. The standards, controls and unknowns were incubated in microtitration wells coated with anti-IGFBP-3 polyclonal antibody. After incubation and washing the wells, another anti-IGFBP-3 polyclonal antibody labelled with the enzyme horseradish peroxidase was applied, followed by a second incubation and washing step. Tetramethylbenzidine (TMB) was then added to the wells and a further incubation was carried out. An acid solution was then added, which causes a colour change from blue to yellow. The degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measured at 450 and 650nm. The absorbance measured was directly proportional to the concentration of IGFBP-3 present in each sample. The IGFBP-3 standards were used to plot the standard curve of absorbance versus IGFBP-3 concentration from which the sample levels of IGFBP-3 were calculated.

All serum samples for this assay underwent a 1 : 100 dilution prior to analysis. 25μl of sample, standard and control were placed into wells, to which 50μl of assay buffer was added. The plates were then incubated for 2 hours at 25°C, shaking at 500-600rpm. The well contents were then aspirated and washed five times with the kit wash solution. 100μl of antibody-conjugate enzyme solution was added to each well followed by a one hour incubation under conditions as described above. The wells were then aspirated and washed 5 times as before. 100μl of TMB Chromagen solution was added to each well with a further 10 minute incubation, was carried out under the same conditions, but avoiding exposure to direct sunlight. Then 100μl of reaction stopping solution (containing 0.2M sulphuric acid) was added to each well and absorbance of the solution within the wells was measured at 450nm.

Precautions were taken so that there were no grossly heamolysed, icteric or lipaemic samples used, as these could give false readings. Repeated freezing and thawing of reagents and specimens was also avoided.

Patient samples containing IGFBP-3 protease activity, especially those from septic patients may have contained immunoreactive IGFBP-3 fragments. The extent of this is unknown, measurement of intact IGFBP-3 in these samples may require preliminary size exclusion chromatography, a procedure not available at the time of analysis.

The assay range was 2-100ng/ml. The sensitivity of the assay was 0.04ng/ml.

The intra-assay CV was 9.6% at 4.62ng/ml, 9.5% at 27.43ng/ml and 7.3% at 74.40ng/ml. The inter-assay CV was 11.4% at 5.64ng/ml, 10.4% at 25.13 ng/ml and 8.2% at 65.55ng/ml.

2.3.7 Thyroid stimulating hormone (TSH)

An immunoradiometric assay from NETRIA (North Thames Region Immunoassay Unit) was employed for the measurement of TSH concentration.

Protocol of method.

A sheep anti-hTSH antiserum and a mouse monoclonal anti-hTSH antibody were used as the basis for IRMA for human TSH. The protocol of the method was identical to that used in the measurement of GH and is detailed in that section. The reagents including stock, assay and wash buffer were identical, the solid phase anti-hTSH was used as supplied, the ^{125}I-labelled-Anti-hTSH was diluted in assay buffer to give approximately 50,000 counts/50μl/100secs.

The standards used were prepared in TSH free animal serum being reconstitited with 2ml distilled water. The standards used were as follows 0.3mU/L, 0.6mU/L, 7.5mU/l, 15.0mU/L, 30.0mU/L, 60.0mU/L.

The sensitivity of the assay was 0.04mU/L. The assay range was 0.3 – 60.0mU/L.

The intra-assay CV was 3.6% at 0.28mU/L, 3.0% at 1.00mU/L and 1.6% at 3.10mU/L. The inter-assay CV was 9.8% at 0.43mU/L, 6.0% at 1.00mU/L and 3.7% at 3.00mU/L.

2.3.8 Total T4

T4 was measured by a solid phase ^{125}I-radioimmunoassay using the Coat-A-Count Diagnostics Product Corporation kit no. TKT41.

Protocol of the method.

This solid phase radioimmunoassay is based on antibody coated tubes and human serum calibrators. ^{125}I-labelled T4 competes for a fixed time (1 hour at 37°C) with T4 in the patient sample for the antibody sites, in the presence of blocking agents for thyroid-hormone binding proteins. As with the insulin assay, the competition reaction was terminated by decanting the

supernatant from the tubes, since the antibody was tube-bound. Each tube was counted for radioactivity in the gamma counter for 1 minute and radioactivity counts were transformed by way of a calibration curve to determine T4 concentration present in each patient sample. All reagents were kit provided and manufacturers guidelines were followed.

The calibration curve had been constructed from kit provided human serum based standards ranging from 12.9 to 309 nmol/L.

The assay range was 12 – 600 nmol/L. The sensitivity of the assay was 3.2nmol/L.

The intra-assay CV was 3.8% at 30.7nmol/L, 2.7% at 94.7nmol/L and 2.8% at 176.6nmol/L. The inter-assay CV was 8.1% at 92.1nmol/L, 4.2% at 145.9nmol/L and 5.9% at 168.9nmol/L.

2.3.9 Cortisol

Cortisol was measured by a solid phase ^{125}I-radioimmunoassay using the Coat-A-Count Diagnostics Product Corporation kit no. TKCO1.

Protocol of the method.

This solid phase radioimmunoassay, wherein 125I-labelled cortisol competes with cortisol in the samples to bind antibody immobilized on the wall of the polypropylene tubes was conducted according to the manufacturers guidelines, using the reagents provided. The principle of this method being identical to that for T4 and insulin, although no binding protein inhibition was necessary as in the case of T4 assay. The incubation period was 45 minutes at 37°C, the incubation was terminated by decanting the supernatant and the tube was counted for radioactivity for 1 minute in a gamma counter. The reading was then transformed by means of a calibration curve to cortisol concentration.

The calibration curve was constructed from kit provided human serum based standards ranging from 27.6 to 1380 nmol/L. The assay range was 14-1500 nmol/L. he sensitivity of the assay was 13nmol/L. The intra-assay CV was 8.4% at 138nmol/L, 5.3% at 529nmol/L and 11.1% at 910nmol/L. The inter-assay CV was 9.1% at 132nmol/L, 8.9% at 527nmol/L and 11.5% at 982nmol/L.

2.3.10 Aldosterone

Aldosterone was measured by a coated tube radioimmunoassay kit from Diagnostic Systems Laboratories no. DSL-8600.

Protocol of the method.

The procedure follows the basic principle of radioimmunoassay, employing competition between the radioactive and non-radioactive antigen for a fixed number of antibody binding sites. The amount of ^{125}I-labelled aldosterone is inversely proportional to the concentration of unlabelled aldosterone present. The assay was conducted according to the manufacturers guidelines, using the reagents provided. The principle of this method being identical to that for T4 and insulin and cortisol, although no binding protein inhibition was necessary as in the case of the T4 assay. The tubes were incubated for 3 hours at 37°C, whilst being rotated at 180rpm. The incubation was terminated by decanting the supernatant and the tubes counted for 1 minute for radioactivity in a gamma counter. The machine reading was then converted by means of a calibration curve to actual aldosterone concentrations.

The calibration curve was constructed from kit provided human serum based standards ranging from 0,25,50,100,250,800 and 1600pg/ml.
The assay range was 25-1500 pg/ml. The sensitivity of the assay was 25pg/ml.

The intra-assay CV was 7.0% at 50.4pg/ml, 3.6% at 254.6pg/ml and 8.3% at 777.9pg/ml. The inter-assay CV was 10.4% at 50.6pg/ml, 7.3% at 257.6pg/ml and 10.0% at 850.9pg/ml.

2.3.11 Catecholamines - Adrenaline and Noradrenaline

Catecholamines were measured by Dr. Elizabeth Newbold at the Dunn Laboratories, Dept. of Endocrinology, St. Bartholemews Hospital, London by high performance liquid chromatography with electrochemical detection as described by Bouloux (143).

The limit of detection was 0.2nmol/l for both adrenaline and noradrenaline and any values up to 100nmol/l may be assayed by this technique. Higher levels of catecholamines require prior serial dilution and reassay.

The intra-assay CV was 6.6% at 5nmol/l for adrenaline and 7.5% at 1nmol/l for noradrenaline.

The inter-assay CV was 10.7% at 3.6nmol/L and 6.8% at 12nmol/l for noradrenaline, 20% at 1.3nmol/l and 11% at 5.6nmol/l for adrenaline. Despite the high CV at lower concentrations for adrenaline, this technique was adopted as the method of choice for catecholamine measurement since it has a superior sensitivity to the double isotope radio-enzymatic assay.

2.3.12 Renin

Plasma renin activity was measured by Nadia Payne at the Middlesex Hospital, London according to the method described by Menard and Catt (144).

The sensitivity of the assay was 0.1 nmol/hr/l. The intra-assay CV was 5.4% at 1.23nmol/hr/l, 5.6% at 8.24nmol/hr/l and 7.5% at 13.8nmol/hr/l. The inter-assay CV was 6.0% at 1.23 nmol/hr/l, 6.2% at 8.24nmol/hr/l and 8.0% at 13.8nmol/hr/l.

Chapter 3 - Clinical details and management of patients recruited in the present study.

3.1 Clinical details of patients

The study was performed on 60 children, 34 boys and 26 girls, all new admissions to the paediatric intensive care unit (PICU). Children were recruited from two centres, 29 from the PICU at The Great Ormond Street Hospital for Children, London and 31 from the PICU at St. Marys Hospital, London. The mean age of all the patients was 4.2 years (range 0.25-16.33 years). The severity rating as defined by predicted risk of mortality or PROM (calculated from the PRISM score), mean value 22.5% (range 0.5 – 99.3%).
These children represented a wide spectrum of basic underlying diseases, the main diagnostic categogories included meningococcal sepsis, surgery and respiratory infection. However, prerequisites for recruitment to the study were onset of illness in the last 24 hours, but with no previous renal, hepatic, metabolic or immunological disease. Ethical approval had been granted for the project and informed written consent from the parent of each subject prior to commencement of the study was obtained on or soon after arrival to the PICUs.
All children recruited at St. Marys had initially been admitted to their District General Hospital where they were initially assessed, stabilized and treated for suspected meningococcal disease, but required intensive care for which they were transferred to the PICU at St.Marys, where the diagnosis was confirmed. Their mean age was 4.4 years (range 0.5-14.4years) and their mean predicted risk of mortality (PROM) was 32.3% (range 0.5-99.3%).
The 29 children recruited to the study at The Great Ormond Street Hospital had a mean age of 4.1 years (range 0.25-16.3 years) and a mean predicted risk of mortality (PROM) of 9.4% (range 0.2-83.0%). They were a

heterogeneous diagnostic group, the main diagnostic categories were respiratory infection (n=13) with a mean age of 3.4years (range 0.3-12.5 years) with a mean PROM of 9.7% range (1.8-34.0%) and post surgical (n=12, mainly gastrointestinal and laryngeal) with a mean age of 5.3 years (range 0.5-16.3 years) and mean PROM of 6.1% (range 2-33%). Other diagnoses fell into the neurological category or into other infectious causes.
The clinical care of the children was provided by the intensive care teams at each PICU with relevant input from surgical, respiratory and neurological teams and consisted of ITU management and disease specific treatment.

3.1.1 Management of patients with meningococcal disease

All children were endotracheally intubated and mechanically ventilated, the sedation and analgesia were administered with continious infusions of midazolam ranging from 0-4μg/kg/min and of morphine ranging from 0-40 μg/kg/hr. Most children received a muscle relaxant in the form of vecuronium. The meningococcal patients received a muscle relaxant at the admitting hospital and for transfer, which was then gradually weaned down.
Intravenous crystalloid fluids in the form of 10% dextrose plus electrolytes were administered at maintenance requirements on day 1 of admission to the PICU.
Nasogastric feeds of a nutritionally complete formula were commenced at 24-48 hours following PICU admission and increased as tolerated. In children where enteral nutrition was contraindicated or not tolerated, total parenteral nutrition was given.
Colloid was given as required to maintain normotension and to ensure adequate peripheral perfusion. The patients recruited from St. Marys with meningococcal disease had the greatest colloid requirement (range 0-220 ml/kg given in the eight hours preceding admission to the PICU, which included initial resuscitation and transfer to St. Marys), being maximal on admission and reducing thereafter.

Vasoactive drugs were given as required to maintain normotension and renal perfusion and included dopamine, dobutamine, adrenaline and noradrenaline and prostacyclin.

Two of the meningococcal patients were given intravenous hydrocortisone for refractory shock at a dose of 0.2 mg/kg/dose tds i.v. Four patients exibiting symptoms and signs of meningococcal meningitis were given dexamethasone at a dose of 0.4 mg/kg bd. (one patient being common to both categories).

In patients who exhibited signs of raised intracranial pressure, mannitol at a dose of 0.25g/kg was given as a bolus together with frusemide 1 mg/kg if urine output was poor. If seizures occurred, these were controlled with phenytoin at 18 mg/kg over 30 minutes.

The antibiotics of choice were cefotaxime or ceftriaxone at 80 mg/kg.

Detailed information on the meningococcal patients is given in Table 3.1.

Table 3.1 Meningococcal patient details

Patient	AGE in years	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
1m	3.79	M	57	Mid 1-2, Mor 20-30, Atr 22.2, Ceftrix,	Dop 10, Dob 5, Adr 0.45	Dex 4	40 hr	6
2m	3.67	F	54.3	Mid 0-2, Fen 0-3.8, Atr 0-19.6,Ceftrix, Frus	0	Dex 2.5	40 hr	6
3m	2.9	M	2.4	Atrac 5, Thio 60, Ceftriaxone, Frus	0	0	8 hr	2
4m	3.2	F	2.3	Mid 0-4, Mor 0-40, Atr 0-20, Mannitol, Ceftrix	0	Dex 2.5	16 hr	3
5m	3.1	F	60.2	Mid 0-2, Mor 0-40, Vec 0-3, Ceftrix, Frus,	Dop 5, Dob 3-20, Adr 0-1	0	40 hr	5
6m	2.85	F	7.8	Cefotax	0	Dex 2, Hydrocort 175	adm	1
7m	2.45	F	5.4	Mid 0-2, Mor 0-42, Atr 0-20, Ceftrix, Frus	Dob 5	0	16 hr	3
8m	0.73	F	41.9	Mid 2, Mor 28-60, Vec 0-2, Ceftrix, Frus	Dob 5-10, Prost 0-5	0	40 hr	6
9m	10.14	M	1.3	Mid 2, Mor 0-40, Vec 0-3, Cefotax, Frus	Dob 0-10, Adr 0-0.3,	0	32 hr	5

Table 3.1 Meningococcal patient details

Patient	AGE in years	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
10m	4.75	F	37.6	Mid 0-3, Mor 0-40, Vec 0-2, Ceftrix, Frus, Valproate	Dob 0-7.5	0	32 hr	5
11m	1.9	M	51.8	Mid 0-2, Mor 0-40, Vec 0-2, Cefotax, Frus	Dop 0-10, Dob 0-10, Adr 0-0.2	0	40 hr	6
12m	0.72	M	14.5	Mid 0-2, Mor 0-40, Vec 0-2, Ceftrix, Heparin	Dob 0-7.5	0	32 hr	5
13m	13.0	M	95.8	Mid 2, Mor 20-40, Vec 2, Cefotax, Frus	Dop 0-3, Adr 1.7-4, Nadr 0-1	Hydrocort*	40 hr	6
14m	3.19	F	26.7	Mid 0-2, Mor 0-60, Vec 0-3, Ceftrix, Frus, Heparin, Mannitol	Dob 0-5	Dex 2.5-4	40 hr	6
15m	5.54	M	66.2	Mid 1.4-3, Mor 28-50, Vec 0-2, Ceftrix	Dop 5, Adr 0.4-1.6, Nadr 0.1-2	Hydrocort*	40 hr	6
16m	2.34	M	74.7	Mid 0-2, Mor 0-30, Ceftrix	Dob 0-5	0	24 hr	4
17m	1.3	M	15.5	Mid 0-3, Mor 0-20, Mannitol, Ceftrix, Vec 0-2	Dob 0-15, Adr 0-1.8	0	40 hr	5
18m	1.4	M	43.7	Mid 0-3, mor 0-20, Heparin, Vec 0-3, Ceftrix, Salbut	Dob 0-10, Adr 0-0.2, Nadr 0-2	0	40 hr	6

Table 3.1 Meningococcal patient details

Patient	AGE in years	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
19m	4.2	M	21.3	Mid 0-3, Mor 0-20, Vec 0-3, Ceftrix	Dop 0-5	0	40 hr	5
20m	3	F	30.5	Mid 0-2, Mor 0-40, Vec 0-2, Mannitol, Ceftrix	Dob 0-5	Dex 4	24 hr	4
21m	3.4	M	2.8	Mid 0-3, Mor 0-20, Vec 0-3, Atrac 0-30, Ceftrix	Dop 0-15, Dob 0-7, Adr 0-0.3	0	24 hr	6
22m	2.5	F	3.6	Mid 0-2, Mor 0-20, Vec 0-2, Atrac 0-20, Heparin, Ceftrix	Dop 0-5, Dob 0-5, Adr 0-2	0	40 hr	6
23m	1	M	99.3	Mid0-3, Mor 0-40, Vec 0-3, Heparin, Ceftrix, Frus,	Adr 0-1.2, Nadr 0-0.6	Dex 4	32 hr	5
24m	1.2	F	9	Mid 0-3, Mor 0-20, Vec 0-3, Heparin, Ceftrix	Dob 0-5	0	8 hr	2
25m	8	F	76	Mid 0-3, Mor 0-20, Vec 0-3, Heparin, Acyclovir, Ceftrix	Adr 0-0.6	0	40 hr	6
26m	14.35	M	7.3	Mid 0-2, Mor 0-40, Heparin, Ceftrix, Vanc	Adr 0-0.5	0	40 hr	5
27m	10.75	F	0.5	Mid 0-3, Mor 0-20, Heparin, Ceftrix, Mannitol	Dob 0-15	0	40 hr	6

Table 3.1 Meningococcal patient details

Patient	AGE in years	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
28m	14	F	15.1	Mid 0-3, Mor 0-20, Vec 0-3, Atrac 0-3, Heparin, Ceftrix	Adr 0-0.3, Nadr 0-0.3	0	40 hr	6
29m	4.5	F	18.2	Mid 0-3, Mor 0-20, Ceftrix	Dob 0-5	0	adm	1
30m	0.5	F	18.2	Mid 0-4, Mor 0-20, Heparin, Ceftrix	Adr 0-3	0	40 hr	6
31m	1.9	M	41.5	Mid 0-3, Mor 0-20, Ceftrix	Adr 0-0.3	0	40 hr	6

Table 3.

Drugs given to patients during their hospital stay.

Mor	Morphine	mcg/kg/hr
Atrac	Atracurium	
Vec	Vecuronium	mcg/kg/min
Mid	Midazolam	mcg/kg/min
Frus	Frusemide	
Ceftrix	Ceftriaxone	
Ceftaz	Ceftazidime	
Amik	Amikacin	
Piptazo	Piptazobactam	
Fluclox	Flucloxacillin	
Ben pen	Benzyl penicillin	
Metronid	Metronidazole	
Ciproflox	Ciprofloxacin	
Vanc	Vancomycin	
Erythro	Erythromycin	
Nebs	Nebulizers	
Salb	Salbutamol	
Carbemez	Carbemazapine	
Lamotrig	Lamotrigene	
Dob	Dobutamine	
Dop	Dopamine	mcg/kg/min
Nadr	Noradrenaline	mcg/kg
Adr	Adrenaline	mcg/kg/min
Hydrocort	Hydrocortisone	
Dex	Dexamethasone	

* denotes not on admission, but during subsequent treatment on PICU
Other drug names are listed in full in patient table.

3.1.1 Management of respiratory patients

All children were endotracheally intubated and mechanically ventilated, the sedation and analgesia were administered with continuous infusions of midazolam ranging from 0-4μg/kg/min and of morphine ranging from 0-40 μg/kg/hr. Most children received a muscle relaxant in the form of vecuronium.

The diagnoses in this category ranged from bronchiolitis to pneumonias, most often on a background of limited respiratory reserve eg. myopathy or cerebral palsy with repeated aspirations. Treatment for each case was disease specific.

Intravenous crystalloid fluids in the form of 10% dextrose plus electrolytes were administered at maintenance requirements on day 1 of admission to the PICU.

Nasogastric feeds of a nutritionally complete formula were commenced at 24-48 hours following admission to the PICU and were increased as tolerated. In children where enteral nutrition was contraindicated or not tolerated, total parenteral nutrition was given.

Colloid was given as required to maintain normotension and ensure adequate peripheral perfusion.

Vasoactive drugs were required to maintain normotension in two cases, which initially presented as pneumonia, but later developed into sepsis syndrome.

Steriods, in the form of dexamethasone, prednisolone and hydrocortisone were required in seven cases, however only three required streroids on admission, the rest were given during subsequent patient management.

Antibiotic treatment, nebulised salbutamol, atrovent, budesonide and intravenous salbutamol were given where appropriate.

Patient details are given in Table 3.2.

Table 3.2 Respiratory patient details

Patient	AGE in years	Diagnosis	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
1r	1	Pneumonia, Downs syndrome	F	1.5	Mid 0-4, Mor 0-40, Vec 0-4, Amikacin, Cefotax, Vanc, Fluconazole, Frus	Nadr 0-0.1	0	96 hr	5
2r	0.25	Bronchiolitis	F	9	Mid 2-4, Mor 10-40, Vec 0-2, Salb 0.5-2.2, Frus	0	0	48 hr	4
3r	0.25	Bronchiolitis	M	15.6	Mid 2-4, Mor 20, Vec 2, Salb 2-6.4, Nebs	0	*Hydrocort	24 hr	2
4r	12.58	Respiratory failure, pneumonia, central core myopathy	M	0.9	Amikacin, Piptazo, Thiopentone, Atrac.	0	0	48 hr	4
5r	4	Pneumonia, respiratory failure, Goldenhaur syndrome	M	1.8	Mid 2-4, Mor 20-40	0	Dex	48 hr	4
6r	2.5	Pneumonitis secondary to aspiration	M	13.6	Mid 1-2, Mor 20-40, Cefurox, Metronid, Valproate, Baclofen, Carbamez, Nebs	0	0	72 hr	2
7r	0.33	Aspiration pneumonia,reflux, tetralogy of Fallot	F	34	Mid 0-2, Mor 0-20, vec 0-2, Amik, Piptazo, Frus	0	*Dex	48 hr	3
8r	0.75	Bronchiolitis/ Aspiration pneumonia	M	15	Mid 2-4, Mor 10-40, Frus, Cefurox, Metronid, Piptazobactam,Amikacin, Nebs	0	0	120 hr	7

Table 3.2 Respiratory patient details

Patient	AGE in years	Diagnosis	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
9r	0.5	Bronchiolitis	M`	3.4	Mid 0-4, Mor 16-40, Vec 0-4, Fluclox, Cefotax, Frus	0	Dex*	48 hr	2
10r	4.25	Tracheitis, pneumonia developed sepsis syndrome	-F	2.7	Mid 0-4, Mor 0-40, Vec 0-3, Cefotax, Erythrom, Fluclox, Frus, Nebs	Dop 0-10, Nadr 0-1.1	Dex*	96 hr	7
11r	8	Aspiration pneumonia cerebral palsy	-M	2.4	Mid 2-3, Mor 10-40, Vec 0-4, Metronid, Cefotax, Valproate, Clonazepam, Lamotrig, Dulphalac	0	0	adm	1
12r	0.75	Respiratory infection/ Tracheal obstruction.	M	7.4	Mid 1.5-4, Mor 20-40, Vec 0-4, Amikacin, Erythrom, Piptazo, Frus, Nebs	0	Pred*	96 hr	5
13r	0.9	Respiratory infection/ Aspiration/ Myopathy	F	8.9	Mid 0-4, Mor 0-40, Vec 0-2, Amikacin, Piptazo, Erythrom, Cisapride	0	Dex*	96 hr	5

3.1.3 Clinical management of post-surgical patients

Eleven children were aneasthetised and intubated endotracheally perioperatively. Mechanical ventilation was continued postoperatively whilst on PICU. One patient (11s) was intubated at the referring hospital prior to transfer and underwent surgery on arrival to Great Ormond Street Hospital. The sedation and analgesia were administered with continious infusions of midazolam ranging from 0-4μg/kg/min and morphine ranging from 0-40 μg/kg/hr. Most children received a muscle relaxant in the form of vecuronium. The surgery performed was elective, mainly gastrointestinal or tracheolaryngeal, in all but one case (11s).

Intravenous crystalloid fluids in the form of 10% dextrose plus electrolytes were administered at maintenance requirements on day 1 of admission to PICU.

Nasogastric feeds of a nutritionally complete formula were commenced at 24-48 hours following admission to the PICU and were increased as tolerated. In children where enteral nutrition was contraindicated (those undergoing gastrointestinal surgery) or not tolerated, total parenteral nutrition was given.

Colloid was given as required to maintain normotension and to ensure adequate peripheral perfusion.

The only vasoactive drug given in this category was dopamine.

One patient required steroids to aid extubation post tracheal surgery. Antibiotics were chosen to cover mainly gastrointestinal microorganisms. PROM (predicted risk of mortality scores) tended to be low in this group, since most of the surgery was elective, resuscitation was not required, patients were continually monitored during surgery and therefore returned to the PICU well hydrated, receiving adequate analgesia and sedation.

Patient details are shown in Table 3.3.

Table 3.3 Surgical patient details

Patient	AGE in years	Diagnosis	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
1s	11.58	Scoliosis surgery	M	0.5	Mor 0-30, Propofol, Amikacin, Fluclox, Diclofenac	0	0	adm	1
2s	16.3	Congenital oesophageal atresia- opn	M	0.2	Mid 0-5, Mor 0-50, Vec 0-5, Amikacin, Ben pen, Metronid, Frus, Piptazo, Diclofenac	Dop 0-5	0	48 hr	4
3s	0.58	Ileostomy, removal of kidney	F	33.3	Mid 0-4, Mor 10-40, Amikacin, Ben pen, Ciproflox, Metronid, Fluconazole, Frus	Dop 0-7	0	48 hr	4
4s	5.83	Oesophageal resection-gastrojejunostomy	M	4	Mid 2-6, Mor 0-40, Vec 0-6, Ben pen, Metronid, Amikacin, Piptazo, Frus, Bupivacain-extradural	0	0	48 hr	3
5s	0.5	Tracheoplasty for subglottic stenosis	M	1.7	Mid 0-2, Mor 0-20, Amikacin, Piptazo, Augmentin,Diclofenac	0	0	36 hr	3
6s	2.5	Diaphragmatic hernia repair	F	6.5	Mid 0-4, Mor 20-40, Vec 0-10, Amikacin, Ben pen, Metronid, Nebs	0	0	36 hr	3
7s	6.17	Dilatation of gastro-oesophageal stricture	M	0.7	Mid 0-3, Mor 10-40, Metronid, Amikacin,Ben pen	0	0	48 hr	4
8s	2.5	Gastric interposition	M	2.9	Moid 0-6, Mor 0-60, Vec 0-4, Metronid, Amikacin, Teicoplanin, Frus, Diclofenac	0	0	48 hr	3

Table 3.3 Surgical patient details

Patient	AGE in years	Diagnosis	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
9s	0.58	Tracheal surgery	M	3.3	Mid 1-6, Mor 10-40, Vec 0-4, Augmentin, Amikacin, Piptaz	0	Pred*, Dex*	48 hr	4
10s	1.41	Gastric interposition	F	1.4	Mid 0-6, Mor 10-60, Vec 0-6, Metronid, Ben pen, Amikacim, Diclofenac	Dop 0-5	0	96 hr	5
11s	10.1	Perforated diaphragmatic hernia - developed sepsis subsequently	M	13	Mid 1-6, Mor 10-40, Metronid, Ben pen, Amikacin, Fluconazole, Frus, Piptazo	Dop 0-20	0	120 hr	7
12s	1.0	Elective craniosynostosis surgery - arrest	M	4.8	Mid 0-3, Mor 0-40, Piptazo, Ceftax, Frus, Nebs	Dop 0-15	0	adm	1

3.1.4 Clinical management of other diagnostic categories

Management of other diagnostic categories was disease specific. Drugs and dosages administered are listed in the patient table. Diagnoses here included neurological conditions eg. status epilepticus, mycoplasma encephalitis.

Patient details are shown in Table 3.4.

Table 3.4 Patient details - other diagnostic categories

Patient	AGE in years	Diagnosis	Sex	PROM %	Drugs	Vasoactive drugs	Steroids	Length of study	No. of samples
1n	12.5	Mycoplasma encephalitis	F	1.0	Propofol 0-3, Piptazo, Amikacin, Phenytoin	0	0	24 hr	2
2n	4	Mycoplasma encephalitis	M	3.3	Mid 0-4, Mor 0-40, Acyclovir, Cefotax, Erythro	0	0	24 hr	3
3n	1.17	Status epilepticus – blind	M	0.8	Mid 0-2, Mor 0-20, Phenytoin, Ceftaz	0	Dex*	24 hr	3
4n	8.8	Encephalitis- generalised infection	M	77	Mid 0-4, Mor 0-80, Vec 0-4.3, Amik, Piptazo, Frus	Dop 0-5, Nadr 0-2.3, Adr 0-2.7.	0	48 hr	3

3.2 Measurements conducted and data collected

Following entry into the study, by obtaining written consent from the parents, relevant details about each child including their age and diagnosis were recorded. Serial blood sample collection was made in each child, as soon as possible after admission to the PICU and was repeated every 6-24 hours until the child was extubated or until the end of the study period (whichever came first). Blood was sampled from indwelling arterial lines inserted by the clinical staff in order to aid patient management. Processing of the blood samples is described in chapter 3. At St. Marys hospital, blood samples were collected 8 hourly over 40 hours. However, at the Great Ormond Street Hospital samples were collected at 12 hourly intervals in children >10kg in weight and every 24 hours in children <10kg in weight.

All measurements were made during periods of clinical stability. Recordings of blood pressure, level of inotropic support and medication at time of sampling were made. In addition, plasma electrolytes and volume of colloid infused were recorded in the patients with meningococcal sepsis.

3.3 Severity Scoring System

Severity of illness is a familiar medical concept, although it is often difficult to define. Numerous severity scoring systems exist, often being disease specific e.g. Glasgow Meningococcal Septicaemia Prognostic Score – (GMSPS), intervention specific e.g. Surgical Severity Score or system specific e.g. Glasgow Coma Score. In the context of intensive care, where multiple diagnostic categories are present, a rational and objective unifying approach to define and quantify severity of illness is through the development of probability models predicting mortality risk (145).

The model to assess illness severity in the present study also needed to be specific to the paediatric age group, widely used, accepted and validated, so that comparisons and correlations with the metabolic and endocrine milieu may be clinically meaningful.

The scoring system to asses illness severity adopted for the present study was the Predicted Risk of Mortality Score calculated from the Paediatric Risk of Mortality (PRISM) score II (146).

The PRISM score had been developed from the Physiologic Stability Index (147) by reducing the number of physiologic variables required for the paediatric ICU mortality risk assessment and by obtaining an objective weighting of the remaining variables. This was conducted by Pollack et al, using univariate and multivariate statistical techniques to the admission day physiological stability index data obtained (n= 1,415 patients, 116 deaths) from four PICUs. The basis for the physiological stability index was based on the hypothesis that physiolgical instability directly reflects mortality risk (148). The resulting PRISM score consists of 14 routinely measured, physiologic variables, and 23 variable ranges. These are listed in chapter 3 along with the relevant scoring system (Table 3.4). PRISM II scoring system is an internationally used and accepted scoring system in PICU. Subsequent modifications and improvements have however resulted in the design of the PRISM III scoring system. This is however, a commercial package and has not been installed by many PICUs including the PICUs at Great Ormond Street Hospital and St. Marys Hospital from where the subjects entered into this study were recruited.

3.3.1 Predicted Risk of Mortality (PROM)

The predicted risk of mortality (PROM) may be calculated from the PRISM score, age and operative status using a linear logistic formula (149). This mortality prediction model was tested and validated in a large sample from six PICUs (n=1,227 patients, 105 deaths). The severity of critical illness in this study is expressed as a percentage.

Table 3.4 PRISM Score

Variable	Age restrictions and ranges		Score
Systolic BP (mmHg)	Infants	Children	
	130-160	150-200	2
	55-65	65-75	
	>160	>200	6
	40-54	50-64	
	<40	<50	7
Diastolic BP (mmHg)	all ages		6
	>110		
HR (beats/min)	Infants	Children	
	>160	>150	4
	<90	<80	
Respiratory rate (breaths/min)	Infants	Children	
	61-90	51-70	1
	>90	>70	5
PaO_2/Fio_2[a]	all ages		
	200-300		2
	<200		3
$PaCO_2$[b] (torr)	all ages		
	51-65		1
	>65		5
Glasgow Coma score[c]	all ages		
	<8		6

Pupillary reactions	all ages	
	Unequal or dilated	4
	Fixed and dilated	10
PT/PTT	all ages	
	1.5 x control	2
Total bilirubin (mg/dl)	>1month	
	>3.5	6
Potassium (mEq/L)	all ages	
	3.0-3.5	1
	6.5-7.5	
	<3.0	5
	>7.5	
Calcium (mg/dl)	all ages	
	7.0-8.0	2
	12.0-15.0	
	<7.0	6
	>15.0	
Glucose (mg/dl)	all ages	
	40-60	4
	250-400	
	<40	8
	>400	
Bicarbonate[d]	all ages	
(mEq/L)	<16	3
	>32	

a Cannot be assessed in patients with intracardiac shunts or chronic respiratory insufficiency; requires arterial blood sampling

b May be assessed with capillary gasses

c Assessed only when there is known or suspected CNS dysfuction; cannot be assessed in patients with iatrogenic sedation, paralysis, anaesthesia,etc. Scores <8 correspond to coma or deep stupor.

d Use measured values.

Chapter 4 - Statistical analysis.

4.1 Database of results

A total of 236 serial measurements of intermediary metabolites, hormones and cytokines were performed on 60 critically ill children. Clinical information on each patient, corresponding to each time point of sampling was also collated.

A database of all results and clinical parameters was compiled in Excel 97, providing over 4000 data points. An extensive database has enabled the study of the following:

1. Aberrations in the metabolic and endocrine parameters on admission to PICU – cross-sectional data
2. Changes associated with age, severity and patient diagnosis
3. Kinetics or rates of change of parameter with time from admission to PICU and factors affecting this – longitudinal data
4. Study of individual patients with time, where the response differed to that expected
5. Interrelationships of different hormone systems
6. Examination of specific hormonal and metabolic axes (eg. GH/ IGF-1 axis, HPA axis and lipid metabolism) in critical illness in childhood.

4.2 Description of changes in metabolic and endocrine milieu at presentation to PICU (t=0)

The raw data were examined and variables which had skewed distributions underwent logarithmic transformation before analysis to normalize the distributions.

Continuous variables were summarised as the mean ± SEM.

ANOVA or analysis of variance was used to compare between diagnostic groups.

4.2.1 Scatter diagrams

Scatter diagrams were used to visualise data and draw preliminary conclusions about the possibility of a relationship between the variables under investigation, namely hormone and metabolite concentrations as the dependent variables and severity of illness, as defined e.g. by PROM or age of patients as the independent variable. Associations between hormones and metabolites at t=0 were also investigated. Examples of the scatter diagrams obtained are shown in Figure 4.1.

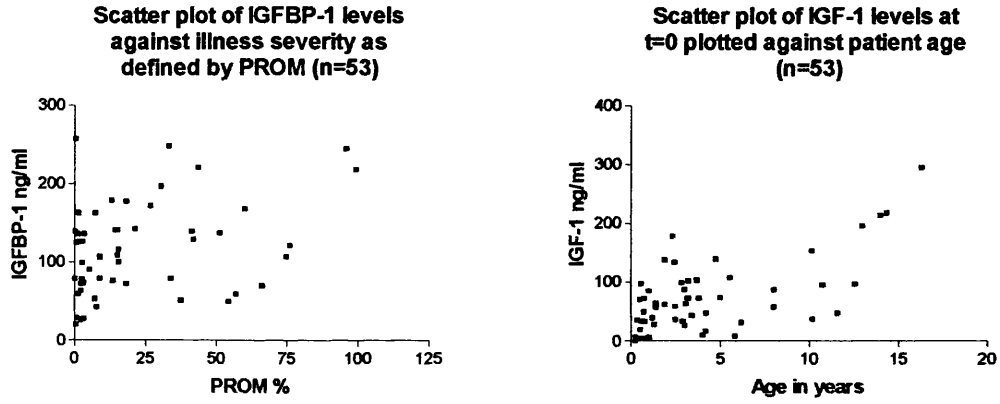


Fig 4.1 Scatter plots of a) IGFBP-1 levels plotted against PROM at t=0, and b) IGF-1 plotted against patient age at t=0

4.2.2 Correlations

The raw data were examined and variables which had skewed distributions underwent logarithmic transformation before analysis to normalize the distributions and to reduce the effect of skewness on the correlations.

Linear association between variables was determined by correlation analysis. The Pearson product moment correlation was used with statistical significance defined as $p < 0.05$. Examples of correlations obtained are shown in Figure 4.2.

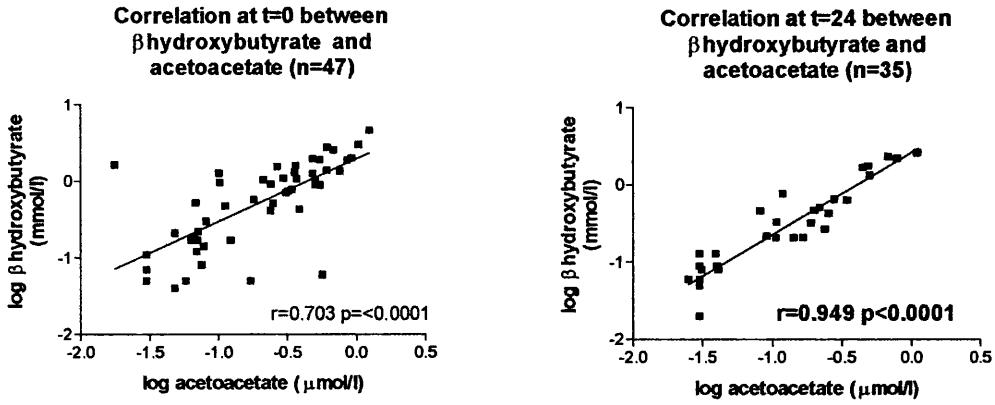


Fig 4.2 Correlations (Pearson product moment) showing the linear association between variables. a) Correlation at t=0 between β-hydroxybutyrate and acetoacetate (n=47) and b) Correlation at t=24 between β-hydroxybutyrate and acetoacetate (n=35).

4.2.3 Logistic regression

To explore whether the meningococcal diagnostic category differed significantly from the other critically ill children in terms of the pattern of metabolic and endocrine disturbance the linear model logistic regression was applied, with the binary outcome variable meningococal or not. Significant differences were quoted as p values.

4.2.4 Factor Analysis

Using the above mentioned parameters factor analysis was attempted. It was not possible to reduce the variables at t=0 to 3 or 4 vectors accounting for 90% of the variation.

4.3 Analysis of longitudinal data

The use of summary statistics in medicine has received more emphasis in the past decade (150).

Scatter diagrams were used to visualise data and review the relationship between hormones and metabolites and time. Each line was drawn to represent serial measurements obtained from each child. Reference ranges were displayed as shaded background Fig 4.3.

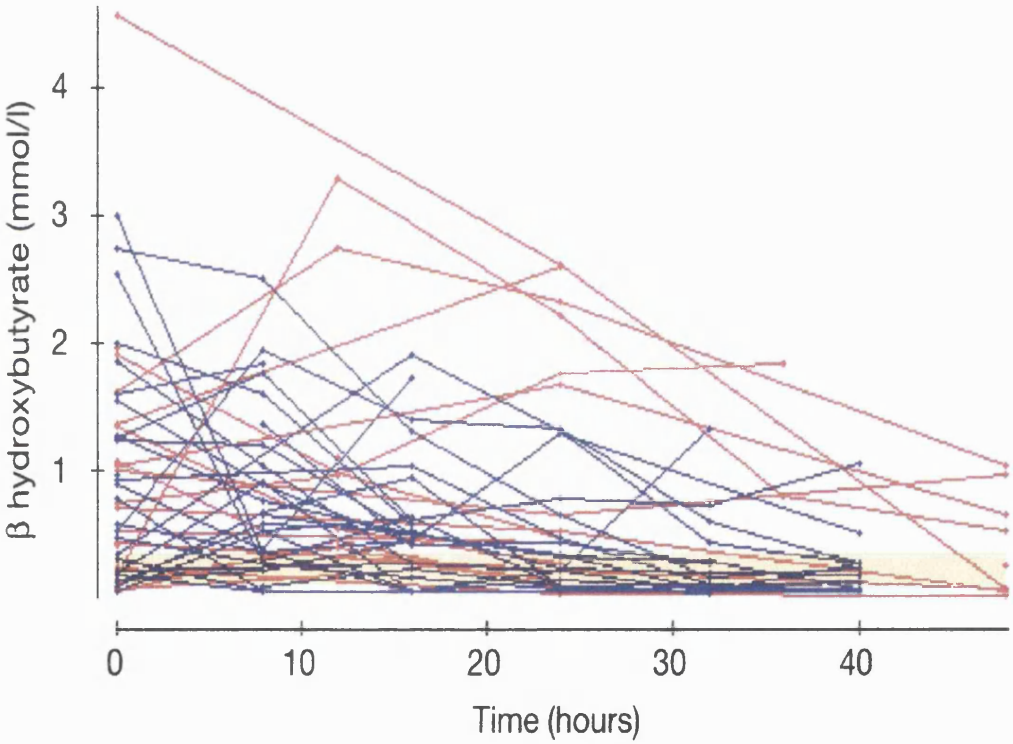


Fig 4.3 Display of longitudinal data. Scatter diagrams were used to display hormone or metabolite level against time. Each line was drawn to represent serial measurements obtained from each child. Reference ranges were displayed as shaded background.

Normal ranges of several of the parameters vary with age. These variables were also plotted against age. Each line was drawn to represent serial measurements obtained from each child. Again, reference ranges were displayed as shaded background. The percentage of values lying outside the normal range was calculated for time points t=0 (on admission to PICU) and at t=24 hours (24 hours post admission to PICU).

The data, given in the original units was therefore clinically meaningful and easily comparable.

Means and standard errors for each of the parameters were calculated at both t=0 and t=24. The paired t test was used to compare values at these time points. Significance level was taken as $p<0.05$.

Differences in parameter level between different diagnostic categories at the two time points were assessed using the 2-sample t-test. Significance level was taken as $p<0.05$.

The rate of change of parameter was assessed by regressing the difference of the values at t=0 and t=24 on the baseline value (t=0). This gave the rate of change of parameter over the first 24 hours from admission independent of the baseline value, thus adjusting for the regression to the mean. By introducing subcategories within this model, the effect of diagnostic category and the effect of age could be examined in turn, the results representing the differences in mean group change.

Repeated measures ANOVA analysis was attempted, in order to describe whether the changes in the variables examined were related to the diagnostic category of the patient. Unfortunately the study had been designed that blood sampling had occurred in the meningococcal cohort at 8-hourly intervals and in the other diagnostic categories at 12 to 24 hourly intervals. Occasinal missing values also contributed to the difficulty and the repeated measures analysis did not compute.

4.2 Computer programmes

The programmes Datadesk 6.1 for Windows (Data Description inc., Ithaca, NY, U.S.A), Excel 97 for Windows and Statistica 5.0 for Windows were used for the data analysis. For graphical display the program Graph Pad PRISM 3 was used.

Chapter 5 - The metabolic and endocrine milieu (MEM) of critically ill children at presentation – lack of correlation with clinical severity scores.

5.1 Introduction

Adaptation to the stress of critical illness evokes a variety of biological responses as a result of disturbance in the body's homeostasis. It has long been recognised that the metabolic and endocrine axes are thought to respond in a coordinated manner in order to improve healing and speed recovery. The acute changes in the metabolic and endocrine axes are triggered by the release of catabolic hormones (30) with an emergence of insulin resistance (18) together with low plasma IGF-1 and T3 levels (32-35). These include hyperglycaemia due to increased hepatic glucose output (36), fat becoming the major body fuel through intensive lipolysis and increased oxidation of fatty acids (37) and accelerated breakdown of muscle proteins (38-40).

In contrast to the wealth of information on the stress response in adults, very little is known about the stress response to critical illness in children.

Severity is known to be a key driver for the stress responses at all ages, determining their magnitude (14). Studies described in the literature to date in children relate different severity of illness scoring systems to the metabolic and endocrine responses in discrete diagnostic categories (114;121-123). Thus the hormonal and metabolic responses to surgery in neonates and children relate to the Surgical Severity Score (14;121); medical illness is known to produce a clear correlation between the increase in plasma cortisol values and illness severity score (114). There is also correlation between the Glasgow coma score and admission hormone concentrations in head injury children (123) and between burn surface area and admission hormone levels in children suffering from burns (122).

The severity scoring systems routinely used in paediatric intensive care units include PRISM score (Prospective Risk of Mortality Score) (147), PROM (Predicted Risk of Mortality Score) (149) and PIM (Paediatric Index of Mortality) (151), which are based on changes in a range of physiological parameters and are thought to be diagnosis-independent. Other severity scores in use are designed specifically for a single diagnostic category for instance the GMSPS score (Glasgow Meningococcal Septicaemia Prognostic Score) (152;153). None of these methods incorporate measurement of endocrine variables and in some only a few metabolic parameters (blood glucose, bicarbonate) are incorporated. It is not known whether these predictive scores reflect patterns of metabolic and endocrine stress.

One of the aims of the present study was to define in a comprehensive manner the profiles of metabolic and endocrine parameters at presentation of critically ill children in relation to:

1. disease entity
2. clinical severity – (as defined by PROM calculated from the PRISM score).

5.2 Patients

The study was performed on 55 children on admission to paediatric intensive care. Group A consisted of 31 children with meningococcal disease, mean age 4.2 years (range 0.5-14.4) with predicted risk of mortality calculated from the PRISM score, PROM mean 32.3% (range 1.3-99.3%), Group B consisted of 13 critically ill children with respiratory infection, mean age 3.4 years (range 0.3 - 12.5), PROM mean 9.7% (range1.8-34.0%) and Group C, consisted of 11 critically ill children following surgery (mainly gastrointestinal and laryngeal) mean age 5.3 years (range 0.5 - 16.3) with PROM mean 6.1% (range 2% - 33%) (Table 5.1). Patient 12s was recruited to the study later and therefore not included in this chapter.

Table 5.1 Details of 55 patients assessed on admission to PICU.

	Diagnosis	Number of patients	Age in years	PROM
Group A	Meningococcal disease	31	4.2 y range (0.5-14.4)	32.3% r (1.3-99.3%)
Group B	Respiratory infection	13	3.4 y range (0.3-12.5)	9.7% r (1.8-34.0%)
Group C	Surgical patients	11	5.3 y range (0.5-16.3)	6.1% r (2.0-33.0%)

Children who had been ill for >24hours, had pre-existing renal, liver, metabolic or immunological disease were excluded from the study. The study was approved by the local Research Ethics Committee and informed parental consent for the studies was obtained.

5.3 Processing of blood samples and laboratory analysis

Blood samples were taken from indwelling arterial cannulae inserted for clinical monitoring and management purposes on or soon after admission to PICU. For the measurement of blood lactate, pyruvate and acetoacetate levels, blood was precipitated in perchloric acid (500µl of 0.7M perchlorate). Blood samples were centrifuged immediately and the plasma fraction separated and stored for measurement of insulin, GH, IGF-1, IGFBP-I, cortisol, aldosterone, T4 and TSH levels. For the measurement of glucagon blood samples were collected into tubes containing trasylol. All samples were stored at –70°C until analyses were performed.

5.4 Statistical analysis

Continuous variables were summarised as the mean ± SEM. Statistical significance was defined as $p<0.05$. Logistic regression of the diagnostic groups, ANOVA and correlations were performed. Skewed distributions were logged before analysis. The programme Datadesk 6.1 for Windows was used for these analyses.

5.5 Results

The data on the plasma or blood levels of intermediary metabolites lactate, pyruvate and acetoacetate and hormones cortisol, aldosterone, GH, IGF-1, IGFBP-1, insulin, glucagon, TSH, T4 are given as mean ± SEM in Table 5.2a and b. These results are subdivided by diagnostic category into Groups A, B and C and then given for all 55 patients collectively. Based on the plasma levels of cortisol in the 55 children from the three groups, all the children presented evidence of endocrine stress with mean plasma levels of 721 ± 56 nmol/l, where an appropriate level of plasma cortisol in response to a stressor is taken as 500nmol/l or above (154). Indeed, many of the other parameters lay outside the normal range (dicussed in detail in chapter 6).

5.5.1 The influence of diagnostic categories

In order to determine whether there was a significant difference in the hormonal or metabolic profile between the meningococcal patients and all other critically ill children, logistic regression was applied. The results, show that plasma aldosterone levels are significantly lower ($p<0.0003$) and plasma cortisol levels higher ($p<0.04$) in Group A (meningococcal disease). No significant differences were found between the groups for any other variables.

In order to establish whether the mean hormone and metabolite levels were different between the three diagnostic categories, ANOVA was applied and the results are given in Table 5.2a and b.

	HPA Axis		GH Axis			Pancreatic hormones		Thyroid Axis	
	Cortisol nmol/l	Aldosterone pg/ml	GH mU/L	IGF-1 ng/ml	IGFBP-1 ng/ml	Insulin µIU/ml	Glucagon pg/ml	TSH mU/l	T4 nmol/l
Group A (n=31)	799.51 ± 75.94	427.5 * ± 88.16	34.75 ± 7.71	84.32 ± 9.81	112.7 ± 9.6	20.38 ± 4.53	126.78 ± 15.36	0.98 ± 0.13	59.05 ± 2.59
Group B (n=13)	634.8 ± 124.8	1373.2 ± 389.9	27.1 ± 6.26	41.69 ± 10.0	99.36 ± 11.2	20.63 ± 4.91	85.00 ± 11.10	2.15 ± 1.05	65.92 ± 7.89
Group C (n=11)	594.0 ± 93.0	1506.9 ± 400.1	20.74 ± 5.7	66.8 ± 26.7	137.81 ± 26.36	10.93 ± 2.74	173.41 ± 75.80	1.26 ± 0.36	55.72 ± 8.28
Groups A +B +C (n=55)	721.81 ± 56.15	843.1 ± 140.8	30.14 ± 4.74	70.81 ± 8.07	113.71 ± 7.71	18.69 ± 2.91	126.09 ± 17.35	1.31 ± 0.27	60.01 ± 2.85

Table 5.2a Hormone levels in critically ill children on admission to PICU. Values given for each group (A= meningococcal diagnostic category, B= respiratory patients, C= surgical patients) are expressed as mean ± SEM.

Using ANOVA the following significant differences were found between the means in the patient groups:

*Aldosterone Plasma aldosterone values were significantly lower in Group A, p=0.0009

No significant differences were found between the groups for and other variables.

	Metabolites		
	Lactate mmol/l	**Pyruvate mmol/l**	**Acetoacetate mmol/l**
Group A (n=31)	1.65* ± 0.33	0.084 ± 0.034	0.398 ± 0.084
Group B (n=13)	1.01 ± 0.17	0.035 ± 0.0039	0.239 ± 0.047
Group C (n=11)	0.79 ± 0.09	0.085 ± 0.036	0.414 ± 0.119
Groups A +B +C (n=55)	1.18 ± 0.14	0.068 ± 0.016	0.348 ± 0.0496

Table 5.2 b

Metabolite levels in critically ill children on admission to PICU. Values given for each group (A= meningococcal diagnostic category, B= respiratory patients, C= surgical patients) are expressed as mean ± SEM.

Using ANOVA the following significant differences were found between the means in the patient groups:

*Lactate Plasma lactate values were significantly higher in Group A, p=0.0290

No significant differences were found between the groups for and other variables.

5.5.2 The influence of clinical severity

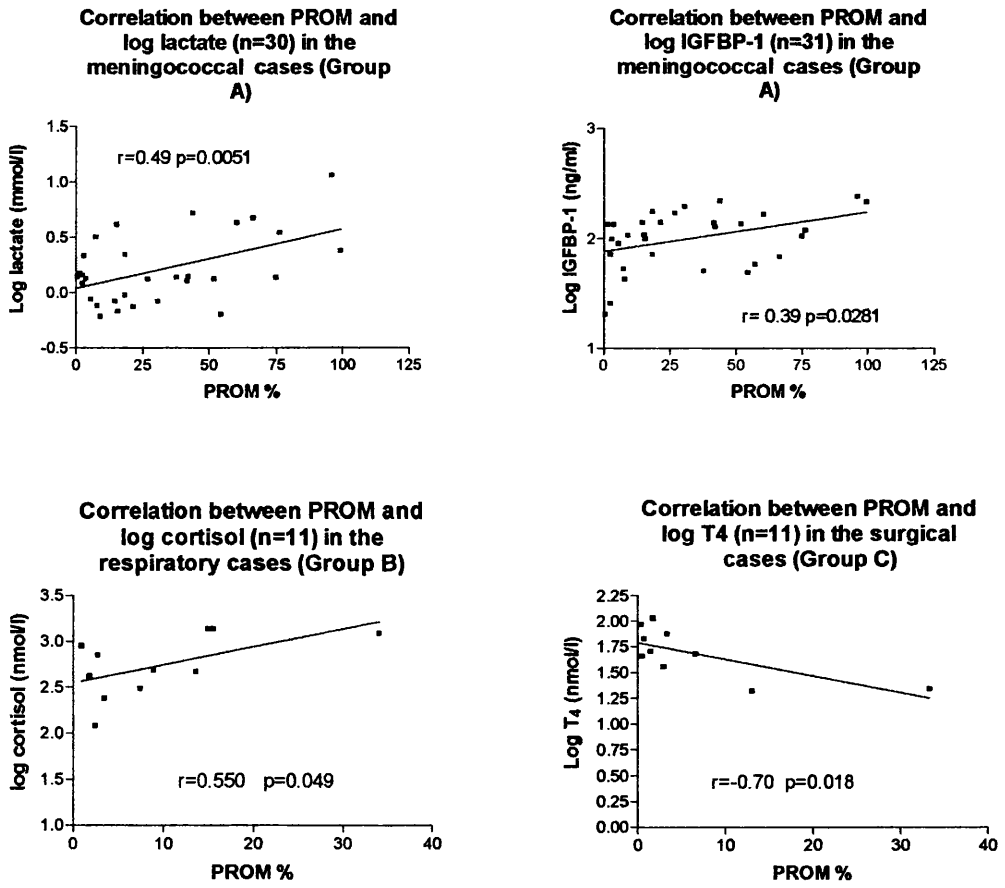
To assess whether there was any correlation between the metabolic and endocrine milieu at presentation and severity of illness as predicted by the PROM score, Paerson Product Moment Correlations were applied to the diagnostic groups A (meningococcal disease), B (respiratory disease) and C (surgical cases) versus plasma lactate, pyruvate, acetoacetate, plasma insulin, GH, IGF-1, IGFBP-I, cortisol, aldosterone, glucagon, T4 and TSH levels. Statistically significant correlations defined as having $p<0.05$ observed in the overall analysis are summarised in Table 5.3. No other significant correlations were found between all other variables and all three

groups. Graphs of the data presenting significant correlations are shown in Fig 5.1.

Table 5.3 Statistically significant correlations between specific metabolic and endocrine variables and the PROM score of severity of critical illness at the time of presentation to PICU.

Patient Group	Correlations between hormone or metabolite concentration and PROM score.		
	Hormone/ metabolite	r	p value
Group A (n=31) (meningococcal disease)	Log lactate	0.49	0.005
	Log IGFBP-1	0.39	0.028
Group B (n=13) (respiratory disease)	Log cortisol	0.55	0.049
Group C (n=11) (surgical cases)	Log T4	-0.70	0.018

Fig 5.1 Graphs of statistically significant correlations between specific metabolic and endocrine variables and the PROM score of severity of critical illness at the time of presentation to PICU.



5.5.3 Correlations between plasma lactate levels and other MEM variables

Plasma concentrations of lactate were compared with plasma levels of all other hormones and metabolites such as pyruvate, acetoacetate, plasma insulin, GH, IGF-1, IGFBP-I, cortisol, aldosterone, glucagon, T4 and TSH levels in the three groups of patients and in all patients taken as a whole (groups A + B + C), at the time of presentation to PICU. Except those listed in Table 5.4, there were no significant correlations between plasma lactate

and other MEM variables. Significant correlations are defined as having $p<0.05$. The graphical data supporting this summary is given in Fig 5.2.

Table 5.4 Statistically significant correlations between plasma lactate concentration and plasma levels of MEM variables at the time of presentation.

Patient Group	**Correlation between log lactate and other MEM variables**		
		r	p value
Group A Meningococcal patients n=31	Log Acetoacetate	-0.53	0.0023
	Log Pyruvate	0.63	0.0001
	Log Glucagon	0.42	0.0233
	Log Insulin	0.36	0.0448
	Log IGF-1	0.43	0.0153
Group B Respiratory patients n=13	Log Aldosterone	0.64	0.0323
Group C Surgical patients n=11	Log GH	-0.83	0.0017
Group A+B+C All critically ill children n=55	Log Pyruvate	0.49	0.0002
	Log IGF-1	0.43	0.0014

Variables examined were as follows: pyruvate, acetoacetate, plasma insulin,

GH, IGF-1, IGFBP-I, cortisol, aldosterone, glucagon, T4 and TSH levels.

No other significant correlations were found between log lactate levels and the other variables.

Graphs of the significant correlations are shown in Fig. 5.2.

Fig 5.2 Statistically significant correlations between plasma lactate concentrations and plasma levels of other MEM variables at time of presentation to PICU

a) in children with meningococcal disease (GroupA).

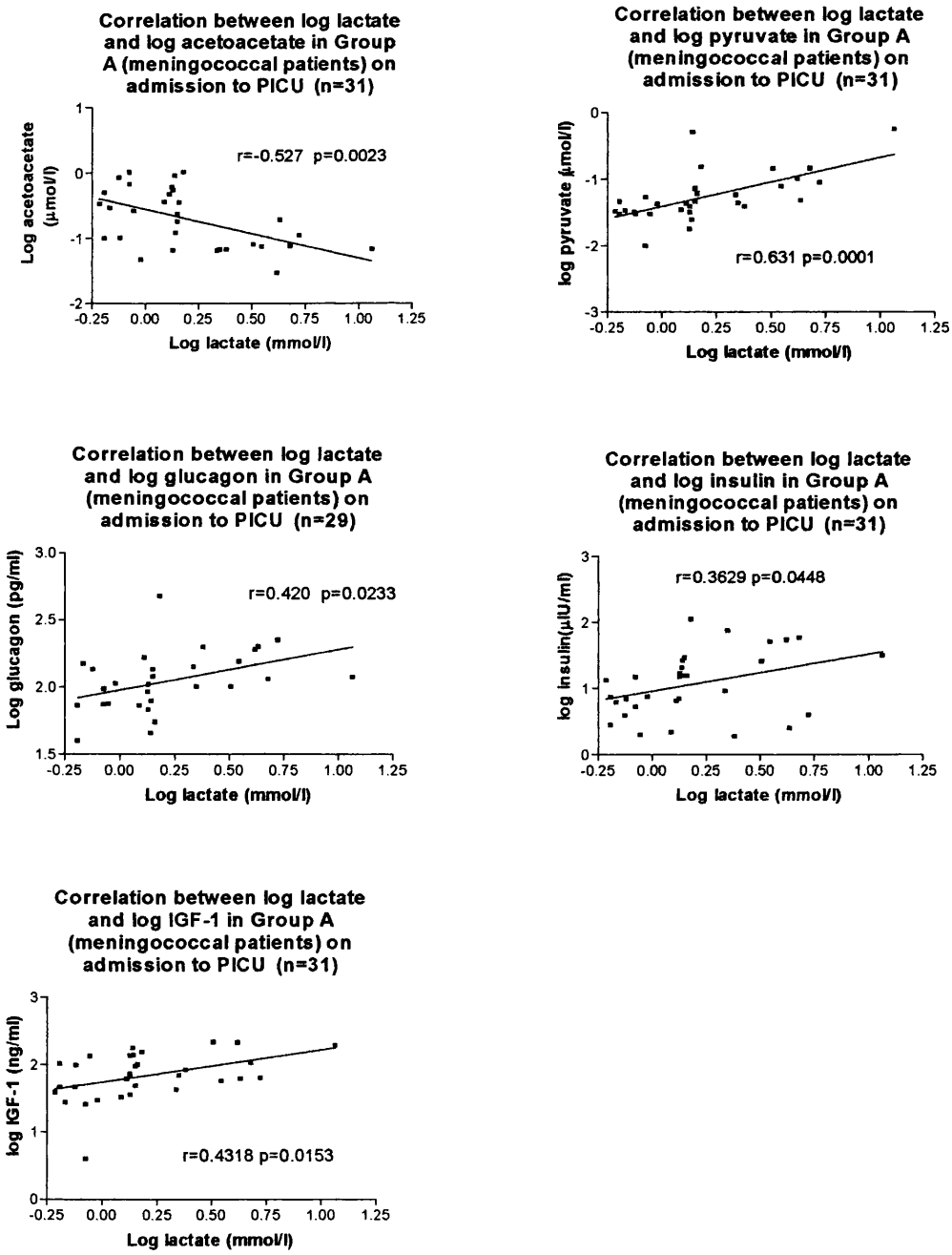


Fig. 5.2 b in critically ill respiratory patients.

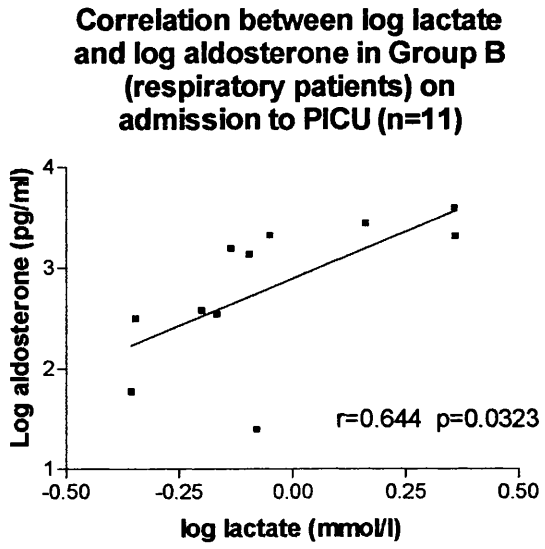


Fig 5.2 c in critcally ill children post surgery.

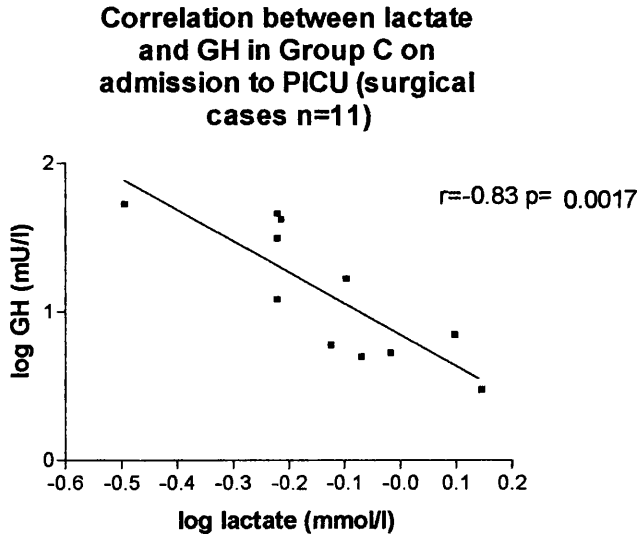
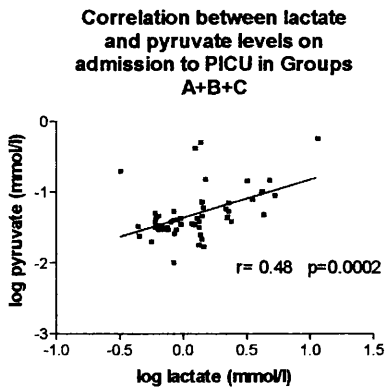
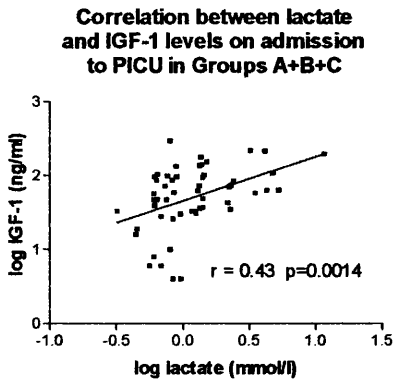


Fig 5.2 d in all 55 critically ill children





5.8 Discussion

As an evolutionary innovation, a multitude of stress responses resulting through different adverse conditions invoke coordinated metabolic, neuroendocrine and immune interactions in order to restore the homeostasis of the body and to improve healing and speed recovery. Whilst this response portrays a survival advantage, the quality and magnitude of the stress responses vary depending on the age (114), the severity of the physiological insult (14), the stimulus (124) and genetic polymorphism (127;128). However, the stress response in both adult and paediatric patients undergoing modern intensive care, could neither be the extension nor the outcome of the fundamental evolutionary process. These patients would not have survived without the intensive care interventions, and the beneficial response to stress of illness may be either exaggerated or inappropriate. Moreover, it may be influenced by organ failure, which may contribute substantially to morbidity and mortality.

At present there are two major problems for the paediatrician in managing the critically ill child. First, little is known of the patterns of the stress response in relation to the diagnosis, age and severity of illness, and second, the present protocols and physiological profile systems for predicting outcome have not been fully assessed in relation to the metabolic and endocrine milieu. A clear understanding of the sequelae of

pathophysiology is necessary, so that appropriate management can be designed and applied to improve outcome. Premature intervention may be detrimental to the patient as reported in recent studies that the GH therapy given to reverse catabolism in critically ill adults leads to increased mortality (140).

Children offer a specific challenge since their homeostatic processes vary with age (114) and their resting energy expenditure rates and protein turnover are higher than in adults (155;156).

5.8.1 Severity of illness scoring systems

Mortality prediction models such as PRISM (90), PROM (149) and PIM (151) are often used to describe severity of illness in groups of patients. Attempts have been made to simplify them for ease of use, to improve accuracy and to reflect patient prognosis for example, the Algren criteria for meningococcal septicaemia (157).

These prognostic scores are based on the assumption that outcome is dependant on the degree to which physiologic homeostasis was deranged prior to or at the time of institution of critical care (158). They do not have, however, sufficient sensitivity or specificity necessary for clinical decisions to be made on individual patients.

PROM is adjusted for patient operative status, but not diagnosis. Current severity scoring systems do not take into account the nature of underlying disease, and do not include endocrine and only few metabolic parameters in their generation.

There is little correlation between indices of metabolic and endocrine stress and PROM, as demonstrated in the results section. Correlations assume a linear relationship. However, there is also no evidence from the present study to suggest that a non-linear relationship exists for either PROM or hormones/metabolites levels. However, the arterial blood lactate levels do

appear to reflect severity to a greater extent, especially in specific diagnostic groups. It is not clear whether these arterial lactate levels reflect generalised impaired peripheral circulation or impaired hepatic clearance, however, whatever the mechanism, they appear to show some correlation with the degree of metabolic and endocrine derangement and may therefore be a useful measure of severity in relation to MEM.

MEM is known to be related to other severity scoring systems such as surgical severity score (14), illness severity score (114), surface area of burn in children (122) at presentation.

There were no deaths in this study, therefore the MEM of survivors and non-survivors could not be compared.

In the light of the above results it is possible to propose that the metabolic and endocrine milieu of critically ill children among the different diagnostic groups may describe aspects of patient severity and outcome not yet addressed by PROM scoring. Development of new scoring systems describing the extent of multiorgan dysfunction, including the metabolic and endocrine systems, may be required to monitor the progress of patients in PICU, whereas the PROM score is inherently sensitive to correction by administration of resuscitative therapy. It is however necessary to define which metabolic and endocrine derangements play a crucial role in recovery and which represent secondary effects.

It remains to be established whether the MEM at presentation, or indeed throughout the intensive care period of treatment, relates to subsequent morbidity and mortality. The process of outcome description, however, in critical illness is still in its infancy (158), and no single measure exists to integrate the impact of morbidity in survivors and mortality for non-survivors. Knowledge of correlates of patient outcome and the MEM might lead to new therapeutic strategies to improve on morbidity and mortality.

Accurate characterisation of disease states, or pathophysiological processes with which they are associated, in a way that accurately defines prognosis remains a goal for the future (147).

5.8.2 Diagnostic group differences

Significant differences have been demonstrated in the MEM at presentation of critically ill children among different diagnostic categories in this study. Of particular interest are the observations that the children with meningococcal disease had lower plasma aldosterone levels despite higher plasma cortisol levels. These distinctive differences and features have not been reported previously.

It can be concluded that there may exist unique endocrine signatures that distinguish different pathophysiological processes. Further studies are therefore required to investigate such mechanisms and functional effects of these differences in order to determine whether these may be of prognostic significance.

The low plasma aldosterone levels as demonstrated in meningococcal sepsis, are unexpected particularly in view of vascular depletion. These may be indicative of failing adrenal reserve. This circumstance has been described in critically ill adults, although not diagnosis specific, where steroidal precursors such as pregnenalone are diverted away from mineralocorticoid synthesis (84) and adrenal androgens (85) towards maintaining glucocorticoid levels. Failing adrenal reserve has in itself been shown to be associated with greater patient morbidity. In children with meningococcal disease basal levels of cortisol are elevated and their response to synacthen stimulation is sub-optimal and is associated with increased vasopressor requirement (159;160). Physiological replacement of hydrocortisone has also been shown to reverse hyperdynamic septic shock (161) in critically ill adults.

Low plasma aldosterone levels may therefore be of prognostic significance, describing evolving adrenal failure, which may be part of multiorgan dysfunction in evolving sepsis. Further studies are required to determine the nature of this phenomenon and the possible benefit of introducing mineralocorticoid replacement. The issue of lower plasma aldosterone in the meningococcal disease category is addressed in chapter 8.

5.8.3 Lactate in critical illness

Bacterial sepsis, haemorrhage and trauma are frequently accompanied by increased blood concentration of lactic acid, which traditionally is attributed to poor tissue perfusion, hypoxia and anaerobic glycolysis. Tissue hypoxia is thought to contribute to the development of multiorgan failure and the severity and duration of the increased blood lactate levels are known to correlate with death (162). Elevated blood lactate can therefore be useful in prognosis. In this study lactate was used as an index of illness severity.

The positive correlations observed between plasma lactate and aldosterone levels in group B (respiratory diagnoses) would appear to support the above hypothesis, where increasing amounts of aldosterone are released in response to increasing levels of hypoperfusion, which is itself associated with tissue hypoxia, increased rates of anaerobic glycolysis and therefore increased lactate production. In group C there was also a positive correlation between plasma lactate and aldosterone levels, although it did not achieve statistical significance ($r=0.409$, $p=0.2407$).

Patients with meningococcal sepsis are known to have poor perfusion, hypotension and olguria due to loss of intravascular circulating volume and decreased cardiac function. These may be expected to activate the renin-angiotensin system and increasing aldosterone release and lead to increasing levels of lactate as a consequence of tissue hypoxia. Yet, it is clear from the results in Group A (meningococcal sepsis), that no such association was found between plasma aldosterone levels and lactate.

There could be two speculative reasons for the present observations.

First, the plasma aldosterone values are lower than expected, given the clinical scenario, and might be, as discussed, a feature of failing adrenal reserve, specifically in this illness category (84;85). The issue of adrenal funcion in meningococcal sepsis is discussed in detail in chapter 8.

The second explanation is that lactate itself may not be a reliable indicator of tissue hypoxia in sepsis. Clinical observations have challenged the accepted notion of equating blood lactate with hypoperfusion (163). Indeed,

in this study, the meningococcal patients were initially resuscitated (Fig 5.3), so that hypotension was corrected with volume replacement and inotrope infusions, urine output was restored, as was patient oxygenation by increasing FiO_2. Despite these corrections lactate levels remained higher in group A then in the other diagnostic categories ($p<0.05$) (see Fig 6.1).

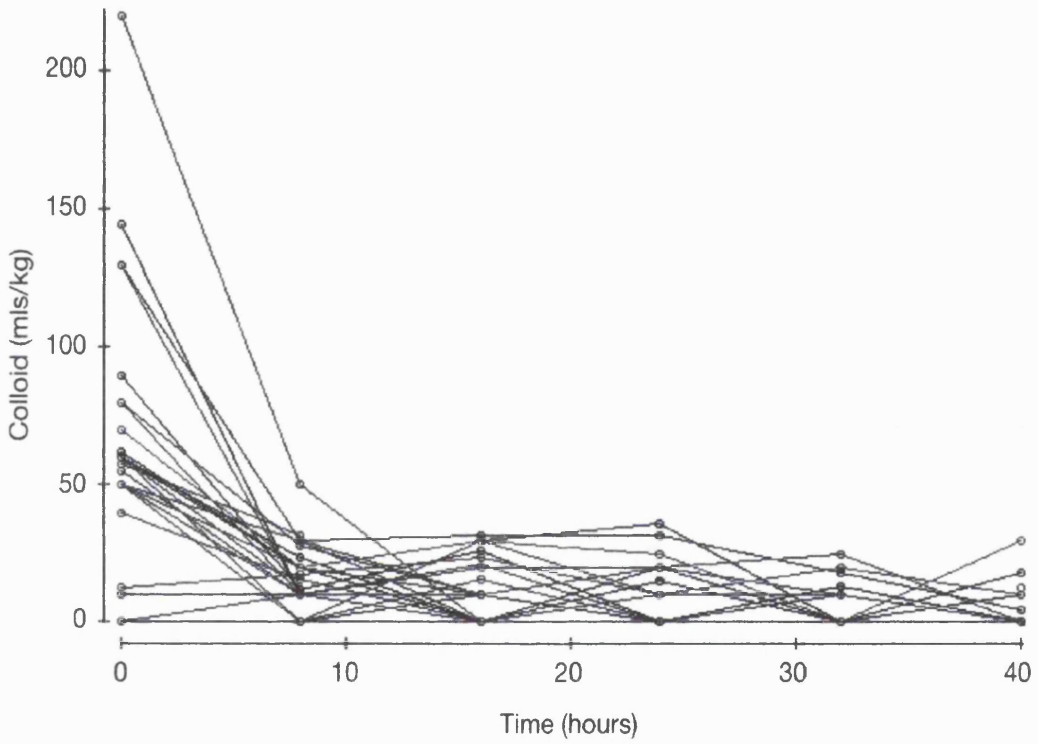


Fig 5.3 Volume of colloid (mls/kg) given in the critically ill patients with meningococcal sepsis so that in conjunction with inotropes hypotension was corrected and urine output was restored. Each line represents a patient.

Until recently, mechanisms of lactate generation by well-oxygenated tissues have received little attention. However in 2002, Luchette et al, reported that hypoxia was not necessarily the sole cause of hyperlactatemia during and after haemorrhagic shock. Their in vitro studies have shown that increased Na^+-K^+ATPase activity during adrenaline treatment or hemorrhage

contributed to increased muscle lactate production (164) through increased aerobic glycolysis.

Within cells, oxidative and glycolytic energy production can proceed in separate, independent compartments. In skeletal muscle and other tissues, aerobic glycolysis is linked to ATP provision for the Na^+-K^+ pump, the activity of which is stimulated by adrenaline. In septic or injured patients, hypokalaemia may reflect increased Na^+,K^+-ATPase activity (165). Vanek et al observed that more than half of their randomly selected trauma patients presented with hypokalaemia, the degree of which was associated with the severity of the insult and with subsequent mortality (166).

This observation agrees with the data obtained in the present study. Patients in group A (meningococcal sepsis) presented in a state of hypokalaemia, which gradually resolved over the time period of study (40hours) as shown in Fig. 5.4. This time scale for the hypokalaemia corresponds to that described by Vanek et al where the serum potassium levels usually decreased within 1 hour of trauma although it recovered faster (within 24 hours) without significant potassium replacement (166).

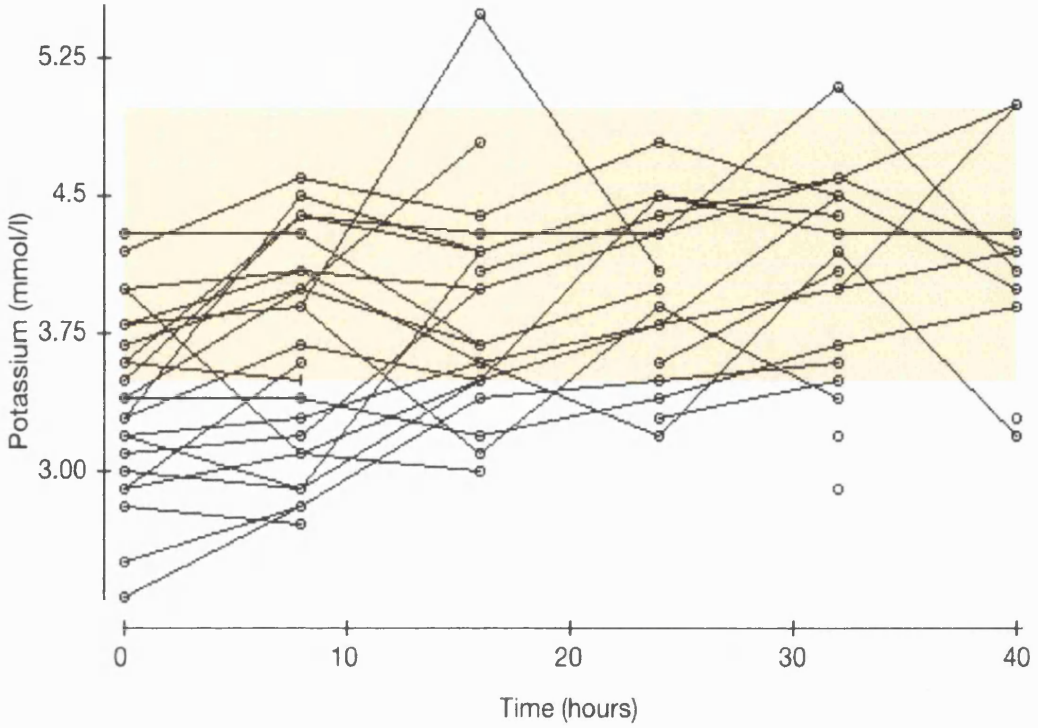


Fig 5.4 Plasma potassium concentration in group A patients (meningococcal sepsis), which gradually resolved over the time period of study (40hours). Each patient is represented by a line. The shaded area represents the normal range for plasma potassium.

It has been proposed that increased blood lactate often reflects increased aerobic glycolysis in skeletal muscle secondary to adrenaline-stimulated Na^+,K^+-ATPase activity and not anaerobic glycolysis due to hypoperfusion (165). This appears to explain why hyperlactacidaemia may not correlate with traditional indicators of perfusion and may not diminish with increased oxygen delivery. This indeed was the case in the patients with meningococcal sepsis, in whom circulating volume was rapidly replaced during the process of resuscitation, mean arteial pressure was maintained with inotropic agents and arterial oxygenation was corrected to normal, yet lacate levels did not correct immediately (see Fig.6.1).

In the present study however, there was no significant correlation between the potassium and lactate levels (r=-0.373, p=0.1891) or between potassium and adrenaline (r=-0.389, p=0.2663) in the meningococcal sepsis patients (group A).

Therefore elevated plasma lactate levels, although related to illness severity may reflect at least a composite of at least two underlying pathophysiological mechanisms. The relationship between lactate and other metabolites and hormones is therefore difficult to interpret, and relates mostly to the predominant pathophysiological mechanism in operation (hypoperfusion or Na^+K^+ATPase activation) in each patient category.

5.84 Significance of correlations between hormone and metabolite levels and severity of illness

A small degree of correlations with severity of illness as defined by PROM have been observed in the present study in diagnostic subgroups of patients. The plasma lactate levels and IGFBP-1 both correlated with PROM in the patients with meningococcal disease on admission to PICU. These observations are consitent with the studies conducted both in critically ill adults (167-170) and children (171). In adult studies in the critically ill elevated levels of IGFBP-1 have been reported to be associated with poor patient outcome (172). However in critically ill children the only group in whom this observation has been made to date is the of meningococcal sepsis (173), which is consistent with the observations from the present study.

In the respiratory subgroup of patients cortisol levels were found to correlate with PROM. This observation again is consitent with the previous studies where cortisol levels were related to illness severity scores in children with bronchiolitis (114). This feature was not observed in the other patient groups. This may be due to a degree of functional adrenocortical insufficiency in the meningococcal sepsis category (as discussed in chapter 8).

The negative correlation between PROM and total T_4 is the result of changes in serum binding of thyroid hormones and alterations in thyroid production rates and metabolic clearance rates (6). This expected negative correlation is only seen in the surgical patients.

Chapter 6 - The longitudinal analysis of the metabolic and endocrine axes.

6.1 Introduction

There is comparatively little data on the on the longitudinal changes of the metabolic and endocrine axes in critically ill children. This may well be due to the complexity of the subject. Critical illness leads to a spectrum of neuroendocrine, metabolic and immunological changes, complicated by multiple hormonal, metabolic and cytokine interactions with the emergence of varying degrees of hormonal insensitivity. This chapter attempts to describe and interpret some of the observations in the metabolic and hormonal milieu made in critically ill children in the first 48 hours of PICU stay. Changes in the plasma levels of cortisol and aldosterone are discussed in chapter 8.

6.2 Materials and Methods

The study presented in this chapter was performed on all 60 children with critical illness enrolled in the project. The diagnoses within this group included mainly children with meningococcal disease, respiratory infection, post surgery (mainly GI and ENT), the combined mean age was 4.4 years (range 0.25-16.33) with a predicted risk of mortality calculated from the PRISM score, mean value 21.2% (range 0.5 – 99.31%). The data on patients with meningococcal disease (n=31) have been compared with critically ill children with other diagnoses (n=29). Patient details are given in Table 6.1.

Blood was sampled from indwelling arterial cannulae inserted for clinical monitoring and management purposes on or soon after admission to PICU. Further samples were collected at 8 hourly intervals in the meningococcal disease category and 12 hourly intervals in the other diagnostic categories. Blood sample collection, preparation and processing was conducted as

described in Chapter 2. The following metabolites and hormones were assayed: blood lactate, pyruvate and acetoacetate concentrations, nonesterified free fatty acid and beta-hydroxybutyrate levels, plasma insulin, GH, IGF-1, IGFBP-I, IGFBP-3, cortisol, aldosterone, T4, TSH and glucagon concentrations.

Table 6.1 Patient details.

Group	Diagnosis	M:F	Age	Severity PROM
A	Meningococcal disease N=31	15:16	4.4 years range 0.5-14.35y	32.3% range 0.5-99.3%
B	Other diagnoses N=29	19:10	4.1 years range 0.25-16.3y	9.4% range 0.2-83.0%
	Respiratory N=13	8:5		
	Surgical N=12	9:3		
	Neurological N=4	3:1		

6.3 Statistical analysis

Graphical display of the data and statistical analysis were performed as described in chapter 4 (under section 4.3 – analysis of longitudinal data).

6.4 Results – Intermediary metabolites

6.4.1 Intermediary metabolites – Lactate and pyruvate

6.4.2 Results on lactate and pyruvate

6.4.2.1 Lactate

The plasma lactate levels obtained in the critically ill children are plotted against time (0-48 hours) (Fig. 6.1). The normal range for healthy children is displayed with a shaded area. A total of 236 lactate values were obtained in the first 48 hours on the patients studied. The maximum and minimum levels of lactate recorded were 11.97mmol/l and 0.32mmol/l respecively.

On admission (t=0) the lactate levels tended to be higher with 30 (50.0%) out of 60 samples were above the reference range, of which 21 (35.0%) were from children with meningococcal disease. At 24 hours however plasma lactate levels had started to fall. Only 10 (22.2%) out of 45 samples, of which 9 (20.0%) were from children with meningococcal disease were above the normal range. Only 1 (1.6%) patient on admission had a lactate level below the reference range. At t=24 there were only 2 (4.4%) of patients with low levels.

The fall in lactate levels over the first 24 hours from the time of admission was significant ($p<0.0001$). There was also a significant difference between the diagnostic categories. Patients with meningococcal disease had higher lactate levels at both time points (t=0, $p=0.0003$; t=24, $p=0.0024$) compared with other critically ill children. However, the rate of fall of lactate, adjusted for regression to the mean, appeared not to show differences between the meningococcal patients and other critically ill children (meningococal disease; $p=0.8222$), or for age ($p=0.4860$).

There were no correlations between age and lactate levels at the both time points. Log lactate correlated with PROM at t=0 ($r=0.626$; $p=<0.0001$) and at t=24 ($c=0.595$; $p= 0.0004$). The reference ranges used for lactate were based on the Great Ormond Street Hospital biochemistry laboratory reference levels for lactate in children.

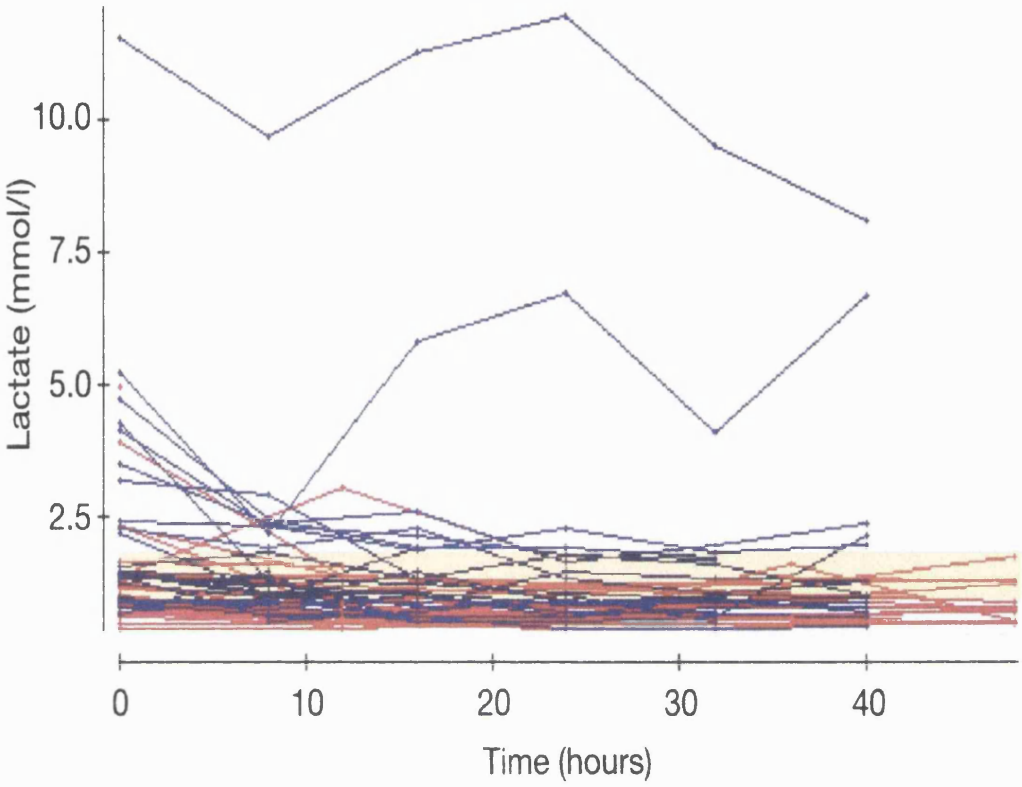


Fig 6.1 Plasma lactate levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the lactate levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis

Normal range of plasma lactate in children.

6.4.2.2 Pyruvate

Plasma pyruvate concentrations over the first 48 hours following admission to PICU, together with reference ranges for normal children are shown in Fig 6.2. 235 plasma samples were analysed for pyruvate levels for the first 48 hours on 60 patients. Pyruvate concentrations ranged from 0.59 mmol/l to 0.003mmol/l. Pyruvate levels in 9 samples (8.1%) were above this reference range and in 112 (47.5%) below this range.

At t=0, there were a total of 60 measurements recorded, of which only 4 (6.6%) were above and 17 (28.3%) were below the reference range. At t=24, there were a total of 45 pyruvate samples analysed, of which only 1 (2.2%) was above and 26 were below (57.7%) the reference range. The difference between pyruvate levels on admission and after 24 hours was significant (p=0.001). There was however no difference between the pyruvate levels in the meningococcal diagnostic category and the other patients at both time points (t=0, p=0.1314; t=24, p=0.3801). There was also no correlation between age and plasma pyruvate level.

The rate of fall of pyruvate adjusted for regression to the mean showed no relation to age (p=0.4217) or diagnostic category (meningococcal p=0.9455).

The reference ranges have been taken from data on intermediary metabolites and hormones in normal children after an overnight fast. These ranges may however vary depending on the age of a child and the duration of fasting (174).

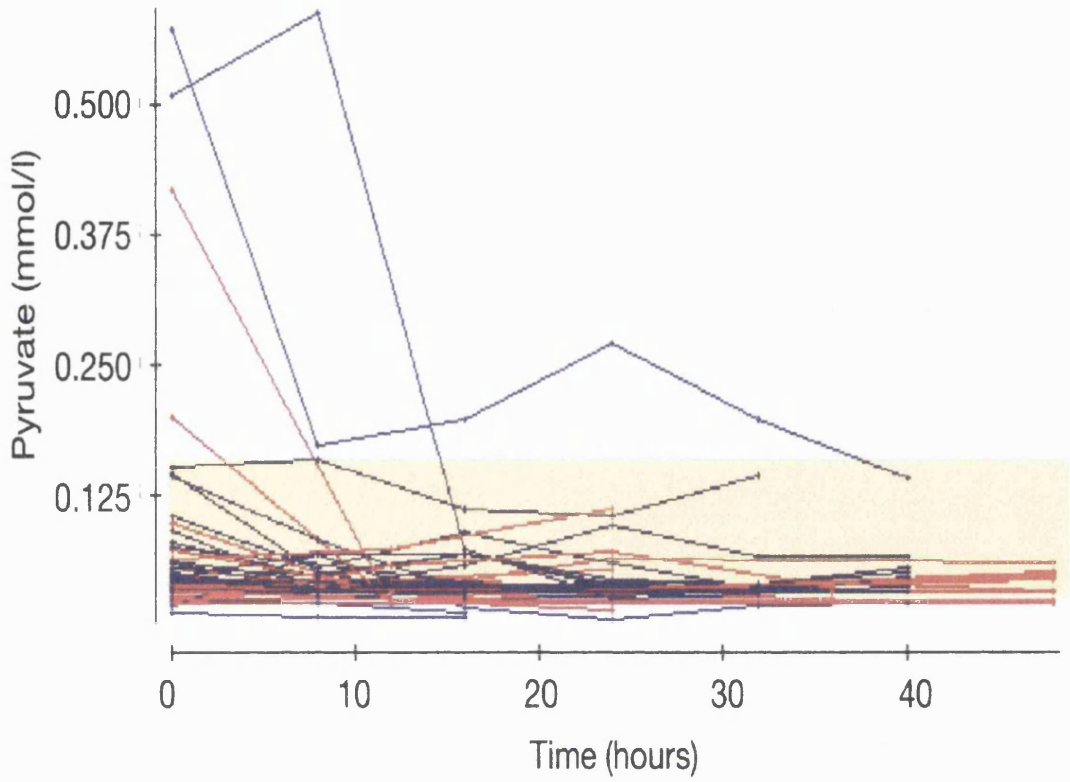


Fig 6.2 Plasma pyruvate levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the pyruvate levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis

Normal range of plasma pyruvate in children.

6.4.2.2.1 Pyruvate instability

The plasma pyruvate concentrations obtained in this study were remarkably low. 47.5% of pyruvate levels recorded in the first 48 hours from admission to PICU were below the reference range (174) and were discordant with the elevated plasma levels of lactate.

In order to address this issue, the stability of pyruvate was tested for extended periods of storage at -70°C. Samples were collected from 2 individuals (fasted overnight) and 100µl aliquots immediately transferred to into pre-cooled tubes containing 500µl of 0.46M perchlorate to deproteinize

the sample and to prevent enzymatic degradation and interconversion of intermediary metabolites. The samples were centrifuged at 12000 rpm for 5 minutes at 4°C, the supernatant being separated and frozen at -70°C. In total 6 separate tubes per individual were collected and stored. These were then sequentially thawed and assayed for pyruvate concentration as described in chapter 2. The data in Fig 6.2a shows the time-dependant degradation of pyruvate in samples stored at –70°C over a twelve week period.

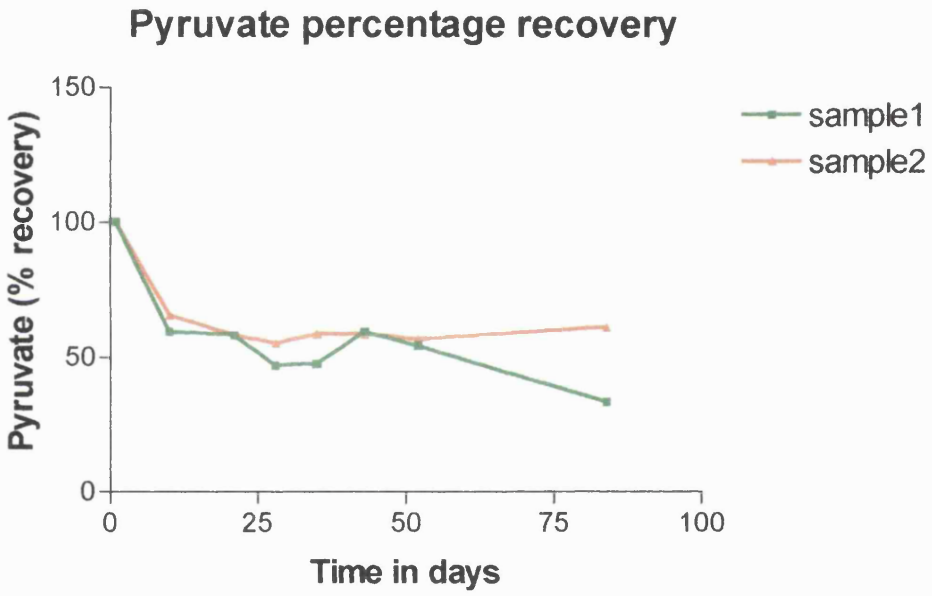


Fig 6.2a Pyruvate percentage recovery plotted against time of sample storage at –70°C.

6.4.3 Discussion

6.4.3.1 Plasma lactate and pyruvate levels in critically ill children

Lactate levels are known to rise in critical illness (47). The present finding in critically ill children is in agreement with previous studies (168;171;175).
Increased production of lactate might be the result of tissue hypoperfusion and anaerobic glycolysis, due to tissue hypoxaemia. Clinically, patients with meningococcal disease present with hypotension and hypoperfusion. The increased levels of lactate were found predominantly in the patients with from meningococcal disease. Indeed, elevated serial arterial lactate measurements have been associated with a poor outcome in patients with systemic inflammatory response syndrome (SIRS), severe sepsis, or septic shock (169).
Serial measurement of arterial lactate concentrations are a prognostic indicator in relation to the incidence of disseminated intravascular coagulation in patients with systemic inflammatory response syndrome (176). Disseminated intravascular coagulation (DIC) may play an important role in the pathogenesis of lactate production.

The plasma pyruvate levels, in the present study were not elevated. In fact 47.5% of values were below the reference range. The determinations of pyruvate levels may not be reliable since it appears extremely unlikely that the patients would have low pyruvate levels in view of elevated lactate.
The reason for this may be that pyruvate is an unstable substance and undergoes rapid degradation. The samples for pyruvate estimation in this study were collected into perchlorate, precipitated, centrifuged, separated and frozen immediately, in order to limit degradation. However, the degradation of pyruvate over several months prior to analysis even at –70°C storage remains a possibility. Fig 6.2a shows pyruvate degradtion over a twelve week period. The majority of samples obtained within the present study were stored for periods up to 24 weeks, as they were batched before

analysis. This explains the low levels obtained in the study and consequently makes the interpretations based on the pyruvate results difficult. Although the results obtained for other metabolites and hormones appeared appropriate, their stability, for completeness should also be addressed.

The lactate/pyruvate (L/P) ratio in this study may therefore be distorted and difficult to interpret. Indeed, the L/P ratio appears abnormally high with 44.25% (104 out of 235), L/P ratios from the first 48 hours of admission to PICU being elevated (>30). Increased lactate /pyruvate ratios have been reported in the case of tissue hypoperfusion and anaerobic glycolysis (177). However, Siegel et al, 1979 found a rise in plasma pyruvate levels in sepsis and consequently the lactate/pyruvate levels remained unchanged (50). Increased plasma lactate concentrations suggest that hepatic lactate uptake and clearance may not match the increasd peripheral production.
Stable isotope studies have demonstrated that both production and uptake are increased (47).

Log lactate correlated with PROM both at t=0 or t=24 in this study. Other investigators have also found correlation between an elevated plasma lactate and mortality (168;169;171;175). Not only are the lactate levels elevated because of increased production, but decreased clearance is predictive of poor outcome independently of other known risk factors such as age and number of organ failures (167). It appears that not only the degree of elevation, but also the duration of elevated arterial lactate is correlated with an increased mortality among medical and trauma patients (162). A study of 95 adult surgical ICU patients demonstrated that patients failing to achieve normal lactate levels had a 100% hospital mortality rate. On the other hand, patients who achieved clearance between 48 and 96 hours sustained a 42.5% mortality rate. Patients normalizing plasma lactate levels in 24 to 48 hours had a 13.3% mortality rate, and those in whom lactate levels returned to normal in less than 24 hours had a mortality rate of 3.9%.

In the present study ten patients continued to have an elevated lactate levels beyond 24 hours (mean severity PROM 55.98% SE ±10.8%). The remaining

patients in whom the lactate levels returned to normal within 24 hours of admission to PICU had a mean PROM score of 17.19%, SE ±2.9%. The difference in patient severity, as measured by PROM, of both groups was significant ($p<0.0001$). There were no fatalities in the study population.

6.4.4 Results and Discussion- Lipid metabolism

6.4.4.1 Non-esterified fatty acids (NEFA)

Plasma NEFA concentrations over the first 48 hours following admission to PICU, together with reference ranges for normal children are shown in Fig 6.3. Plasma NEFA concentrations ranged from 3.12 to 0.05mmol/l. 199 measurements were obtained over the first 48 hours from admission to PICU. Of these only 36 estimations (18%) were above the reference range and 7 (3.5%) were below the reference range.

49 samples, each relating to a patient, obtained on admission, were analysed for NEFA levels; 12 of which (24.5%) were elevated. No patients exhibited values below the reference range. Of the 38 measurements recorded at 24 hours after admission, 5 (13.2%) were above the reference range and 1 (2.6%) was below the reference range.

The reference ranges have been taken from data on intermediary metabolites and hormones in normal children after an overnight fast. These ranges may however vary depending on the age of a child and the duration of fasting (174).

Although NEFA levels decreased with time, the difference between the plasma NEFA levels at t=0 and t=24 was not significant ($p= 0.1191$). There were no significant correlations between age and severity (as defined by PROM) and plasma NEFA levels at both time points.

There were no differences in NEFA level between the meningococcal patient group and the other diagnostic categories at the two time points t=0, $p=0.2148$; t=24, $p=0.4223$).

The change in plasma NEFA levels over the first 24 hours after admission, adjusted for baseline (t=0), appeared not to be influenced by age (p=0.5728) or diagnostic category, although the plasma NEFA levels tended to fall more rapidly over this time period in the meningococcal sepsis patients, this did not achieve statistical significance (p=0.0886).

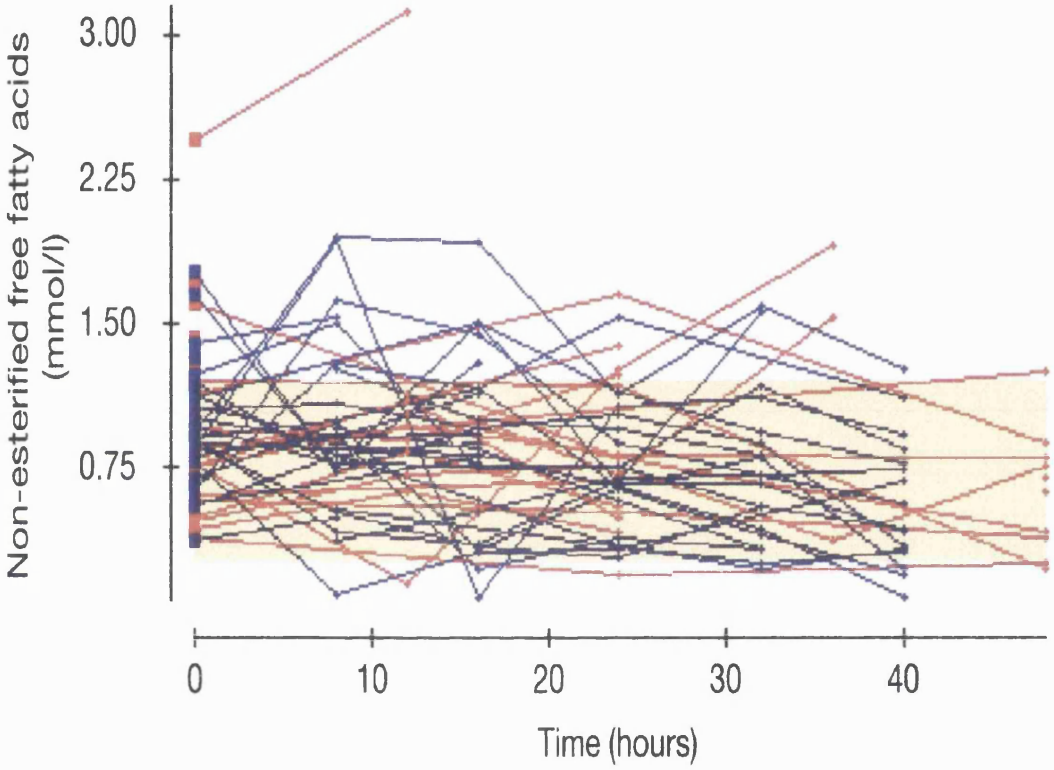


Fig 6.3 Plasma NEFA levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the NEFA levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis

Normal range of plasma NEFA in children.

6.4.4.2 Acetoacetate

The plasma acetoacetate concentrations over the first 48 hours following admission to PICU, together with reference ranges for normal children are shown in Fig. 6.4. 236 samples obtained in the first 48 hours on the 60 patients were analysed for acetoacetate concentrations. Plasma acetoacetate concentrations ranged from 1.35 mmol/L to 0.0156 mmol/L, both maximum and minimum values were obtained in surgical patients.
The reference ranges have been taken from data on intermediary metabolites and hormones in normal children after an overnight fast. These ranges may however vary depending on the age of a child and the duration of fasting (174).

115 measurements (48.7%) of the acetoacetate levels were above and 121 (51.3%) of the measurements were within the normal reference range. No patients exhibited values below the reference range. The patients were exhibiting ketosis.

On admission 37 patients from a total of 60 (61.6%) had plasma acetoacetate concentrations above the reference range. 16 patients (26.6%) with elevated plasma acetoacetate levels had meningococcal disease. 25 out of 60 patients (41.6%), of which 14 (23.3%) had meningococcal disease exhibited acetoacetate values within the normal reference range.
At 24 hours post admission to PICU, 22 patients from a total of 46 (47.8%) had acetoacetate levels above the reference range. Only 5 patients (10.9%) with meningococcal disease had elevated plasma acetoacetate levels. The remaining 24 patients (52.2%) of which 11(23.9%) had normal levels at t=24. It is noteworthy that of the plasma acetoacetate levels in all but one case, the meningococcal category returned to within the normal range by 40 hours post admission to PICU. This is not the case with the other diagnostic categories.
Using the paired t test analysis, it was not possible to demonstrate a statistically significant fall in plasma acetoacetate levels over the first 24

hours from admission to PICU (p=0.0633). There appeared to be no difference between the diagnostic categories in the presenting level of acetoacetate, although by 24 hours a difference was detected between the meningococcal disease category and other patients (p=0.0488).

There was no correlation between age or severity, as defined by PROM and plasma acetoacetate levels.

The change in plasma acetoacetate levels over the first 24 hours from admission adjusted for baseline value (t=0), appeared not to be influenced by age (p=0.5728) or diagnostic category (meningococcal patients p=0.7684).

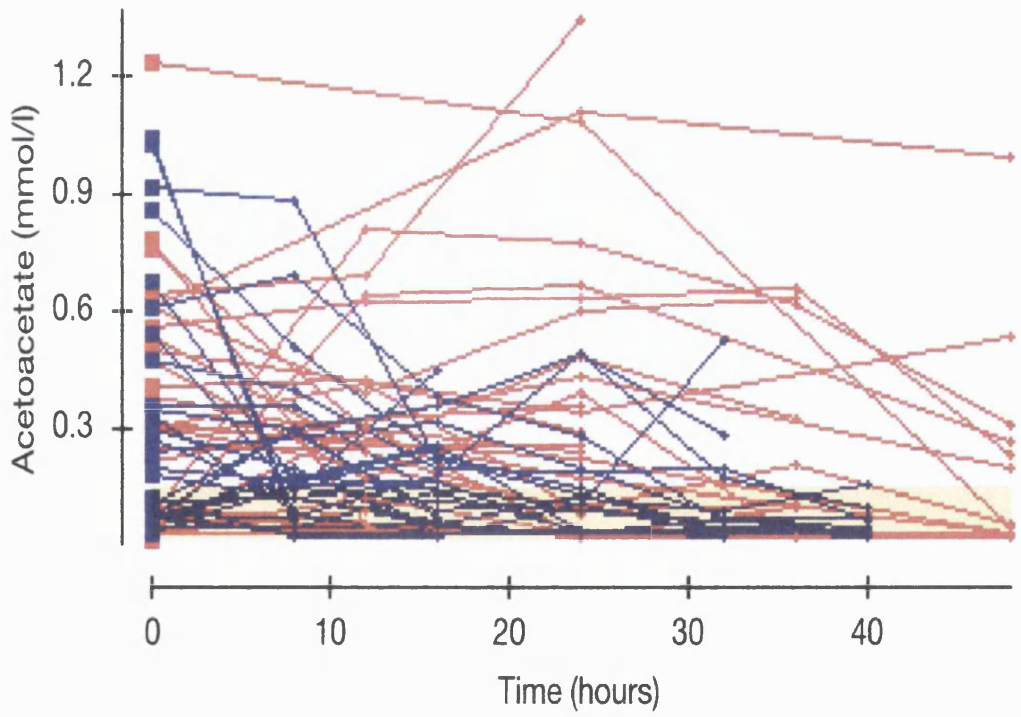


Fig 6.4 Plasma acetoacetate levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the acetoacetate levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma acetoacetate in children.

6.4.4.3 β-hydroxybutyrate

Plasma β-hydroxybutyrate concentrations over the first 48 hours following admission to PICU, together with reference ranges for normal children are shown in Fig 6.5. 196 plasma samples obtained in the first 48 hours from the 60 patients were analysed for β-hydroxybutyrate concentrations. Plasma β-hydroxybutyrate concentrations ranged from 4.58mmol/l to 0.01mmol/l. Of these 105 (53.6%) were above and only 1 value (0.5%) was below the reference range. The reference ranges have been taken from data on intermediary metabolites and hormones in normal children after an overnight fast. These ranges may however vary depending on the age of a child and the duration of fasting (174).

On admission the β-hydroxybutyrate levels tended to be higher with 32 (66.6%) out of 48 being elevated. At 24 hours the levels had started to fall in 17 (49.5%) out of 37. In no case were the levels of β-hydroxybutyrate below the reference range at the t=0 and t=24 time points.
Despite the observed fall in plasma β-hydroxybutyrate levels over the first 24 hours, this did not achieve statistical significance (p=0.0775). There were no differences between the meningococcal patients and the other diagnostic categories at both time points. The change in β-hydroxybutyrate levels adjusted for baseline, appeared not to be influenced by age (p=0.5462) or diagnostic category (meningococcal disease p=0.1223).
There were no significant correlations between age and severity (as defined by PROM) and plasma β-hydroxybutyrate levels at both time points.

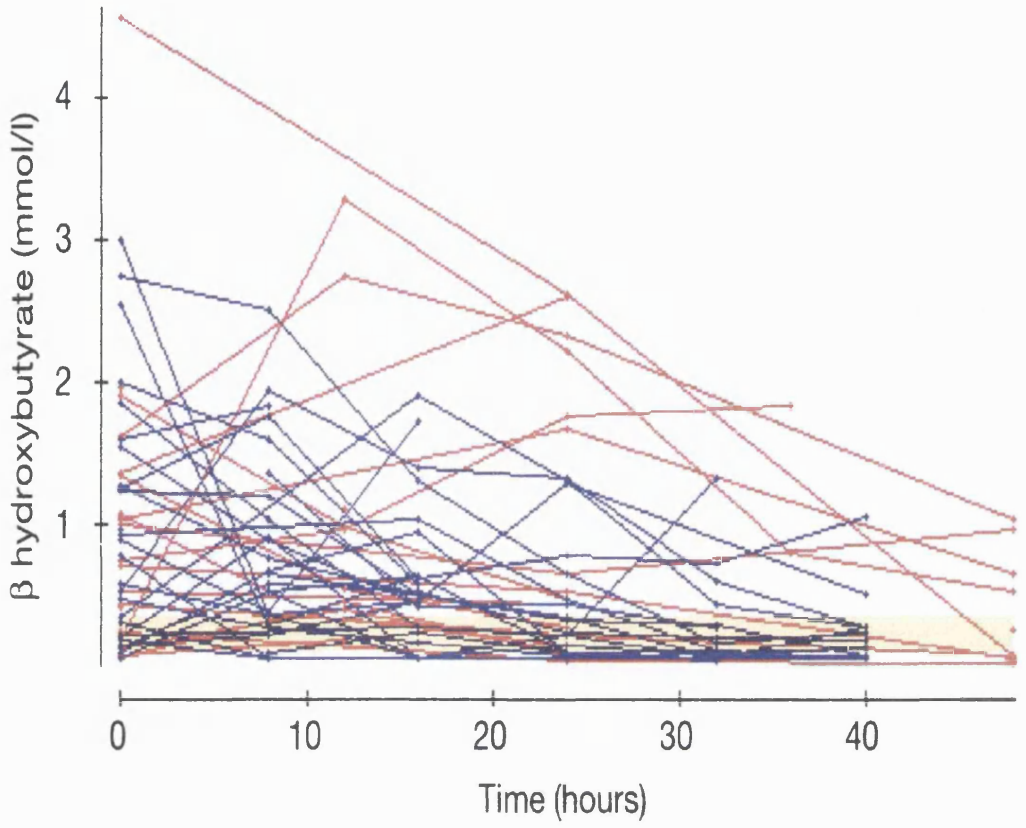


Fig 6.5 Plasma β-hydroxybutyrate levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the β-hydroxybutyrate levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma β-hydroxybutyrate in children.

6.4.5 Discussion: Lipid metabolism

The data on plasma levels of NEFA in patients during the first 48 hours of intensive care suggest that although NEFA levels were normal in most cases and only slightly elevated in some, the patients presented with marked ketosis.

It is known that rates of adipose tissue lipolysis are increased in critically ill patients, thus increasing the systemic supply of free fatty acids. This increase in the availability of free fatty acids is probably mediated by various factors including an increase in counterregulatory hormones and cytokines (178).

However, there are difficulties in interpreting metabolic responses to stress through measurement of concentrations of metabolic intermediaries in discrete blood samples (179). This is because the concentration reflects the net balance between the rate of production of fuel and its consumption. Little change in absolute levels may mask an increase in fuel turnover. Further investigation using stable-isotope techniques are required to resolve this issue.

In the present study 18% of plasma NEFA values were elevated in the first 48 hours from admission to PICU. This is comparable to the data reported by Matthews at al, in critically ill head injured children, where 12% of NEFA values were elevated (180).

In neonates undergoing cardiac surgery, the NEFA levels were found to rise immediately post surgery and return to baseline within 12 hours. The rise was far greater (0.8mmol/l) in the group that received only nitrous oxide anaethesia but reduced to 0.2mmol/l in the group receiving halothane (134). In older children undergoing surgery, the rise in NEFA levels was related to the magnitude of surgery (121). The mean NEFA levels obtained in the subgroup of children undergoing major surgery related closely to the maximal NEFA response to critical illness in children in the present study, where only 9 patients showed maximal plasma NEFA levels greater then the

mean maximal response of 1.23mmol/l obtained by Ward-Platt (121). The plasma NEFA levels in critically ill children fell within the first 24 hours, but was not statistically significant (p=0.1191). The rate of fall appeared not to be related to the diagnositic category of the patients or their age.
There also appeared to be no correlation between plasma NEFA levels at t=0 and t=24 and severity, as assessed by PROM.

Metabolic and hormonal interactions are known to be multiple and complex.
In health, among the metabolic effects of insulin, the effect on lipolysis is probably the most sensitive, with suppression of lipolysis at insulin concentrations of 10 mU/l or above (181). Distortions of these interrelationships occur in the acute stress response and include aspects of reduced insulin sensitivity (47;142). The inhibitory influence of insulin on lipolysis appears to have been lost. In the presence of elevated circulating insulin concentrations (see Fig. 6.10), plasma NEFA levels were unsuppressed. The data from the present study thus support evidence from previous findings (65;182).

6.4.5.1 Marked ketosis in critically ill children

The patients in the present study demonstrated marked ketosis. Total ketone body levels are also known to rise in children undergoing surgery (183). The level of increase was approximately 0.1mmol/l in the neonatal cohort (183) and approximately 0.6mmol/l in older children undergoing major surgery (121). The ketogenic response to surgery is therefore age dependant. However, in this study no relationship was found between the absolute level of acetoacetate or β-hydroxybutyrate at t=0 or t=24 and the patients age. Ward-Platt also noted that the degree of rise in ketone bodies was related to the severity of the surgery (121). In the present study of critically ill children no relationship was found between PROM and plasma acetoacetate or β-hydroxybutyrate levels at t=0 or t=24. The β-hydroxybutyrate levels were elevated in 66.6% of the patients on admission and fell to 49.5% by 24 hours. Acetoacetate levels displayed a similar trend, although both parameters did not show a statistically significant fall over this time period (β-

hydroxybutyrate p=0.0775, acetoacetate p=0.0633). The rate of fall was unrelated to the diagnostic category or age of the patients.

The reversal of levels of ketone bodies to within the normal range signifies that their production and utilization are in equilibrium. This may indicate an improvement in the clinical condition of the patients with time. Cogo et al, 2002 in stable-isotope turnover studies found that protein turnover but not lipolysis correlated with a persisting critically ill condition in children (184).

The children recruited into the present study continued to be ventilated and required intensive therapy despite normalization of circulating ketone bodies.

6.4.5.2 Abnormal metabolic pattern found in one patient

Strikingly, one patient with meningococcal disease had extremely elevated lactate levels (11.5 mmol/l on admission and 11.97 mmol/l at 24 hours). Assessing the relationship between NEFA and ketone bodies in the study group at the different time points revealed that this relationship was grossly distorted in this patient, suggesting a defect in β–oxidation (Fig 6.6). Patients with β-oxidation defects characteristically present with episodes of hypoglycaemia or sudden death during early infancy, most commonly during an intercurrant illness, which induced an episode of fasting. This patient however had been well prior to developing meningococcal sepsis and at the age of 13, and had made a full recovery from this ICU admission. He had however, been severely unwell with the one of the highest PROM scores recorded in this study.

Medium chain acyl coenzyme A dehydrogenase (MCAD) deficiency is the most common inborn error of fatty acid oxidation with a frequency of 1 in 12,000 and a carrier frequency of 1 in 500 in the general population. The patient was therefore recalled and blood spots were collected on a Guthrie card for tandem mass spectrometry for analysis of acyl carnitines (185). The results of this analysis did not support the diagnosis of β-oxidation defect. However, levels of acyl carnitines were low, which may account for the observed pattern found in this patient during the episode of severe critical

illness. (Analysis was conducted by Dr. S. Krywawych, Dept. of Biochemistry Great Ormond Street Hospital).

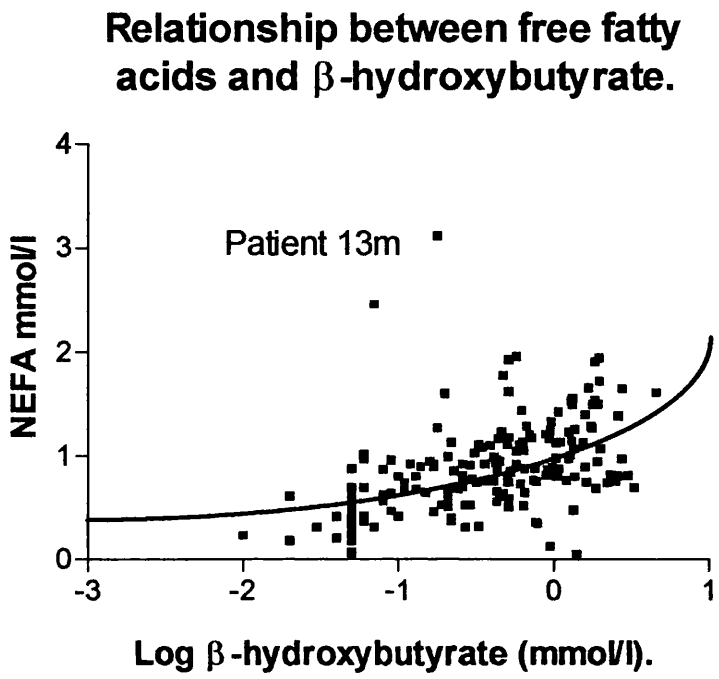


Fig 6.6 Relationship between free fatty acids and β-hydroxybutyrate in critically ill children.
Points from patient 13m fell well above the line consistent with free fatty acid oxidation defects.

6.5 Hormonal Axes – Longitudinal analysis

6.5.1 The GH – IGF-1 Axis

6.5.1.1 Results Growth hormone

The plasma growth hormone levels obtained in the critically ill children between admission and 48 hours are shown in Fig. 6.7. The reference range is displayed as a shaded area, the cut-off corresponds to the level of GH considered to be appropriate post stimulation (using the NETRIA assay) (186). A total of 235 GH values were recorded in the first 48 hours on the 60 patients studied. The maximal level of GH was 325 mU/l and the minimum was 1.3 mU/l.

On admission the GH levels tended to be higher with 22 (36.6%) out of 60 being above 35mU/l. At 24 hours however the GH levels started to fall and 9 (20.0%) out of 45 cases were above 35 mU/l. However, this did not achieve statistical significance (p=0.3908).

There appeared to be no difference in the meningococcal diagnostic group and other patients, in the GH levels at presentation (p=0.6535), at 24 hours (p=0.1942) and the rate of fall (adjusted for regression to the mean)(p=0.3595). The rate of fall was not influenced by age of patient (p=0.5234).

There appeared to be no correlation between age and severity and GH values at t=0 and t=24. However, when the age variable was changed from continuous into discrete bands of age <2years and >2 years, the GH values obtained in the <2year age group were significantly different to those in older children (t=0; p=0.0317, t=24; p=0.0004).

The reference range for adequate rise in GH using the NETRIA assay was obtained from Nanduri et al, 1996 (186).

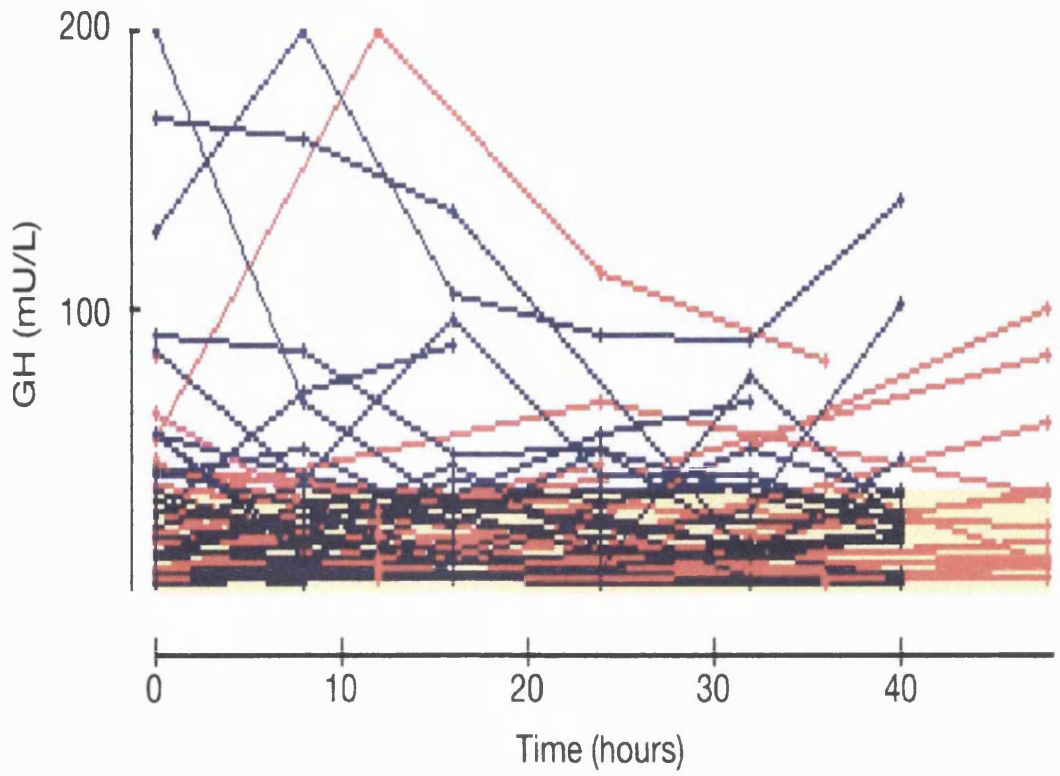


Fig 6.7 Plasma GH levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the GH levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Reference range for adequate rise in GH using the NETRIA assay was obtained from Nanduri et al, 1996 (186).

6.5.1.2 IGF-1

The plasma IGF-1 levels assayed in the critically ill children between admission and 48 hours are shown in Fig. 6.8a. Fig. 6.8b shows the same data, but plotted against age, since IGF-1 levels are age dependant. These are displayed against a shaded area, the cut-off corresponds to 3SD above and below the mean for the ages displayed. The reference data are derived from Mitchell et al, 1999 (187). A total of 233 IGF-1 estimations were made

in the first 48 hours on the patients studied. The maximal value was 346 ng/ml and the minimum was below lower limit for assay detection.

On admission the 12 (20.7%) out of the 58 IGF-1 values were below the -3SD line. 17 values (29.3%) were above the mean. There was very little change in the distribution of values at 24 hours, where 8 (18.2%) out of 44 IGF-1 values were below the –3SD line, with 13 (29.5%)values being above the mean. There was no difference in the means of the two time points (p=0.8608).

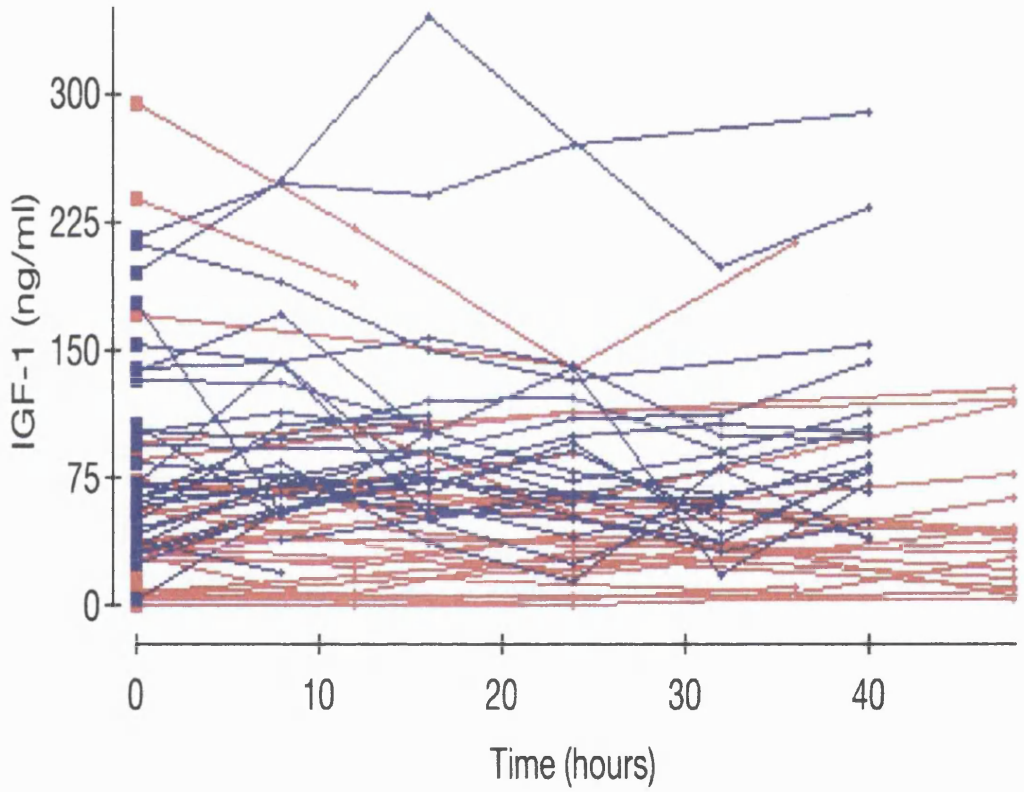


Fig 6.8a Plasma IGF-1 levels in critically ill children shown over the first 48 hours from admission to PICU – plotted against time. Each line represents the IGF-1 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

The log of IGF-1 correlated with patient age at both time points (t=0; r=0.422, p=0.0004 and t=24; r=0.397, p=0.0100). However there was no significant correlation with illness severity as defined by PROM.

Interestingly, patients with the meningococcal disease appeared to have higher IGF-1 values at both time points (t=0; p=0.0108, t=24; p=0.0151).

There was no difference in the rate of change of IGF-1 (adjusted for regression to the mean) dependant on patient diagnosis (meningococcal group p=0.1883) or age (p=0.9079).

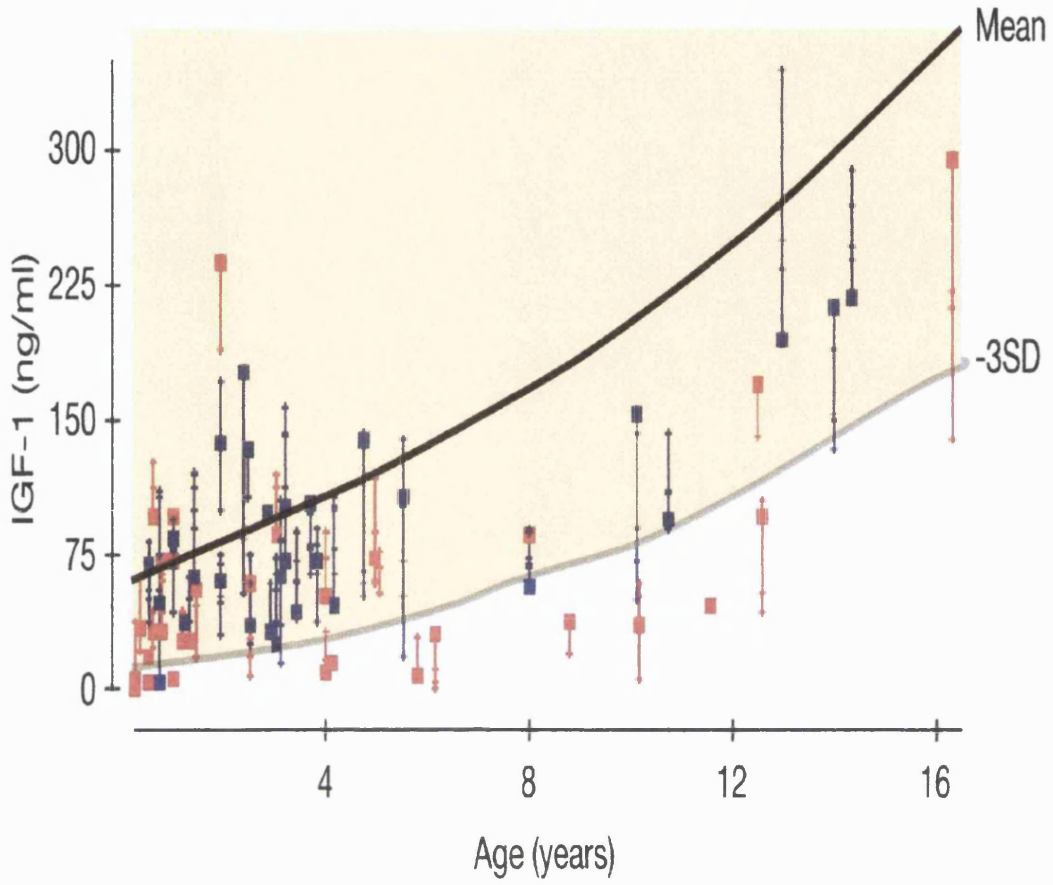


Fig 6.8b Plasma IGF-1 levels in critically ill children shown over the first 48 hours from admission to PICU – plotted against age. Each line represents the IGF-1 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma IGF-1 in children.

6.5.1.3 IGFBP-3

The plasma IGFBP-3 levels obtained in the critically ill children are shown against time (0-48 hours) in Fig. 6.9a. Fig 6.9b shows the same data, but plotted against age, since IGFBP-3 values are age-dependant. These are displayed against a shaded area, the cut-off corresponds to 3SD above and below the mean for the ages displayed. The reference data are derived from Mitchell et al, 1999 (187). A total of 178 IGFBP-3 values were obtained in the first 48 hours on the patients studied. The maximal value was 3.2mg/ml and the minimum was 0.28 mg/ml.

On admission 15 (50.0%) out of 30 IGFBP-3 values were below the –3SD line. 2 values (6.6%) were above the mean. At 24 hours however, 10 (40.0%) out of 25 IGFBP-3 values were below the –3SD line, with no values above the mean. There was no significant difference in the means of the two time points (p=0.1625).

Interestingly, patients with meningococcal disease appeared to have higher IGFBP-3 values which became significantly different by 24 hours (t=0; p=0.2265, t=24; p=0.0003).

The log of IGFBP-3 correlated with patient age on admission to PICU (t=0; r=0.493, p=0.0120), but not at t=24. However there was no significant correlation with illness severity as defined by PROM.

The difference in the rate of change of IGFBP-3 (adjusted for regression to the mean) dependent on patient diagnosis (meningococcal group p=0.0876) or age (p=0.0812) did not achieve statistical significance.

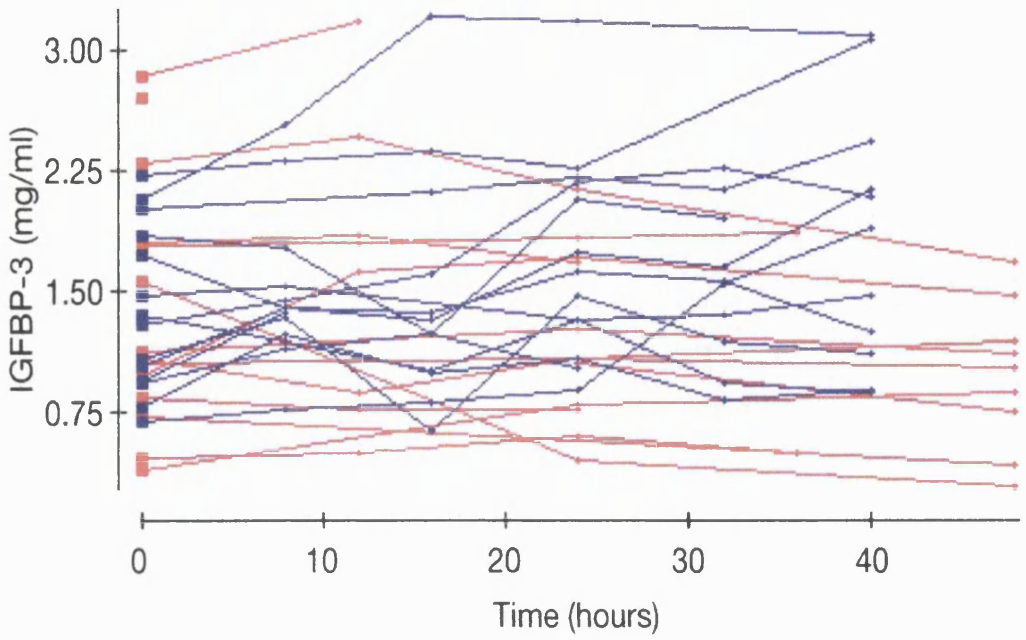


Fig 6.9a Plasma IGFBP-3 levels in critically ill children shown over the first 48 hours from admission to PICU – plotted against time. Each line represents the IGFBP-3 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

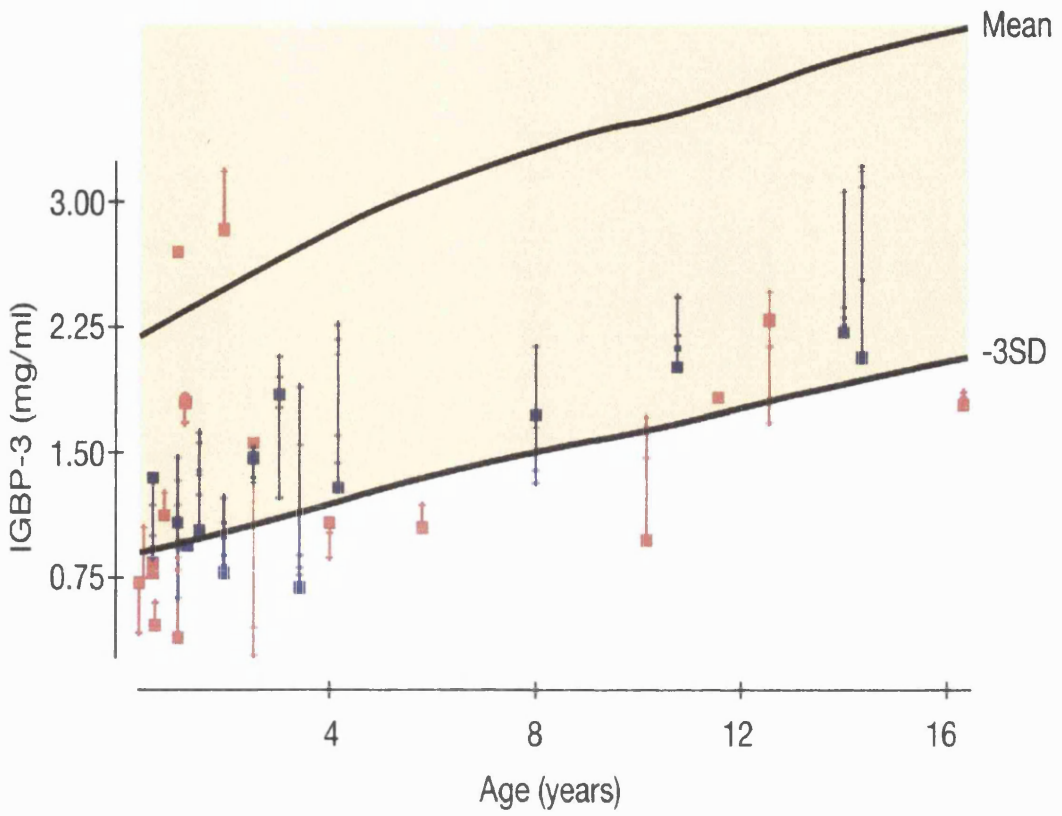


Fig 6.9b Plasma IGFBP-3 levels in critically ill children shown over the first 48 hours from admission to PICU – plotted against age. Each line represents the IGFBP-3 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma IGFBP-3 in children.

6.5.2 Discussion on the GH-IGF-1 Axis data

A large number of investigators have described a state of GH insensitivity during the acute phase of critical illness (32;188). The initial stress response comprises of an increase in basal but blunted pulsatile GH secretion, with a fall in circulating levels of GH-dependent IGF-I and IGFBP-3 and a rise in IGFBP-1 concentrations (189).

The importance of trough and peak levels relates to both the metabolic influences of GH action (troughs influencing the lipolysis and insulin antagonism), whereas the peaks are related to somatotropic properties. Pulsatile GH is known to have a greater effect on IGF-1 gene expression than a continuous GH infusion, thus loss of pulsatility of GH in the acute phase of critical illness may partly explain the lower IGF-1 and IGFBP-3 levels found in the critically ill children. However, decreases in the serum levels of IGF-1, IGFBP-3 and ALS are also known to occur with disruption of the ternary complex (190). The decrease in IGFBP-3 is mediated by an increase in IGFBP-3 protease activity (191). In the present study, the GH-IGF-1 axis behaved as expected with elevation of GH levels, low IGF-1 and IGFBP-3 levels and elevated IGFBP-1 values. ALS and IGFBP-3 protease were not measured.

Data from critically ill adult patients showed a twenty-five fold increase in GH levels on the first day following major trauma, with subsequent fall in IGF-1 by 50-60% and IGFBP-3 by 55-75% and up to a three fold increase in IGFBP-1 levels during the period of ITU stay (192).
The increase in GH levels in the present cohort of PICU patients, however, was not as marked.
Pituitary GH is normally released in pulses every 180-200min (86). Dynamic measurements (half-life 19 minutes) are necessary to interpret changes in GH secretion. Unfortunately, 20-minute sampling for GH was not part of the study design, making interpretation of these widely spaced growth hormone measurements difficult.

This may explain why in some cases there was an apparently insufficient GH response during the stress of critical illness. 65.1% of patients on admission to PICU had GH levels less than 35 mU/l. None of these children were known to be GH-insufficient before the episode of critical illness. The relatively low GH levels detected in this cohort may be due to the infrequent sampling, where trough levels were measured and GH peaks missed. Iatrogenic factors may also have played a role in depressing GH release (inotropes, namely dopamine) (103), particularly in the patients under four months of age (91). The GH levels showed no correlation with the PROM score, a measurement of the severity of illness.

Recently, de Groof et al have reported extremely high GH levels in nonsurvivors compared with mean GH levels in survivors during a 6-hour GH profile in critically ill children with meningococcal sepsis. The non-survivors also showed no low trough values between the peaks of GH. This was thought to be in response to endotoxin and cytokine load (173). When compared to the data obtained from the present study, the GH levels obtained were considerably lower and comparable to the GH levels reported in the meningococcal survivor cohort of children described by de Groof (173).

It is noteworthy, that non-survivors in the critically ill children cohort studied by de Groof et al had extremely high GH levels (173). The findings of de Groof et al appear to be in line with the increase in mortality observed in critically ill adults on administration of GH as an anabolic agent (140). High plasma GH levels, whether endogenous or iatrogenic appear to be associated with non-survival, although the underlying mechanism remains unresolved. GH and many cytokines in fact interact with the same receptor superfamily and share a number of postreceptor signalling pathways (193). High GH levels may therefore be associated with dysregulation of these interactions, which may influence the patients immunological, hormonal and metabolic responses to critical illness (194) contributing to a cascade of adverse events leading to death.

6.5.2.1 IGF-1 and IGFBP-3

Both IGF-1 and IGFBP-3 levels were depressed in the course of the critical illness in the children examined in the present study. This finding is in agreement with the de Groof observations in meningococcal patients. The IGF-1 and IGFBP-3 levels were significantly lower than the IGF-1 and IGFBP-3 levels were found in the children compared with healthy children. Their non-survivors exhibited lower IGF-1 and higher IGFBP-3 protease than the survivors with little difference in the IGFBP-3 levels (173). In the present study however, there were no deaths and therefore the comparisons between survivors and nonsurvivors were not possible. There was however no relationship between the level of IGF-1 and severity of critical illness as defined by PROM either in the whole cohort studied or in group A- the meningococcal sepsis category. This may be, not necessarily a lack of relationship between IGF-1 and severity, but a limitation of the severity of illness scoring system.

IGF-1 production may be reduced in cases of hypoperfusion of the liver and in cases of elevated cytokines, in particular TNF-α, that may inhibit hepatic IGF-1 and IGFBP-3 production (195). Increased destruction of the ternary complex may occur due to elevated protease levels in meningococcal sepsis. However, contrary to the above observations, in the present study, both the IGF-1 (on admission and 24 hours post admission) and the IGFBP-3 levels (within 24 hours of admission) were found to be higher in the patients with meningococcal disease than in the other critically ill children. The changes observed in the GH/IGF-1 axis are most likely due to multiple factors. Continued increases in cortisol (see Fig.8.10) and insulin (see Fig 6.10) may alter both the central and peripheral responsiveness of the GH/IGF-1 axis (196), as well as local factors, such as cytokines, which appear to act peripherally to alter the GH/IGF-1 axis (190).

6.5.3 Results on insulin levels

The plasma insulin concentrations over the first 48 hours following admission to PICU, together with reference ranges for normal children are shown in Fig. 6.10. 230 insulin levels were determined on samples collected in the first 48 hours on the 60 patients. Plasma insulin concentrations ranged from a maximum of 200 µIU/L down to 1.3 µIU/L. 112 measurements (48.7%) of the insulin levels were above and only 13 (5.7%) were below the normal reference range. On admission 29 patients from a total of 60 (48.3%) had insulin values above the reference range. Only 6 patients (10.0%) had insulin levels below the reference range. At 24 hours 19 out of 44 patients (43.2%) had elevated values and only 2 (4.5%) had values below the reference range. Two peaks in insulin levels were detected in two patients with meningococcal sepsis. These two patients did not receive any insulin or glucose boluses at the time, however the corresponding blood glucose levels were not recorded.

There was no significant change between the insulin levels on admission and 24 hours later ($p=0.8582$). There was no correlation with age or PROM and insulin levels at $t=0$ and $t=24$. Patients in the meningococcal sepsis did not differ in their insulin levels from the other diagnostic categories at both time points. There were no differences in the diagnostic categories or age, in the rate of change of insulin adjusted for regression to the mean.
The reference ranges have been taken from data on intermediary metabolites and hormones in normal children after an overnight fast. These ranges may however vary depending the blood glucose level (174).

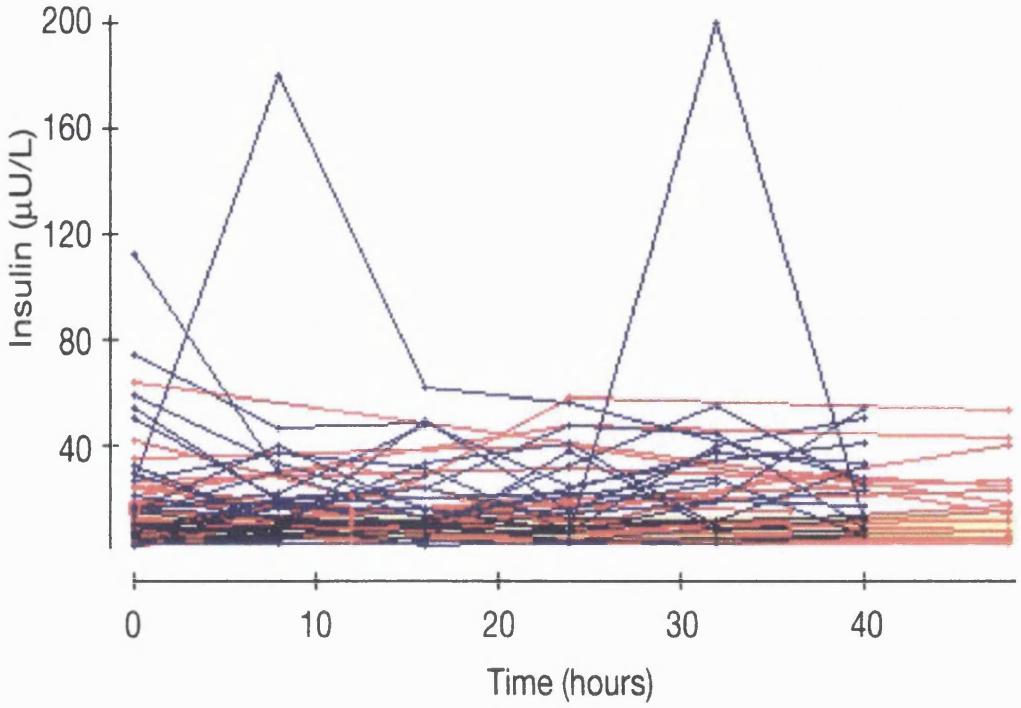


Fig 6.10 Plasma insulin levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the insulin levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Reference range of plasma insulin in children.

6.5.4 Discussion on insulin levels in critically ill children

Insulin levels in critical illness have been shown to be either within (197) or above (198) the normal range. The present observations on plasma insulin levels in critically ill children are comparible with observations made in adults. The insulin levels remained relatively unchanged over the period studied.

It was not possible to determine the degree of insulin resistance, since the project design did not incorporate the concurrent measurement of plasma glucose level, and therefore the insulin/glucose ratio in relation to age, severity or diagnosis could not be addressed.

However, the insulin responses have been shown to differ with age of patient (183), presumably relating to the metabolic maturity of the study population. Quantitative differences in insulin response have been demonstrated in preterm and term neonates, with preterm babies showing no change postoperatively in plasma insulin levels (133) and term infants showing an immediate rise (14). No age relationship or variation was observed in the present study, the patients studied were of an older age group (age range 0.25-16.3 years), with no preterm or term neonates.

In healthy older children it has also been shown that insulin secretion is related to age and that an insulin-resistant state occurs during puberty, coinciding with Tanner stage II (199).

It is not known how the insulin profile differs in the critically ill pubertal child from that of the prepubertal individual. In this study the number of patients in the pubertal age range was small (n=6), pubertal staging was not recorded, as this was not part of the study design.

The severity of illness has also been shown to influence the insulin response. Hawdon found the sick stressed hyperglycaemic neonates had significantly lower plasma insulin concentrations than did clinically stable hyperglycaemic babies, who had appropriately raised insulin levels (200). Further evidence was provided by observations made by de Groof et al, 2002 in which critically ill children with meningococcal sepsis demonstrated lower median serum insulin and glucose levels in non-survivors, as compared to survivors (173). However the authors did not find any correlation between insulin or glucose level and PRISM score. The observations are in agreement with the present study where there was no relationship between severity of illness, as defined by the PROM score and

the insulin levels at presentation, at 24 hours or even the rate of change of insulin within the first 24 hours of PICU support. Corresponding glucose values were unavailable to assess whether insulin resistance changes with severity of illness.

Stress hyperglycaemia is a common event in acute critical illness. The absolute levels of insulin do not reflect the degree of insulin resistance experienced by the patients, which itself may relate more closely to illness severity. Recently it has been suggested that maintaining normoglycaemia with intensive insulin treatment in a heterogeneous population of critically ill adult patients decreases morbidity and mortality. The correction of the prevailing stress hyperglycaemia resulted in a 40% reduction in mortality, especially due to multiple-organ failure with a proven septic focus. Intensive insulin therapy also reduced overall in-hospital mortality by 34 percent, bloodstream infections by 46 percent, acute renal failure requiring dialysis or haemofiltration by 41 percent, the median number of red-cell transfusions by 50 percent, critical-illness polyneuropathy by 44 percent, and patients receiving intensive insulin therapy required less prolonged mechanical ventilation and intensive care (142).

This study in adults shows striking improvement in patient outcome. It is most important to conduct a similar study in the paediatric and neonatal populations (to address the effects of prolonged periods of stress hyperglycaemia in the critically ill child) in order to establish whether modulation of the stress response with intensive insulin therapy is able to improve morbidity and mortality.

The anabolic effect of insulin on muscle protein metabolism has been recognized since the initial clinical use of insulin therapy in type 1 diabetes about sixty years ago. In children with type 1 diabetes insulin is also known to influence the GH axis, by decreasing IGFBP-3 proteolysis (201) in insulin-dependant diabetic children, restoring ALS levels (202) and increasing IGF-1 levels (203) to that of normal subjects. In the present study of critically ill

children however, despite elevated insulin values, IGF-1 and IGFBP-3 levels were low. In children with burns insulin therapy alone did not influence ALS or IGF-1 levels, additional GH was required to restore IGF-1 levels to normal (204).

Normally circulating insulin regulates IGFBP-1 through inhibition of gene transcription in the liver (205). The elevated insulin levels in the present study do not appear to have had this effect since IGFBP-1 measurements remained elevated throughout the study (Fig. 6.11).

It appears that the influence insulin exerts on the GH axis in critical illness and burns may be diminished, contributing to the picture of insulin-insensitivity.

Unfortunately, the design of the present study, as part of a larger study, did not include the measurement of plasma glucose levels. It was therefore not possible to assess the insulin/glucose ratio or to comment on the degree of insulin insensitivity with repect to glucose.

6.5.5 Results on plasma levels of IGFBP-1

The plasma IGFBP-1 concentrations over the first 48 hours following admission to PICU are shown in Fig 6.11. There are no reference ranges shown, as there is little normative data on IGFBP-1 levels in healthy children. 232 IGFBP-1 measurements were obtained in the first 48 hours on the 60 patients. Plasma IGFBP-1 concentrations ranged from a maximum of 546 ng/ml down to 2.5 ng/ml.

There was no significant change between the IGFBP-1 values on admission and 24 hours later (p=0.2305). There was no correlation with age or PROM and insulin levels at t=0 and t=24. Patients in the meningococcal sepsis category did not differ in their IGFBP-1 levels from the other diagnostic categories at both time points (t=0, p=0.5507; t=24,P=0.3233). There were

no differences in the diagnostic categories or age, in the rate of change of IGFBP-1 adjusted for regression to the mean.

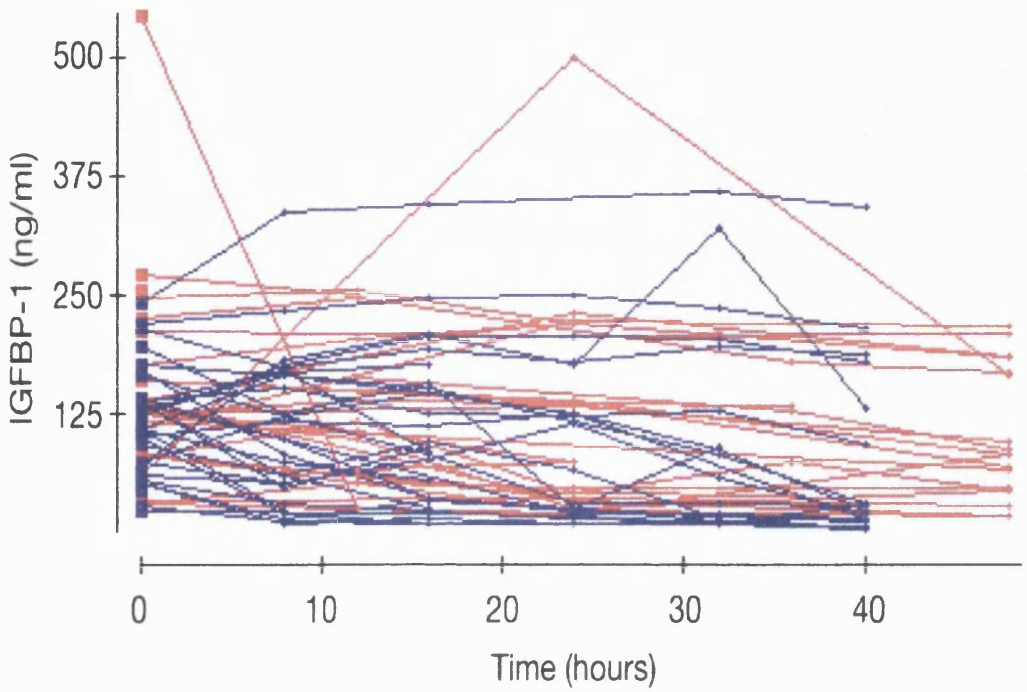


Fig 6.11 Plasma IGFBP-1 levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the IGFBP-1 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

6.5.6 Discussion on plasma IGFBP-1 levels

IGFBP-1, which is regulated by metabolic status, has been found to be elevated on admission to intensive care in critically ill adults (192) but may fall in response to nutritional support. IGFBP-1 is also negatively regulated by insulin (172). Increased IGFBP-1 levels are thought to reflect hepatic insulin resistance since the liver is the major source of IGFBP-1 (206). Initial evidence suggests that the level of IGFBP-1 may be predictive of outcome in

critically ill adult patients, suggesting a possible prognostic role for this protein (172).

Until recently, there was little data on the prognostic value of IFGBP-1 measurements in critically ill children. In 2002, de Groof at al (173) described the acute stress response in children with meningococcal sepsis and showed considerably higher IGFBP-1 levels in non-survivors, as compared to survivors (173). They observed a fall in the IGFBP-1 levels over 48 hours from admission in the surviving cohort. In the present study IGFBP-1 levels did not appear to alter significantly over the first 48 hours of critical illness.

The de Groof data showed IGFBP-1 correlation with the PRISM score as a measure of patient severity (173). However, there was no correlation in the present study between IGFBP-1 and PROM. This appears surprising, since the PROM score is derived from the PRISM score.

The explanation may allude to the differences in sample populations. The de Groof cohort were younger than patients within the present study, had a single diagnosis and nearly 30% of the patients studied had died (173). The children recruited to the present study fell into multiple diagnostic categories, in whom different pathophysiological processes and iatrogenic factors were operating and potentially exerting different influences on IGFBP-1 synthesis. There had been no deaths in the present studies cohort and therefore a potentially different sample population, which may influence the results.

During unfavourable metabolic conditions (e.g. such as those prevailing in critical illness), the hepatocytes appear to alter the production of IGF regulatory proteins by increasing cAMP production, which stimulates IGFBP-1 and suppresses IGF-1 and ALS production (207;208). The IGFBP-1 in critical illness undergoes posttranscriptional modification and appears in a highly phosphorylated form (192), that shows higher affinity for IGF-1. The inhibitory effect of IGFBP-1 on IGF-1 is therefore potentiated in critical illness.

As mentioned earlier, the data on normal ranges of IGFBP-1 levels in healthy children are lacking. IGFBP-1 levels are known also to vary with

patients age and tend to reach their lowest level as the child approaches puberty (209;210). Ethnic differences in the IGFBP-1 levels have also been established (211). This makes interpretation of IGFBP-1 levels more difficult.

Cytokines such as TNF-α are also known to stimulate IGFBP-1 production in vitro (190). The consequent changes in IGFBP-1 are complex. Further detailed analysis is required in order to establish the reliability of IGFBP-1 levels as a prognostic indicator at different ages, in different disease states (of varying cytokine load) in the critically ill child.

6.5.7 Glucagon - Results

The plasma levels of glucagon in 60 patients over the first 48 hours from admission to PICU are shown in Fig.6.12. A total of 226 measurements were recorded in the time, of which 60 (26.5%) were above the reference range for normal healthy children and 27 (11.9%) were below. The maximum glucagon levels were 828pg/ml and the minimum 20pg/ml. On admission there were 61 values recorded for glucagon. 19 (31.6%) of these were above the reference range and 4 (6.6%) below. At 24 hours the total number of recorded measurements of glucagon fell to 44, of which 10 (22.7%) were above the reference range and 5 (11.3%) were below. This fall was statistically significant (p=0.0030). There were no correlations between age and severity. There were no differences in the diagnostic categories or age, or in the rate of change of glucagon adjusted for regression to the mean. The normal range was defined as 40-130 pg/ml (laboratory reference range).

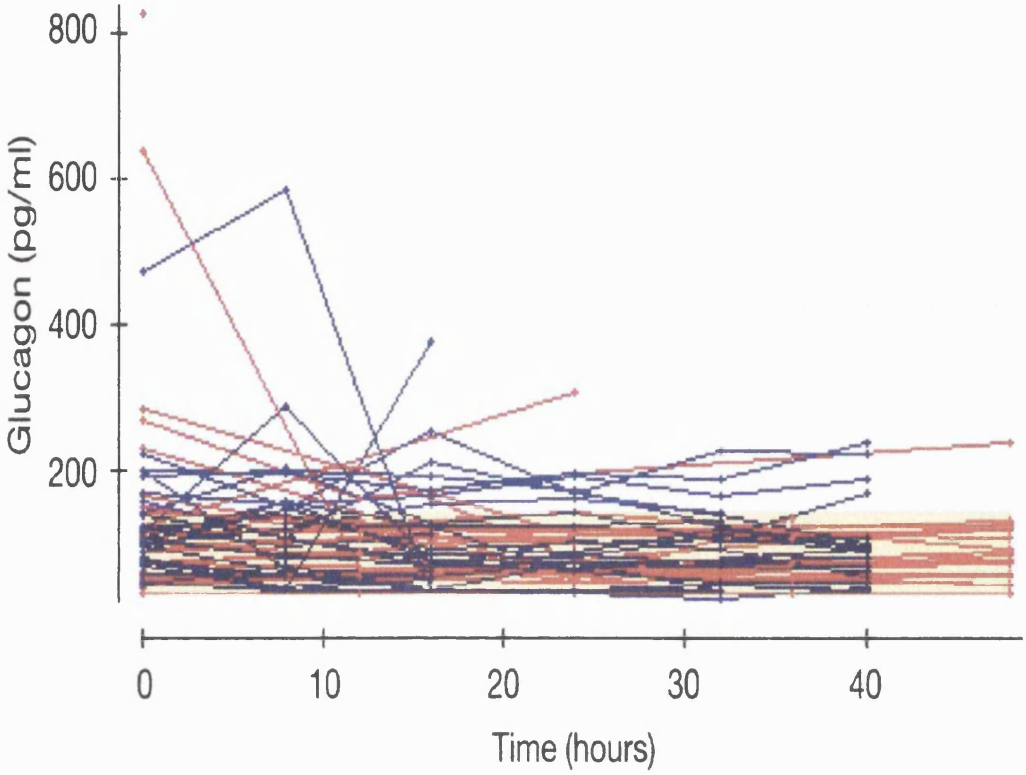


Fig 6.12 Plasma glucagon levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the glucagon levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma glucagon.

6.5.8 Glucagon – Discussion

The endocrine response to stress is complex. Elevations in the serum concentrations of the "classic" stress hormones, adrenaline and cortisol, occur following many kinds of physiologic challenge and are accompanied by elevations in GH and glucagon levels (212).

However, although elevated plasma glucagon levels are cited in many texts on stress responses in adults (213;214), relatively little is known about the glucagon stress response in critical illness in childhood.

The data available on the glucagon response in children undergoing surgery, show variation with age. In two studies of term neonates, (14;183) no change in serial measurements of glucagon was found after surgery. However, infants and children undergoing major surgery demonstrated a small but significant rise in glucagon, compared with baseline, at the end of surgery (121). When validating the surgical stress score Anand et et al, 1988 found that severe stress evoked a substantial elevation of glucagon concentration (14).

The results of the present study, show a 33% of plasma glucagon levels being elevated on admission, this falling to 22.7% by 24 hours. Contrary to the observations by Anand et al (14), who found a graded response with respect to stress score in newborn infants, in the present study in critcally ill children there was no correlation with severity as defined by PROM and plasma glucagon levels either on admission or within 24 hours. There was also no difference in the plasma glucagon levels and the age of the infants and children examined either at t=0 or t=24. There were however no neonates in the present study and the ages ranged from 0.25 to 16.33years. It appears that critical illness in infancy and childhood does not evoke age specific responses in glucagon.

It has been known that important differences exist in glucose metabolism between severe infection and other causes of critical illness. It appears that the infection may induce a block in hepatic glucose output (124). Although the precise mechanism underlying this finding remains unclear, there was also no difference between the diagnostic categories (including meningococcal sepsis group) and plasma glucagon levels on admission or at t=24.

6.5.9 The Thyroid Axis

6.5.9.1 TSH

The plasma TSH levels over the first 48 hours following admission to PICU, together with reference ranges are shown in Fig. 6.13. 234 TSH measurements were obtained in the first 48 hours on the 60 patients. TSH concentrations ranged from 14.1 mU/l to 0.15 mU/l. Only 3 TSH values (1.2%) were above this reference range and the rest 66 (28.2%) were below the reference range. At t=0, there were a total of 60 measurements recorded, of which only 2 (3.3%) were above the reference range and 15 (25%) were below. At t=24, there were a total of 42 TSH levels recorded, none of which were above the reference range and 12 were below (28.5%).
A significant negative correlation was found however between log TSH and severity of illness as defined by PROM at both time points (t=0; r=-0.192, p=0.0391 and t=24; r=-0.445,p=0.0025). Ther was no correlation with patient age and TSH levels. The reference range used for TSH was 0.5 to 5.0 mU/l (laboratory reference range).

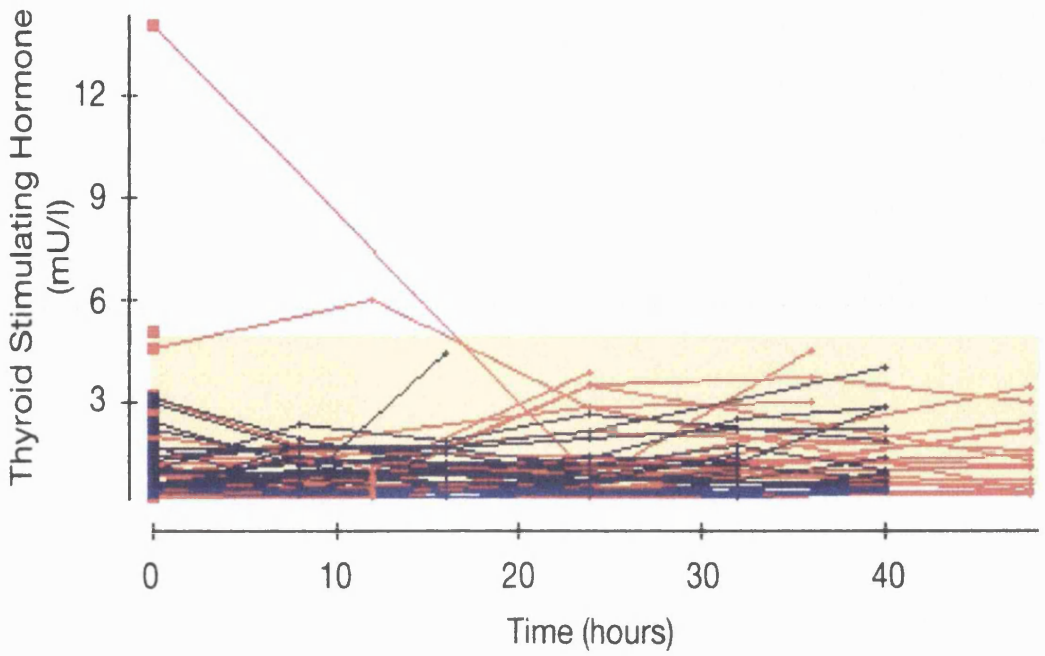


Fig 6.13 Plasma TSH levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the TSH levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma TSH in children.

6.5.9.2 Total thyroxine (T_4)

The plasma thyroxine levels obtained in the critically ill children over the 0-48 hour period are shown in Fig 6.14. They are displayed against a shaded area, which corresponds to the normal range. A total of 236 T_4 measurements were made in the first 48 hours on the patients studied. The maximal and minimal values of T_4 recorded were 157 nmol/l and 15 nmol/l respectively.

On admission 60 T_4 measurements were made of which no measurements were above the reference range and 36 (60%) were below. At 24 hours 43

T_4 measurements were made, of which 29 (67.4%) were below the reference range and none above the reference range.

There was no correlation between the severity of illness as defined by the PROM and levels of total T_4 on admission or at 24 hours. There was no significant correlation between patient age and the plasma T_4 measurements.

Levels of T4 were significantly different at 24 hours than on admission to PICU and rate of change of T4 within the first 24 hours was independent of the absolute total T4 level on admission. The results obtained did not appear to support any difference between the rate of fall of T4 and age of patient, their clinical severity or their diagnosis. The normal range was defined as 60-160 nmol/l (laboratory reference range).

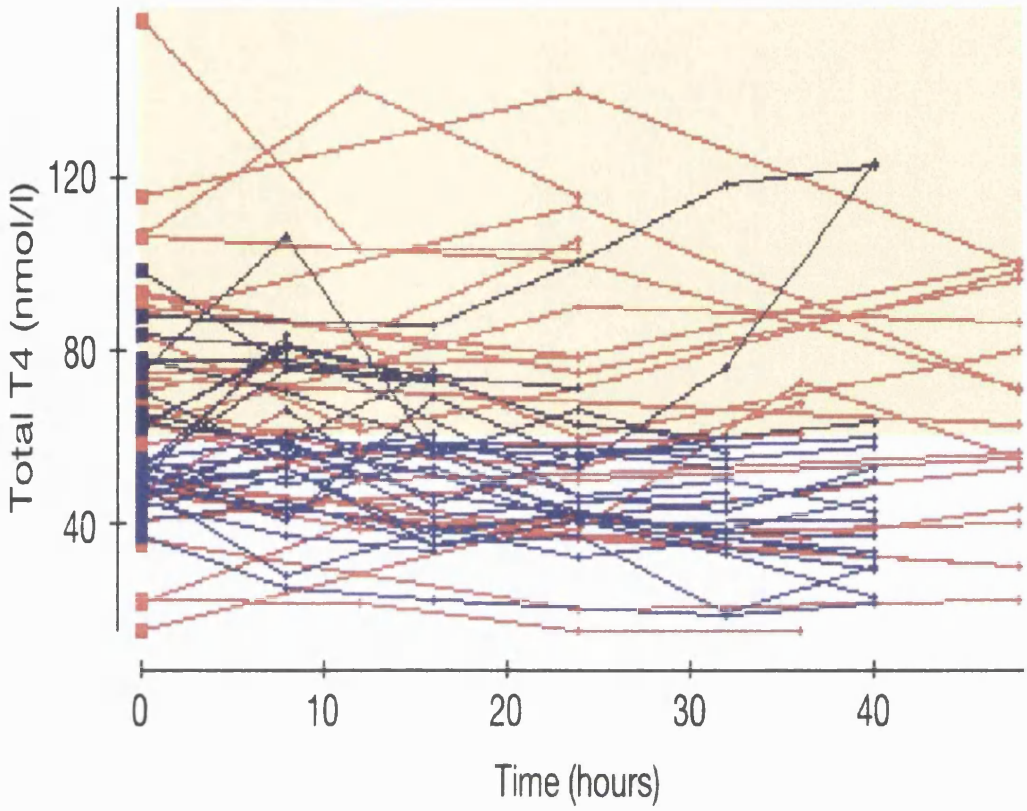


Fig 6.14 Plasma total T_4 levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the total T_4 levels in one patient.

Critically ill children - other diagnoses

Critically ill children - meningococcal sepsis.

Normal range of plasma total T_4.

6.5.10 Discussion

6.5.10.1 Time related changes in the thyroid axis

During illness the serum concentrations of thyroid hormones are often found to be abnormal. The degree of aberration is dependant on the severity of

illness (215), duration of illness (2) and may also be influenced by certain iatrogenic factors (216). This results from changes in the sensitivitiy of the feedback loops (217), in production rates, metabolic clearances and levels of serum binding proteins, all of which have been documented in the critically ill patient.

The observations made in the present study indicate depressed levels of TSH and total T_4 throughout the first 48 hours from admission to PICU, with the T_4 levels were showing a trend returning to the normal range with time.

The biological activity of the thyroid axis is also influenced by decreased extrathyroidal generation of T3 from T4. The resultant low T3 or sick euthyroid syndrome has been well described (218).

Unfortunately, in this study no T3 or rT3 measurements were made to further assess the thyroid axis and its response in childhood to critical illness.

6.5.10.2 Thyroid axis and severity of illness

The correlation between thyroid axis status and patient outcome was examined by Uzel et al, 1986, in critically ill infants with infections (217). Significantly lower initial and subsequent T_4 levels were observed in the group of infants who died, than in the survivors. Indeed, according to Nylen et al, 2001 thyroid hormone levels can be as predictive of outcome as the commonly used severity parameters (i.e. APACHE) (219). A significant negative correlation was found in the present study between log TSH and severity of illness as defined by PROM at both time points (t=0; r=-0.192, p=0.0391 and t=24; r=-0.445,p=0.0025).

However in the present study, where there was no correlation between the severity of illness as defined by the PROM and levels of total T_4 on admission or at 24 hours, although T4 levels were depressed. Several factors may account for this.

It is known that the administration of pharmacological doses of dopamine significantly reduces serum TSH levels and thyroid hormone secretion in normal and critically ill adult patients, probably owing to a direct inhibition of

pituitary TSH (220) with a secondary effect on thyroid gland secretion (216). Several of the patients recruited in the present study required dopamine infusions, which may have led to iatrogenic suppression of the thyroid axis, thus exacerbating the influence of the critical illness on the thyroid axis and distorting the correlation between severity score and hormone level.

Van den Berghe and her group have carried out extensive work on the pattern of changes in anterior pituitary function in critical illness, namely anterior pituitary activation in the early phase of critical illness, followed by low circulating thyroid hormone levels during prolonged critical illness which appear to be positively correlated with the reduced pulsatile TSH secretion (221). The observations made during the course of the present study demonstrate low TSH levels, both on admission and at t=24. Although it is possible to establish the onset of a critical state in patients requiring major elective surgery, it is far more difficult to define the exact timing of the onset of illness in the other diagnostic categories. The process of thyroid axis response may well have begun prior to admission to PICU, consequently the results may well show a more advanced response than expected.

In critically ill adult patients with multiple organ failure, the effect of the non-thyroidal illness on the thyroid axis may be observed within 24 hours of admission to ICU. Suppression of the TSH response to TRH has been described and been found to correlate with severity of illness and outcome (222).

Since suppression of TSH in response to critical illness is a rapid phenomenon, this would help to explain the suppressed TSH levels found in the critically ill children in present study.

The observed decrease in serum concentration of T_4 and TSH are not compatible with a negative feedback loop and suggest a major change in set point regulation of the hypothalamic-pituitary-thyroid axis. Post mortem investigations have demonstrated a decreased expression of thyrotropin-releasing hormone in the hypothalamic paraventricular nucleus of patients

with a decreased serum T3 level. In critical illness, serum T3 may even become undetectable without giving rise to an elevated concentration of serum TSH (223).

Further studies are required in critically ill children to address the TSH responsiveness to TRH and its relation with T3.

In the present study, levels of total T4 were significantly lower at 24 hours than on admission to PICU and rate of fall of T4 within the first 24 hours was independent of the absolute total T4 level on admission. Diminished protein binding of T_4 in plasma is known to occur in critical illness, resulting in subnormal total plasma T4 levels (6), and may account in part for the observation.

Chapter 7- Interrelations between hormones and metabolites in critical illness in children.

7.1 Introduction

Profound changes are known to occur in both the hormonal and metabolic systems as a result of the stress of critical illness. The acute responses are thought to be adaptive, having evolved to optimise survival in the face of illness or injury.

The initial endocrine response to illness or injury consists primarily of an activated release of anterior pituitary hormones and peripheral inactivation of anabolic pathways, which is thought to provide substrates for survival while anabolism is postponed (5).

Patterns of insulin insensitivity (142), GH insensitivity (189) and sick euthyroid syndrome (35;224) have been well described in adults who are critically ill. Specific metabolic and endocrine responses have been observed in children post surgery (117;121;183), following burn injury (225) and after severe head injury (180).

The aim of the analysis performed in this chapter was to examine the hormonal and metabolic interrelations in critically ill children and to follow their progression with time. Comparisons were made to the interrelationships observed in health, in critically ill adults and in subgroups of seriously ill children. This chapter describes and discusses the:

1. Correlations between hormones and metabolites involved in lipid metabolism ;
2. The interrelationships within the GH-IGF axis.

This chapter is divided into 3 subsections: section I will describe and discuss correlations between hormones and metabolites versus age of patients and severity of illness as defined by the PROM score; section II will describe and discuss the interrelations of hormonal mediators of lipolysis and correlations between various fatty acid metabolites and hormones; and section III will

describe and discuss the interrelations and correlations between the GH-IGF-1 axis, insulin, aldosterone and lactate.

7.2 Materials and Methods

This section of the study was performed on all 60 children with critical illness enrolled in the project, as described in chapter 6. The diagnoses within this group included mainly children with meningococcal sepsis, respiratory infection, post surgery (mainly GI and ENT), the combined mean age was 4.4 years (range 0.25-16.33) with a predicted risk of mortality calculated from the PRISM score, mean value 21.2% (range 0.5 – 99.31%).

Common time points for sampling of the meningococcal patients and other critically ill children were on admission and 24 hours later. In order to examine the maximal number of patients, data from these 2 time points was used for analysis. Blood sample collection, preparation and processing was conducted as described in Chapter 2. The metabolites and hormones assayed and correlated were as follows: blood lactate, pyruvate and acetoacetate concentrations, nonesterified free fatty acids (NEFA) and β-hydroxybutyrate levels, plasma insulin, GH, IGF-1, IGFBP-I, cortisol, aldosterone, T4, TSH and glucagon.

7.3 Statistical analysis

Scatter diagrams were used to visualise data and review the relationship between age, severity, hormones and metabolites on admission and 24 hours later. Skewed distributions were logged (log 10) before analysis. Linear associations between variables were explored by correlation analysis. The parametric method Paerson Product Moment was used with statistical significance being defined as $p < 0.05$. The programme Datadesk 6.1 (Data Description inc., Ithaca, NY, U.S.A) for Windows was used for the analysis.

7.4 Results and Discussion

7.4.1 Section I: Correlations between age and severity and hormone and metabolite levels at t=0 and t=24 hours

		t=0		t=24	
		r	p value	r	p value
Age	log IGF-1	0.422	0.0004	0.397	0.01
	log IGFBP-3	0.493	0.0120	0.293	n.s.
Severity	Log Lactate	0.626	<0.0001	0.595	0.0004
	Log TSH	-0.192	0.0391	-0.445	0.0025

Table 7.1 Summary of the positive and negative statistically significant correlations between age and severity and hormone and metabolite levels at t=0 and t=24 hours.

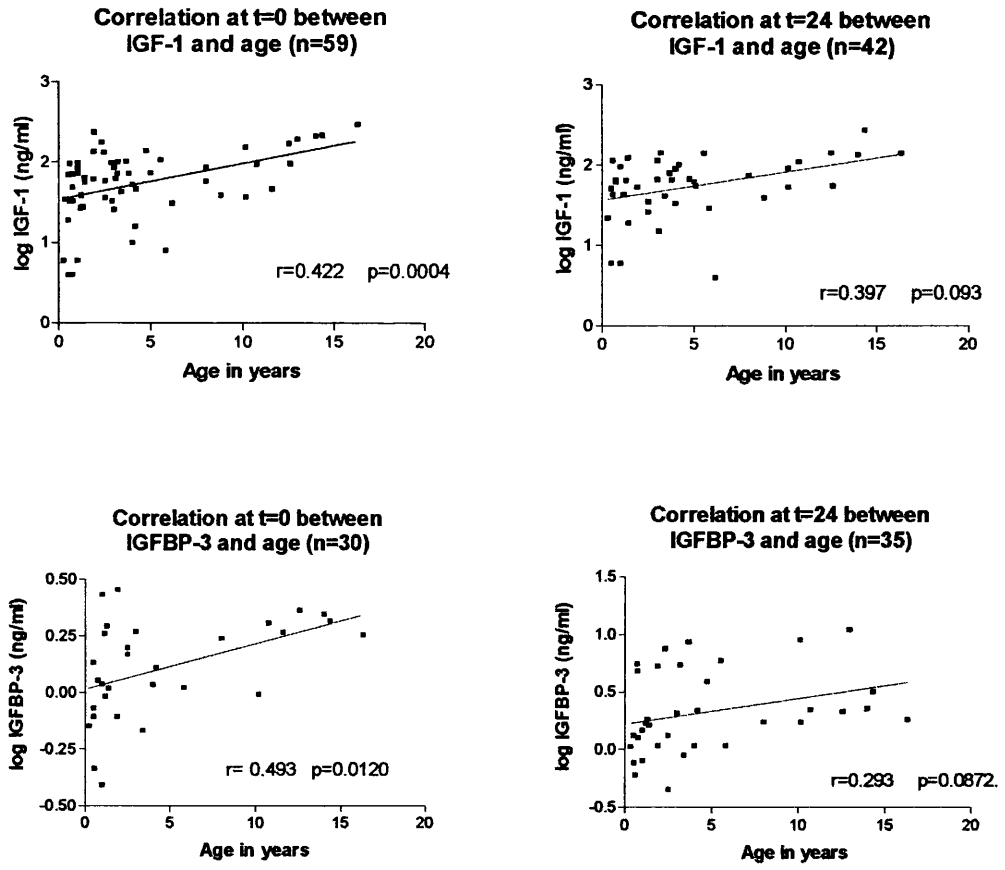
The correlation analyses were performed between the log of all metabolites and hormone concentrations versus age and severity of the critical illness score (PROM). Only variables, which showed statistically significant correlations are listed in Table 7.1. The actual graphic presentations are given in Fig 7.1. No other metabolic and hormonal variable showed significant correlation.

GH secretion undergoes age-related changes in secretory patterns in both human and animals (226). Under physiological conditions, IGF-1 and IGFBP-3 generally follow the age-related changes in GH secretion (227), except in neonate, where IGF-1 levels are low despite GH hypersecretion (196). At puberty both IGF-1 and IGFBP-3 levels are known to rise (187),

paralleling GH secretion, but then tend to fall thereafter. The relationship is not strictly linear, however at t=0 there were significant correlations with age of both IGF-1 and IGFBP-3. Similar correlation at t=24 was less marked, presumably due to the increasing GH insensitivity that is observed in the critically ill patients.

Levels of both IGF-1 and IGFBP-3 are known to vary with age (187).

Fig 7.1 Summary of the positive and negative statistically significant correlations between age and severity and hormone and metabolite levels at t=0 and t=24 hours.



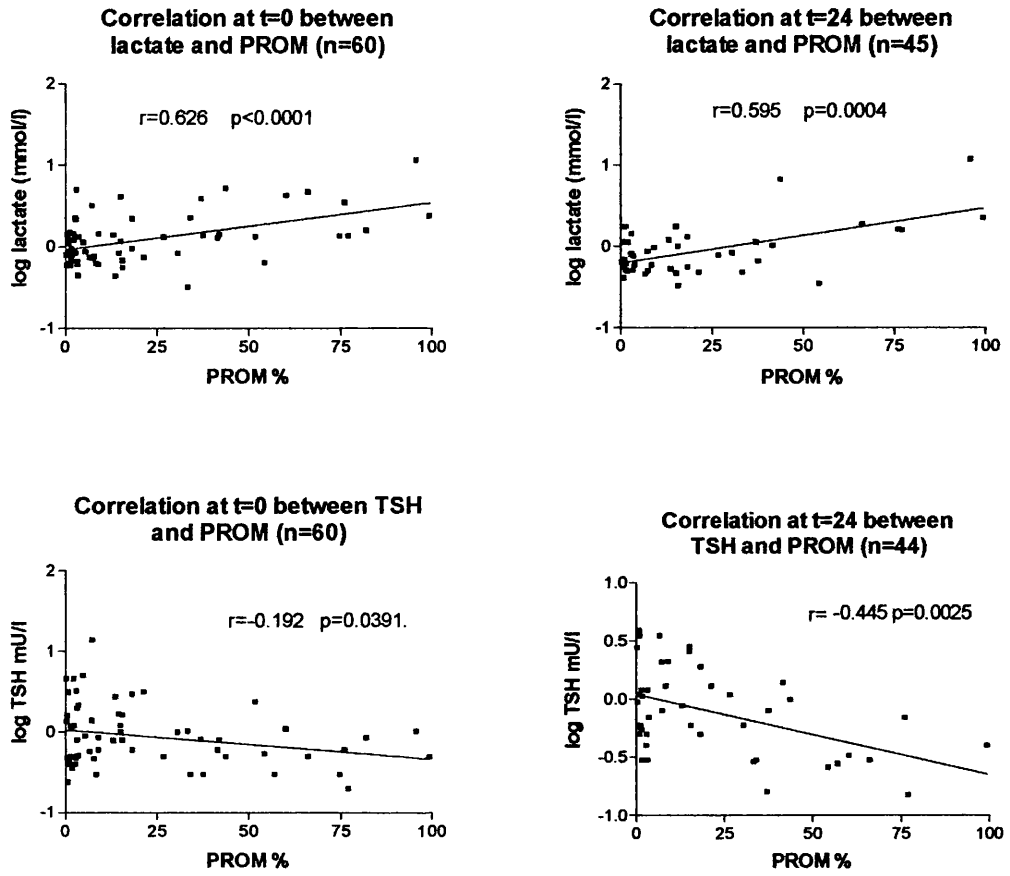


Fig 7.1 (continued).

The positive correlation between plasma lactate levels and illness severity was obtained at both t=0 and t=24. It is not only the magnitude of rise in plasma lactate that is of prognostic significance, but also the duration of elevated plasma lactate (>24 hours) that is known to correlate with patient mortality (171) in critically ill children and adults (170). These issues have been discussed in detail in both chapters 5 and 6.

The negative correlation between plasma TSH levels and illness severity observed in the present study has been previously been recognised in adult studies, where the degree of T3 suppression with concomitantly low TSH correlated positively with the severity and duration of the disease and correlated negatively with outcome. An endocrine prognostic index based on

ICU admission measurements of thyroxine, thyrotropin, and cortisol concentrations was even reported as being a superior discriminator of patient outcome than the APACHE II score for assessing critical illness severity in adults (34).

7.4.2 Section II: Interrelation between hormonal mediators of lipolysis

7.4.3 Introduction

7.4.3.1 Fatty acid oxidation

Metabolism of fatty acids occurs primarily within the liver, where dietary fatty acids are oxidised to ketone bodies, β-hydroxybutyrate and acetoacetate. These compounds cannot be utilized by the liver cells effectively and are exported to the blood. The ketone bodies are readily utilized by various organs including the brain, due to the availability of an enzyme to convert ketone bodies to acetyl-CoA. This final product of fat oxidation is then utilized by the Tricarboxylic Acid Cycle (TCA or Krebs cycle) ultimately producing ATP and glucose in these organs. Organs other than liver are not capable of producing ketone bodies but except for the brain, are capable of oxidising long-chain fatty acids to energy (ATP) since the process of β-oxidation is tightly linked to the TCA cycle and respiratory chain.

7.4.3.2 Fat metabolism – Catabolic and anabolic pathways

Triacylglycerol undergoes hydrolysis by a hormone sensitive lipase to form free fatty acids and glycerol. The free fatty acids formed by lipolysis can be reconverted to acyl-CoA by acyl-CoA synthase and reesterified to glycerol-3-phosphate to form triacylglycerol in the tissues. However, when the rate of reesterification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma. They are an important source of fuel for many tissues especially in critical illness.

The rate of release of free fatty acids from adipose tissue is affected by many hormones that influence either the rate of esterification or the rate of lipolysis. Insulin administration is followed by a fall in circulating plasma free fatty acids and in vitro insulin inhibits the release of free fatty acids from adipose tissue, enhances lipogenesis and the synthesis of acyl-glycerol,

increases the oxidation of glucose to CO_2 via the hexose-monophosphate shunt. Insulin has been shown to increase the activity of 3 enzymes, pyruvate dehydrogenase, acetyl-CoA carboxylase and glycerol phosphate acetyltransferase, which explains the increase in fatty acid and acyl-glycerol synthesis. On the other hand, a principal action of insulin in adipose tissue is to inhibit the activity of hormone sensitive lipase, reducing the release of both free fatty acids and glycerol.

Other hormones such as adrenaline, noradrenaline, glucagon, adrenocorticotrophic hormone (ACTH), α- and β- melanocyte stimulating hormone (MSH), thyroid stimulating hormone (TSH), growth hormone (GH) and vasopressin accelerate the release of free fatty acids from adipose tissue and raise the the plasma level of free acids by increasing lipolysis of the triacylglycerol stem. Many of these hormones activate the hormone sensitive lipase and increase glucose utilization. For an optimum effect, most of these lipolytic processes require the presence of glucocorticoids and thyroid hormones.

Adipose tissue contains a number of lipases, one of which is hormone sensitive triacylglycerol lipase, which catalyses the rate-limiting step of lipolysis. Insulin has a profound antilipolytic effect both in vivo and in vitro and antagonizes the effect of the lipolytic hormones. Glucocorticoids promote lipolysis through synthesis of new lipase protein by a cAMP dependant pathway. These findings help explain the role of the HPA axis in enhancing fat mobilization. Human adipose tissue is unresponsive to most of the lipolytic hormones except catecholamines. Under physiological conditions, it is likely that the main lipolytic stimulus in adipose tissue is due to the liberation of noradrenaline through sympathetic activity.

Diversion towards lipolysis (catabolic pathway) as opposed to lipogenesis (anabolic pathway) in the overall metabolism in critical illness ensures energy supply of in the form of free fatty acids to the vital central organs at the expense of the peripheral organs. Ketogenesis occurs, the final product acetoacetate is reversibly convertible to β-hydroxybutyrate and spontaneously to acetone. The correlations between plasma levels of acetoacetate and β-hydroxybutyrate and the initial levels of free fatty acids

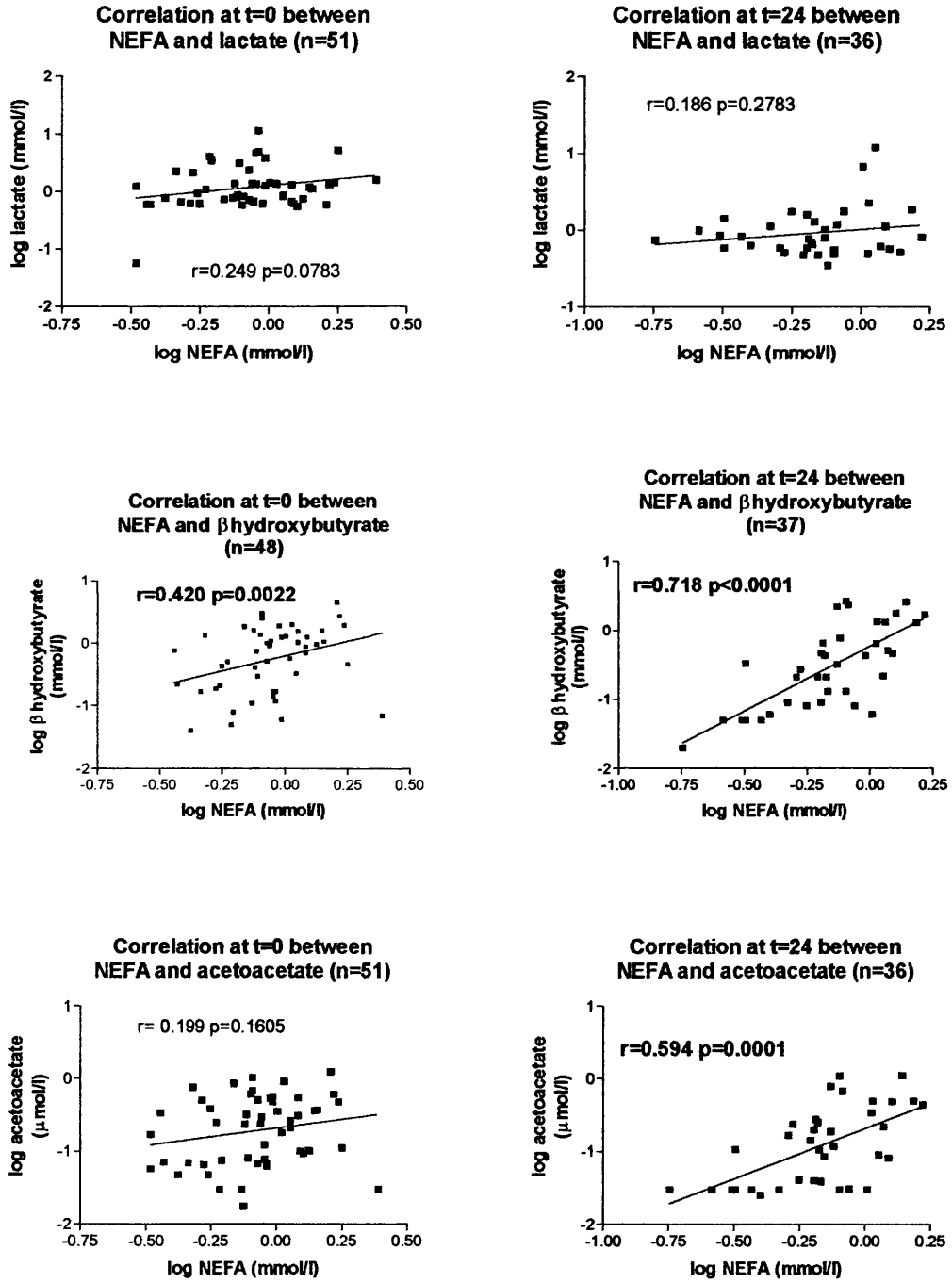
and between hormones stimulating these pathways as well as the appropriate enzymes carrying out the intermolecular convesions of the precursor-substrate molecules have a significant importance in the sequelae of impairment or recovery during the course of intensive care therapy.

7.4.4 Fat metabolism – Results

Table 7.2 Summary of the correlations between plasma concentrations of non-esterified free fatty acids, ketone bodies, lactate and of pituitary, adrenal, and pancreatic hormones at the time of admission to PICU (t=0) and after 24 hours (t=24).

Intermediary metabolites and hormones as variables		t=0		t=24	
		r	p value	r	p value
Log NEFA	Log Lactate	0.249	n.s.	0.186	n.s.
	Log BOHbutyrate	0.420	0.0022	0.718	<0.0001
	Log Acetoacetate	0.199	n.s.	0.594	0.0001
	Log Insulin	-0.212	n.s.	-0.655	<0.0001
	Log GH	-0.203	n.s.	-0.148	n.s.
	Log Cortisol	0.171	n.s.	0.440	0.0065
	Log glucagon	-0.001	n.s.	-0.007	n.s.
Log Acetoacetate	Log BOHbutyrate	0.703	<0.0001	0.949	<0.0001
	Log Insulin	-0.207	n.s.	-0.474	0.0015
	Log GH	-0.240	n.s.	-0.291	n.s.
	Log glucagon	-0.357	n.s.	-0.202	n.s.
Log BOHbutyrate	Log Insulin	-0.451	0.0010	-0.500	0.0019
	Log GH	-0.259	n.s.	-0.362	0.0302
	Log glucagon	-0.413	0.0036	-0.194	n.s.

Fig 7.2 Summary of the correlations between plasma concentrations of non-esterified free fatty acids, ketone bodies, lactate and of pituitary, adrenal, and pancreatic hormones at the time of admission to PICU (t=0) and after 24 hours (t=24).



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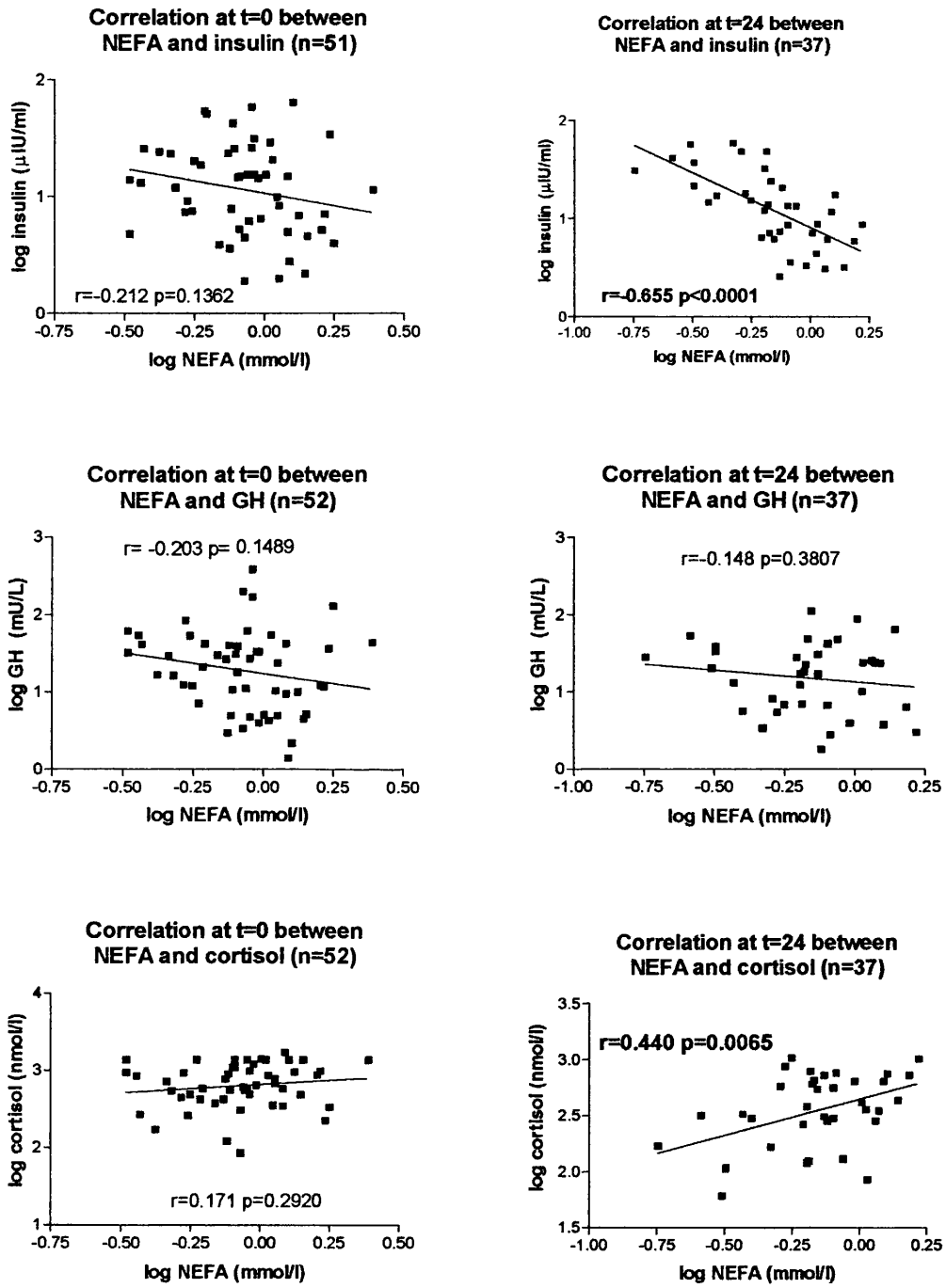

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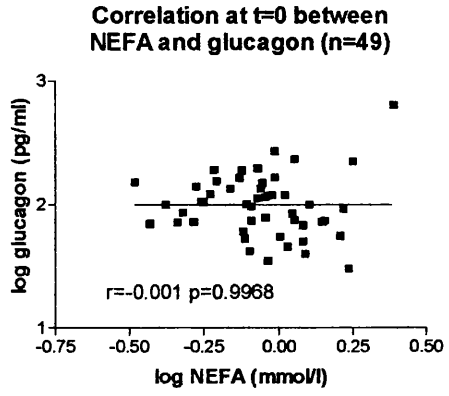

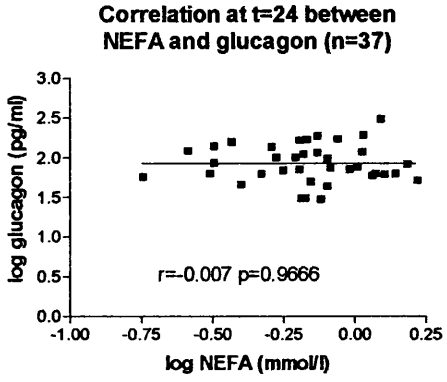

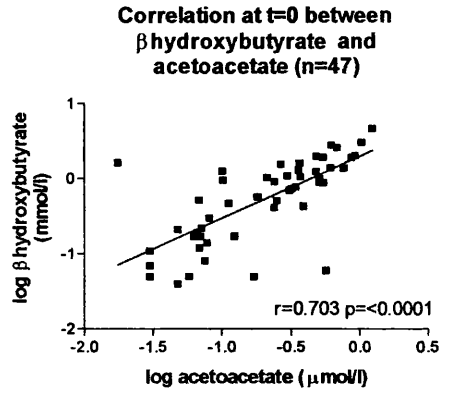

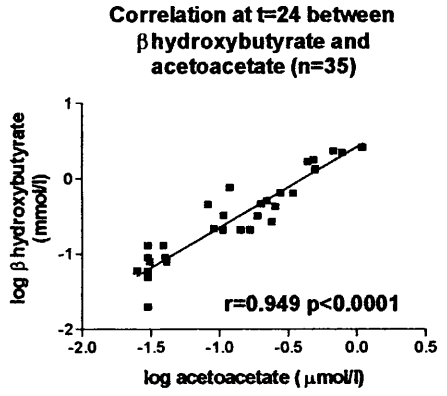

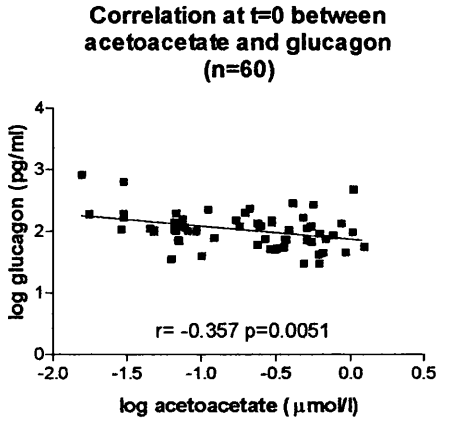

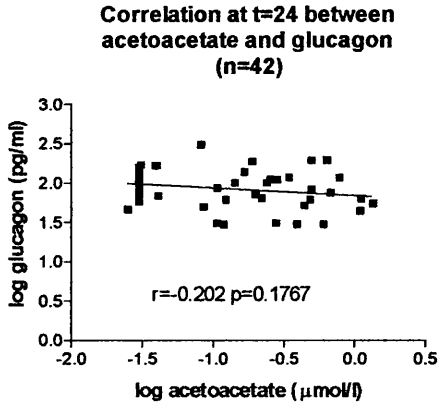

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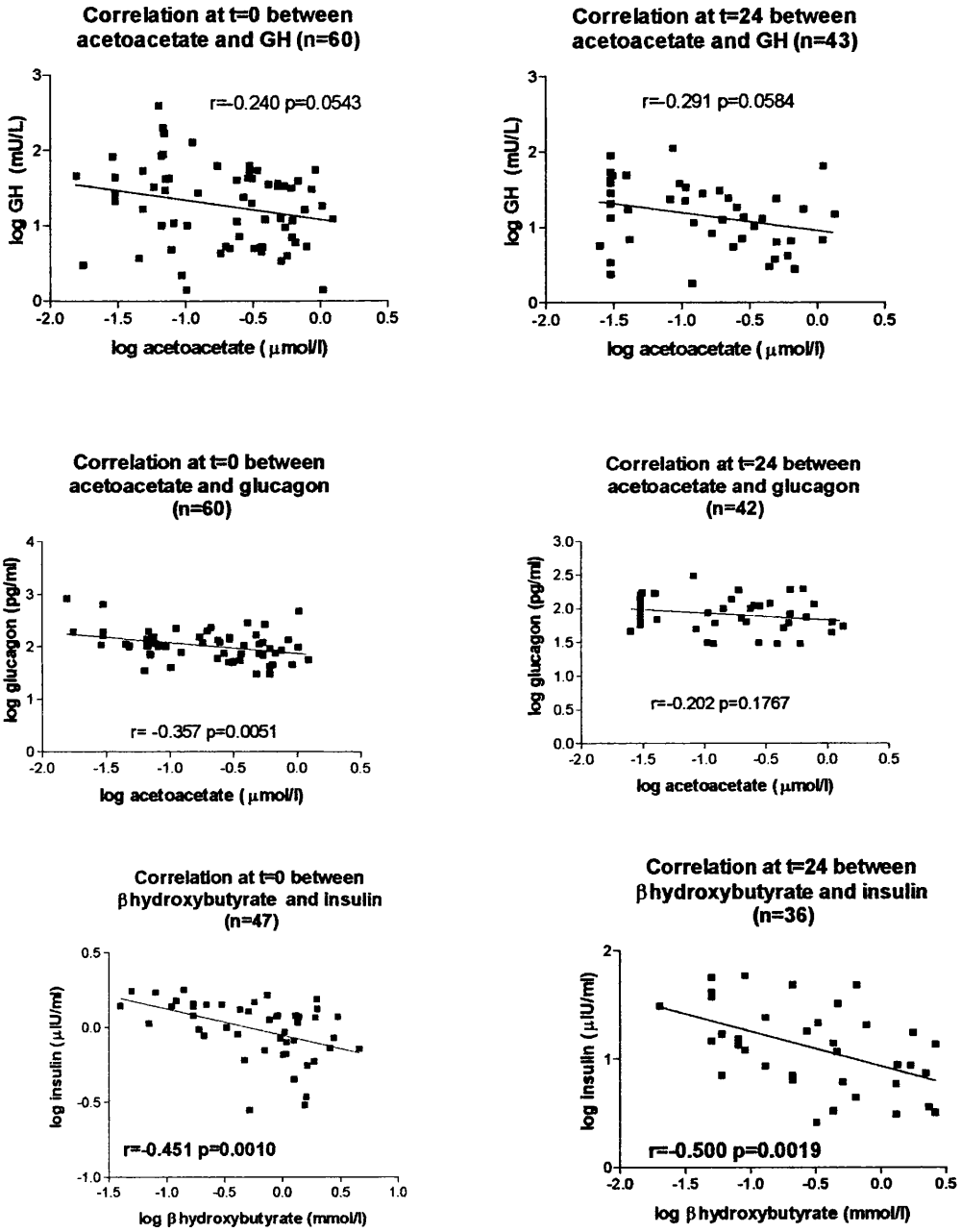

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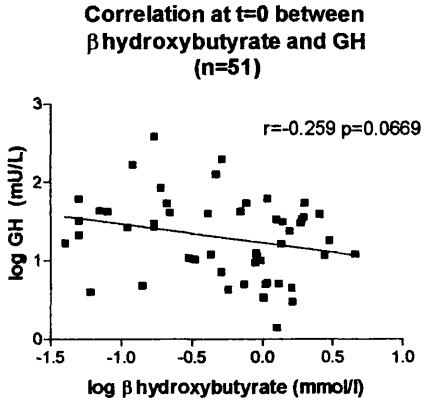
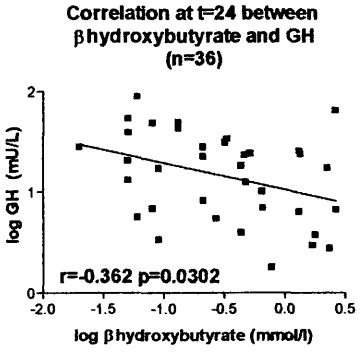
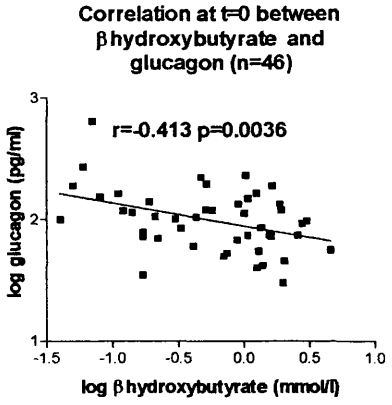
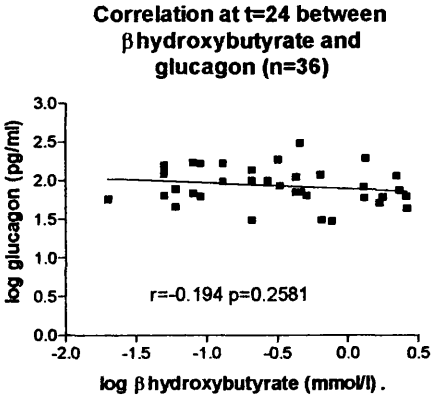








7.4.5 Discussion – Fatty acid metabolism

The plasma levels of catecholamine concentration were not determined, as this was not part of the study design. Therefore it is not possible to assess correlations between NEFA and catecholamines.
Insulin is the main inhibitor of lipolysis (53). Insulin regulation of lipolysis is in itself complex, showing regional heterogeneity of insulin-regulated FFA release in vivo, where visceral adipose tissue lipolysis is more resistant to insulin suppression than is leg lipolysis in humans (228).
In this study, there was no significant correlation between plasma insulin and plasma NEFA levels at t=0. The expected negative correlation became however apparent at t=24 ($r=-0.655$, $p<0.0001$). Data from critically ill head injured children appear to support the present finding, where a significant negative relationship between plasma NEFA and insulin levels has been described (229).
However, when examining data provided by turnover studies in critically ill children, there does not appear to be any relationship between plasma insulin levels and the rate of lipolysis (184).

Other hormones known to modulate lipolysis include cortisol, growth hormone, vasopressin, α- and β-MSH, glucagon and ACTH, although the latter two are known to be effective mainly in vitro (53). Physiological levels of glucagon are thought not to influence lipolysis in humans, as assessed by microdialysis in abdominal adipose tissue (230). In the present investigation there was no association between plasma levels of glucagon either at t=0 or t=24. Plasma levels of ACTH, vasopressin and α- and β-MSH were not assayed in this study.

The effects of glucocorticoids on lipid mobilization are still thought to be controversial (231). Results of in vivo studies on lipid metabolism have proved complex, some older studies reporting no effect of cortisol on lipolysis (232), whilst more recent data appear to support the fact that

physiological hypercortisolemia may contribute to the increased rates of lipolysis observed in humans during stress (233). In Cushings syndrome, however, central adiposity is thought, at least in part, to be due to an inhibiting effect of glucocorticoids on the lipolytic activity in vivo (234). More recent in vitro work on human adipose tissue however suggests that cortisol inhibits lipolysis (231). In line with the fact that steroid hormone receptors are found in human adipose tissue (235) the effect of cortisol is thought to be direct on the adipose tissue.

In the present study there was no correlation between plasma cortisol and plasma NEFA levels on admission to PICU. However at 24 hours there was a positive correlation ($r=0.440$, $p=0.0065$) between plasma cortisol and plasma NEFA levels (Fig 7.2). It appears that in the critically ill children, cortisol may be associated with lipolysis, although this effect is only observed after 24 hours.

In animals GH is known to stimulate basal lipolysis, but also alters the ability of adipocytes to respond to lipolytic factors such as catecholamines (236). In man, GH is known to reduce adipose tissue mass (237) and increase the release of free fatty acids (238). Recent in vitro work on human adipose tissue has shown that the lipolytic effect of GH is strengthened in the presence of cortisol (231).

The acute effects of GH, determined by iv injection of GH into dogs, are known to include a significant, but transient rise in free fatty acids levels at 20 min, and a subsequent elevation in ketone body concentrations at 120-180 min (239).

However, in the present study there was no significant correlation between plasma NEFA levels, plasma acetoacetate and β-hydroxybutyrate levels and GH on admission to PICU. At 24 hours post admission, plasma NEFA levels and plasma acetoacetate levels again showed no significant correlation with GH, yet the β-hydroxybutyrate levels correlated negatively with GH ($r=-0.362$, $p=0.0302$). These findings are supported by recent stable isotope turnover studies, where critically ill children failed to show any association between plasma GH levels and lipolysis by measuring palmitic acid and glycerol turnover (184).

Net output of NEFA from adipose tissue is the balance of lipolysis or breakdown of triacyl glycerols and the reseterification of NEFA. Plasma lactate levels are frequently known to be elevated in the critically ill and are thought to influence the process of reesterification. Increased lactate concentrations give rise to increasing cytosolic NADH/NAD+ ratios and increased production of α- glycerophosphate within adipose tissue, thus, promoting reesterification (229). In the current study there was no correlation between plasma lactate and plasma NEFA levels either on admission to PICU or 24 hours after admission.

NEFA is known to enter the liver down a concentration gradient, it is then transported into the hepatic mitochondria as acyl carnitine and converted to acetyl CoA. Ketone bodies are then formed from acetyl CoA and released into the circulation (240). Carnitine palmitoyl- transferase catalyses the formation of acyl carnitine. This enzyme is inhibited by malonyl CoA, whose intracellular concentration is increased by insulin and decreased by glucagon. (47).

There was no significant association between insulin or glucagon and plasma acetoacetate levels at t=0, on admission to PICU. However a negative relationship with insulin was observed (r=-0.474 ,p=0.0015) as expected within 24 hours of admission. There was no correlation between glucagon levels at t=24. β-Hydroxybutyrate levels were however more closely associated with insulin levels, showing the expected negative relationship (r= -0.451, p=0.001) at t=0 and (r=-0.500, p=0.0019) at t=24. Surprisingly glucagon levels showed a negative relationship (r=-0.413, p=0.0036), and not the expected positive relationship to β-hydroxybutyrate levels at t=0. The peripheral insulin/glucagon ratio shows no correlation to the plasma β-hydroxybutyrate levels. It is important to note that the peripheral insulin and glucagon levels do not necessarily reflect portal levels and there is little information on portal insulin and glucagon in critical illness. There may be multiple hormonal interactions occurring in the critically ill

children and therefore it is not possible to tease out the influences of individual hormones on lipid metabolism as is the case in clamp studies.

A linear relationship is known to exist between plasma NEFA levels and the log of ketone bodies (241), lack of correlation between high NEFA and low ketone bodies may suggest a defect in β-oxidation. In this sample of critically ill children, there was a significant relationship between NEFA and β-hydroxybutyrate at t=0, ($r=0.420, p=0.0022$) and an even tighter relationship at t=24 ($r=0.718$ and $p=<0.0001$). There was no significant correlation between NEFA levels and plasma acetoacetate at t=0, but there was a significant correlation by t=24 ($r=0.594$ and $p=0.0001$). Tighter association is observed between plasma levels of β-hydroxybutyrate since it is a more stable substance than acetoacetate, which requires precipitation at the bedside with perchlorate and may have undergone a degree of degradation despite appropriate processing. The β-hydroxybutyrate/acetoacetate ratio tends to rise also with the duration of stress. Plasma levels of β-hydroxybutyrate and acetoacetate however showed good correlation ($r=0.703, p<0.0001$ at t=0 and $r=0.949$, $p<0.0001$ at t=24).

The effect of individual hormones on different aspects of lipid metabolism (lipolysis and β–oxidation) is a complex field to address in the context of critical illness, since the patients are subject to changing levels of hormones and metabolites as well as altered responsiveness to the above mentioned hormones. Indeed more precise techniques to address lipid turnover and fat oxidation include the use of stable isotopes and indirect calorimetry. A recent study in critically ill children addressed the issue of lipid turnover. Despite all the known hormonal influences on aspects of lipid metabolism, in critically ill children, overall the lipid turnover measurements did not correlate with any endogenous hormone levels or with duration of critical illness (184).

7.4.6 GH-IGF axis

7.4.6.1 Hormonal and metabolic control of the GH –IGF axis

As described in chapter 1, IGF-1 is a GH dependant hormone, that itself exerts negative feedback on GH secretion both at the level of the pituitary, where it inhibits GH synthesis and release and at the level of the hypothalamus, where it stimulates somatostatin and inhibits GHRH-secreting neurons, both of which result in reduced GH release (242).

Both in vivo and in vitro, insulin, possibly through binding IGF-1 receptors can also decrease GH release (243). Glucagon however can only stimulate GH release when given intramuscularly, the underlying mechanism remains obscure (244).

It has been documented that spontaneous GH secretion is reduced in hypothyroidism and increased in hyperthyroidism. Thyroid hormones are needed for GH gene transcription, translation and GH secretion. Thyroid hormones are known to exert an effect at the hypothalamic level, as well as peripherally where they are required for normal IGF-1 synthesis and release (245;246).

The duration of exposure to glucocorticoids governs the response of the GH axis. Acutely, they are able to stimulate, but the response becomes blunted after 12 hours or more. Indeed chronically elevated glucocorticoids block spontaneous and stimulated GH secretion, possibly via hypothalamic mechanisms. In hypocortisolaemia, however, GHRH induced GH secretion is also blunted and may be restored by glucocorticoid replacement(247;248).

Metabolites are also known to affect the GH axis. Insulin-induced hypoglycaemia forms the basis of the classical stimulatory test, the underlying mechanism being that of a counter-regulatory response to a fall in plasma glucose. The oral glucose load being used as an inhibitory test where both basal and GHRH-stimulated GH secretion is inhibited (249). However, this effect is lost in conditions such as acromegaly, diabetes, liver

cirrhosis, malnutrition, anorexia, psychosis and is not present in the newborn. Peripherally glucose, in conjunction with amino acids, allows GH sensitivity and thus IGF-1 synthesis and release.

Elevated free fatty acids are known to exert an inhibitory effect on GH secretion, whereas lipid lowering agents eg. nicotinic acid are able to enhance both basal and GHRH stimulated GH release (196). Elevated free fatty acid levels may therefore play a role in the GH hyposecretion that is found in obese individuals. However, in catabolic states, GH secretion is often elevated in spite of concurrently elevated free fatty acid levels.

GH secretion is also known to be influenced by oestrogen or aromatized testosterone (250). This is however beyond the scope of the present study.

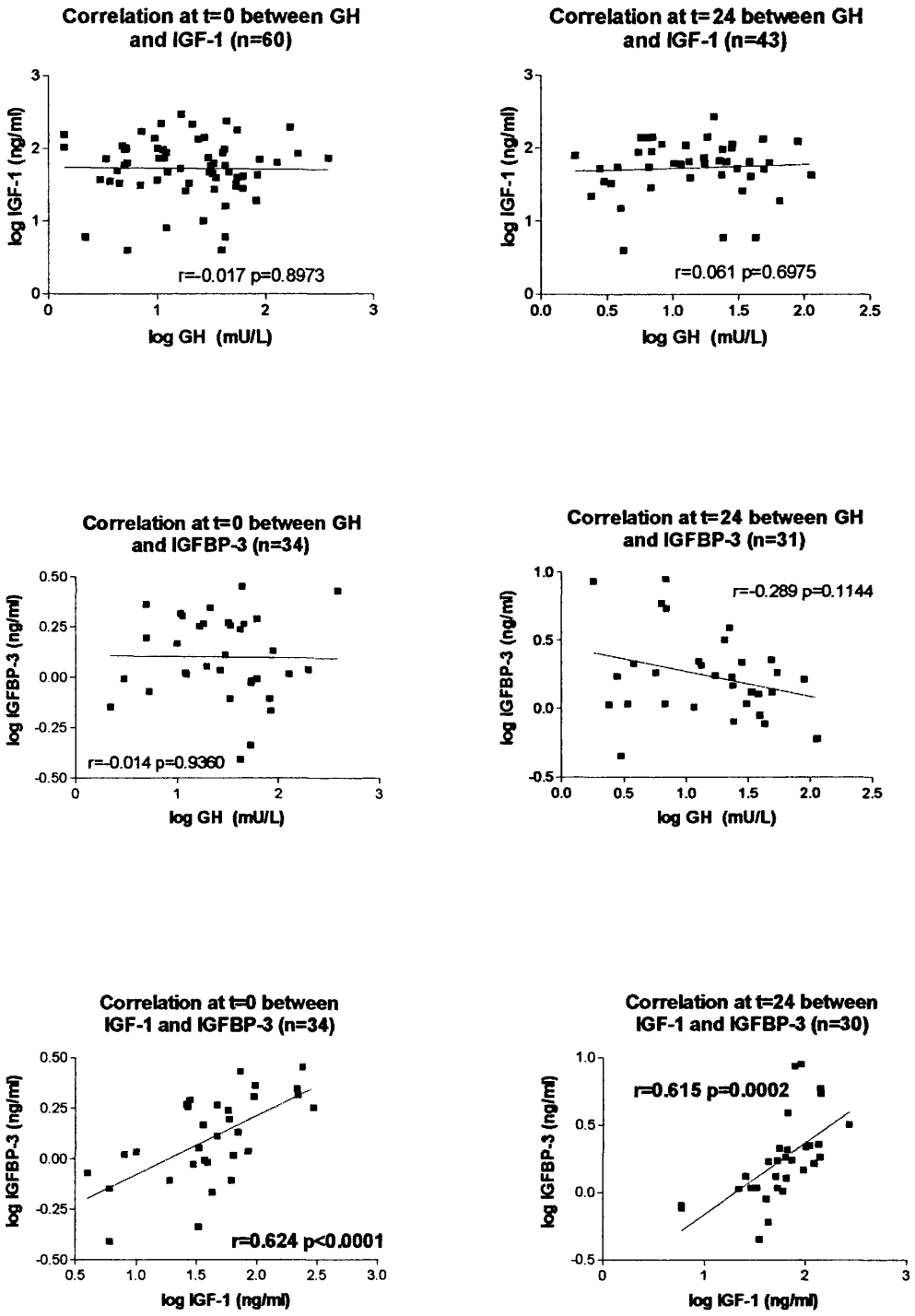
7.4.6 GH-IGF-1 axis - Results

The data on plasma concentrations at t=0 and t=24 of different components of the Gh-IGF axis and some other hormones and metabolites were compared. The results are presented in graphic form in Fig 7.3, and the data are summarised in Table 7.3. No other significant correlations were found between the GH-IGF-1 axis and other hormones.

Table 7.3 Summary of the correlations between plasma concentrations of GH, IGF-1, IGFBP-3, IGFBP-1, Insulin, lactate and aldosterone at the time of admission to PICU (t=0) and after 24 hours (t=24).

Hormones as variables		t=0		t=24	
		Correlation	p value	Correlation	p value
Log GH	Log IGF-1	-0.017	n.s.	0.061	n.s.
	Log IGFBP-3	0.014	n.s.	-0.289	n.s.
Log IGF-1	Log IGFBP-3	0.624	<0.0001	0.615	0.0002
	Log Insulin	0.315	0.0119	0.335	0.0280
	Log Lactate	0.376	0.0026	0.313	0.0490
Log IGFBP-3	Log Insulin	0.454	0.0069	0.218	n.s.
Log IGFBP-1	Log Insulin	-0.193	n.s.	-0.575	<0.0001
	Log IGFBP-3	-0.157	n.s.	-0.405	0.0239
Log Aldosterone	Log IGF-1	-0.119	n.s.	-0.453	0.0026
	Log IGFBP-1	0.324	0.0124	0.479	0.0013
	Log IGFBP-3	0.209	n.s.	-0.719	<0.0001

Fig 7.3 Summary of the correlations between plasma concentrations of GH, IGF-1, IGFBP-3, IGFBP-1, Insulin, lactate and aldosterone at the time of admission to PICU (t=0) and after 24 hours (t=24).



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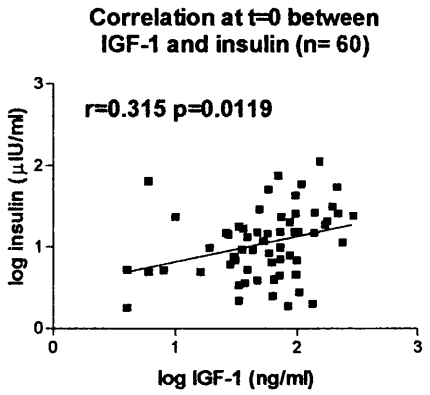

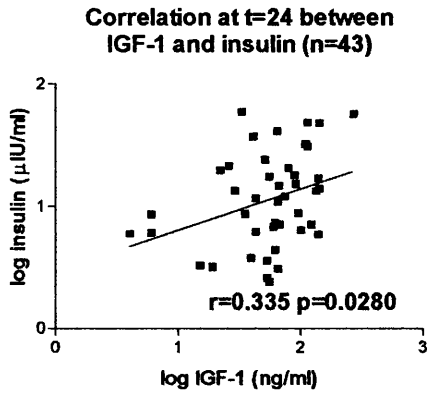

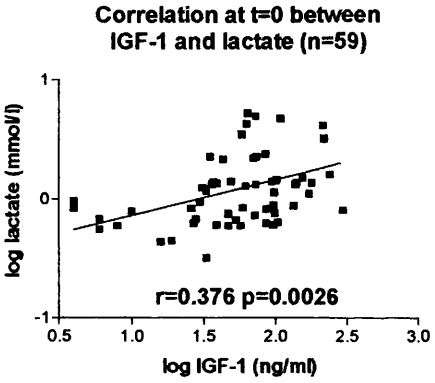

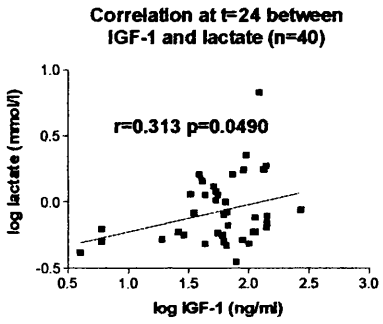

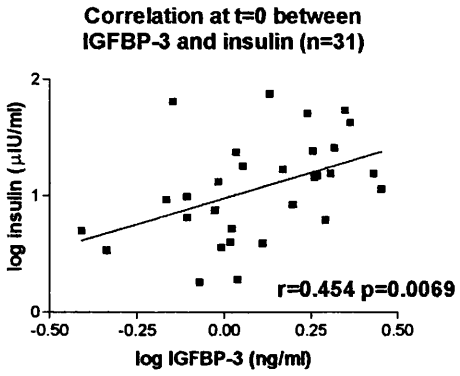

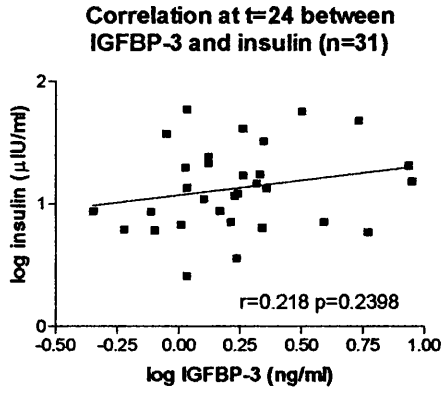

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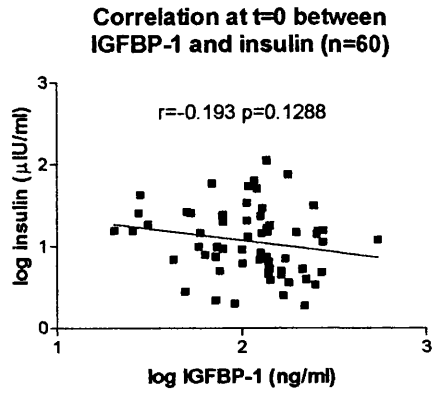

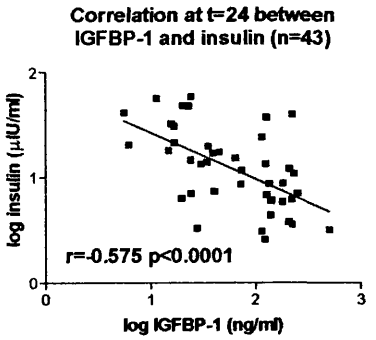

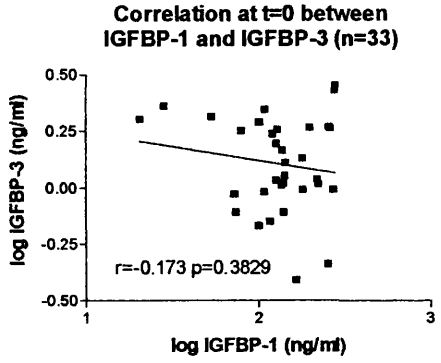

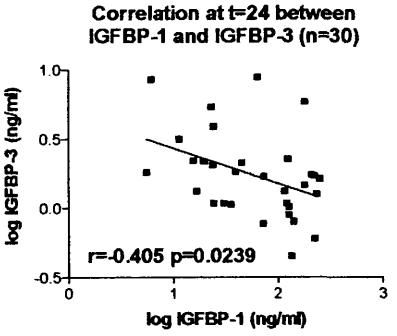

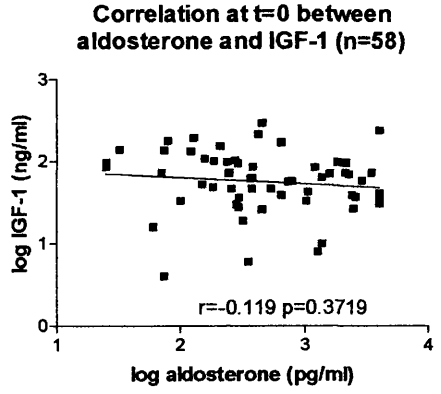

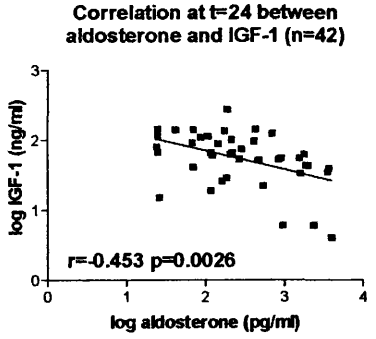

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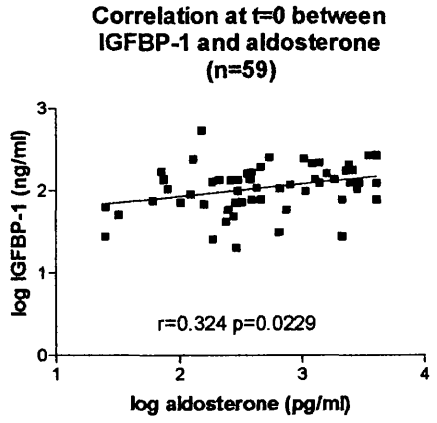
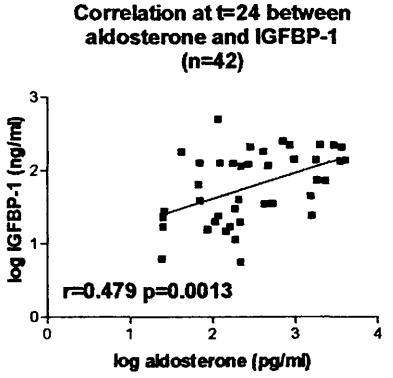
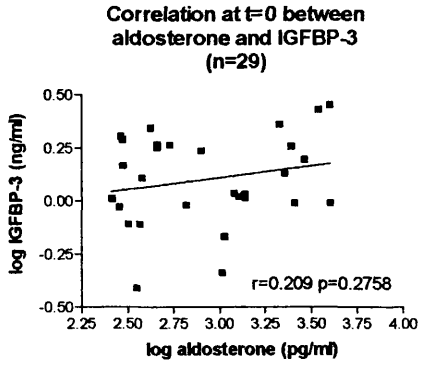
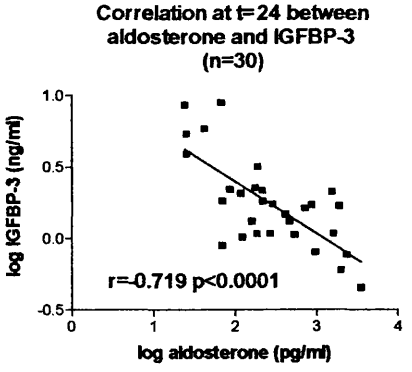








7.4.7 GH- IGF-1 axis – Discussion

In critically-ill patients the basal GH secretion pattern is known to change, resulting in an increased basal level, but loss of oscillatory activity (32). Pulsatile GH administration is known to induce a greater effect on IGF-1 gene expression than continuous infusion (88) and this loss of pulsatility may in part explain the GH resistance that is observed in critical illness. As expected, in the present study there was no association between the plasma levels of GH and plasma levels of IGF-1 at both t=0 and t=24.

The production of IGFBP-3, the major carrier protein for IGF-1, is known to be induced by GH (251). However, severe illness is known to be associated with an increase in IGFBP-3 protease activity (191), and a change in the binding affinity between IFGBP-3 and IGF-1, which results in disruption of the ternary complex (190). The data obtained in the present study appear to support the above, since there was no discernable correlation between plasma levels of GH and plasma levels of IGFBP-3 at t=0 and t=24. However the close correlation between IGFBP-3 and IGF-1 remained intact, the correlations at t=0 and t=24 being highly significant ($r=0.624$, $p=<0.0001$ at t=0 and $r=0.615$, $p=0.0002$ at t=24). This is in agreement with the observation made by Baxter et al, 1989, that circulating levels of IGFBP-3 are present in equimolar amounts to the sum of IFG-1 and IGF-II concentrations (252).

Insulin therapy in children with uncontrolled diabetes mellitus is known to decrease IGFBP-3 proteolysis (201) thus restoring the IGF-1 levels comparable to the normal subjects (203). However, this phenomenon was not observed in children suffering from burns. Insulin alone was not able to influence the levels of IGF-1 or ALS (204). In the present study on critically ill children there was a significant relationship between insulin and IGF-1 at both time points ($r=0.315$, $P=0.0119$ at t=0 and $r=0.355$, $p=0.0280$ at t=24) and between insulin and IGFBP-3 at t=0 ($r=0.454$, $p=0.0069$). Insulin may therefore be able to limit the IGFBP-3 proteolysis initially, but, with time, the

overwhelming degree of proteolysis that is observed in critically ill and septic patients may far outweigh the anti-proteolytic effects of insulin.

Insulin is also known to control the IGFBP-1 levels exerting a negative effect (253;254). De Groof et al, 2002 in their study on children with meningococcal sepsis were unable to demonstrate any correlation between insulin and IGFBP-1 levels on admission and at 24 and 48 hours (173). In the present study although there was no correlation at t=0, by t=24 there was a significant negative correlation between plasma levels of insulin and plasma IGFBP-1 levels ($r=-0.575$, $p<0.0001$). In animal studies, the effect of insulin on IGFBP-1 gene expression requires acute exposure to GH and consequently in states of GH insufficiency, the negative influence of insulin on IGFBP-1 expression is lost (253) One possible explanation therefore for the observed lack of correlation between insulin and IGFBP-1 at t=0 in the present study, may be due to a high level of GH resistance mimicking in part the GH insufficiency states described by Baxter.
There also appears to be a negative correlation between IGFBP-1 and IGFBP-3 at t=24 ($r=-0.405$, $p=0.0239$).

The most surprising, consistent correlations were found in the case of aldosterone and the GH-IGF-1 axis. Although there was no correlation with GH, plasma aldosterone levels correlated positively with IGFBP-1 at t=0 ($r=0.324$, $p=0.0124$) and at t=24 ($r=0.479$, $p=0.0013$) and negatively correlated at t=24 with both IGF-1 ($r=-0.453$. $p=0.0026$) and IGFBP-3 ($r=-0.719$, $P=<0.0001$). There have been very few studies in this area to date, and logically these findings are difficult to explain.
One plausible explanation may be the altered regulation of the angiotensin-aldosterone pathway. It has been shown that multiple signal transduction systems regulate angiotensin II type 1 (AT1) receptor mRNA expression in bovine adrenocortical cells, including IGF-1 which has been shown to increase the levels of AT1 receptor mRNA (255). This mechanism if operationally important in this clinical scenario, would lead to a positive and not negative correlation between IGF-1 and aldosterone.

Further studies are therefore required to elucidate the mechanism underlying these newly described associations between plasma aldosterone and IGF-1, IGFBP-1 and IGFBP-3 and their clinical implications in critically ill children.

Chapter 8 - Adrenocortical status and function in critically ill children. A comparative study of plasma cortisol and aldosterone.

8.1 Introduction

The hypothalamo-pituitary-adrenal (HPA) axis in coordination with the immune system play a central role in the response to the stress of critical illness as a result of infection, trauma or surgery. Bacterial infection not only stimulates the immune system to produce pro and anti-inflammatory factors to combat the disease but also directly and indirectly can affect the neuroendocrine axis. Since different categories of critically ill children, had been recruited to the present study (namely meningococcal sepsis patients, respiratory infection patients, surgical patients and other catgories of critically ill children), the present study provided an excellent opportunity to explore the adrenocortical status and role in the paediatric intensive care scenario and to investigate the impact of meningococcal sepsis as compared to other disorders on the adrenocortical response.

As described in a previous chapters (chapter 5 and 7), analysis of plasma aldosterone levels in meningococcal sepsis produced some rather surprising findings. Therefore this chapter is fully devoted to the detailed analysis and discussions on plasma levels of cortisol and aldosterone in the critically ill children with particular emphasis on the meningococcal sepsis category. The introduction gives an overview of the structure and function of the adrenal gland, the biosynthesis, metabolism, secretion and effects of cortisol and aldosterone as well as a brief overview of the main diagnostic category of meningococcal sepsis.

8.1.1 An overview of the adrenal gland

The mature human adrenal gland weighs about 5-7g, with the cortex comprising about 90% and the medulla about 10% of the total mass. The innermost medulla is fairly homogeneous, however, the cortex is most distinctive even when viewed under low power magnification, since this reveals three concentric zones: the outermost zone or zona glomerulosa, the thick middle zone or zona fasciculata and the thin inner zone or zona reticularis. Based on the embryonic origin and type of hormones produced, the cortex and medulla are best thought of as separate endocrine glands.
Each adrenal gland is encased in a connective tissue capsule that extends septae into the substance of the gland. The organ is richly vascularised and capsular blood vessels, nerves and lymphatics penetrate along the connective tissue septae. Numerous small arteries from several sources form a plexus in the outer layer of the cortex, subsequently penetrating the gland branching into a capillary bed within the cortex itself and finally coalescing into veins at the cortico-medullary junction. Medullary arteries penetrate the cortex without branching, then form a capillary bed within the medulla. However, blood from both the medullary and cortical veins empties through a single central large vein that anastamoses with either the renal vein or vena cava.

8.1.2 Developmental maturation of the adrenal gland in the human

Human fetuses have a large adrenal cortex, devoted largely to the production of androgens, which in the early stages is not subdivided into zones. However, fetal cortex gradually involutes and undergoes developmental maturation throughout the fetal and early childhood period with the two outermost zones, the glomerulosa and fasciculata differentiating

by the age of 3 years. Eventually, by mid-childhood, the adrenal cortex develops into the mature configuration with the zona glomerulosa comprising 15% of the cortex, the zona fasciculata 75% and the innermost zona reticularis 10% of the gland.

8.1.3 Hormones of the adrenal cortex

Some 50 steroids have been isolated and crystallized from adrenal tissue to date. Most of these are intermediates with only a small number being secreted in significant amounts. The adrenal cortex makes 3 general classes of steroid hormones. There is some overlap in biological activity, since naturally occurring glucocortocoids tend to have a degree of mineralocorticoid activity. Cortisol is the predominant glucocorticoid in humans, its main site of production being the zona fasciculata. The zona reticularis mainly produces androgens although there is a degree of overlap with the zona fasciculata producing some androgens and cells within the reticularis producing some cortisol. The exclusive site of aldosterone production is the zona glomerulosa, due to the localization of the enzyme aldosterone synthetase. It is the most potent hormone of the mineralocorticoid class. Fig 8.1 depicts the different sites of adrenal steroid production.

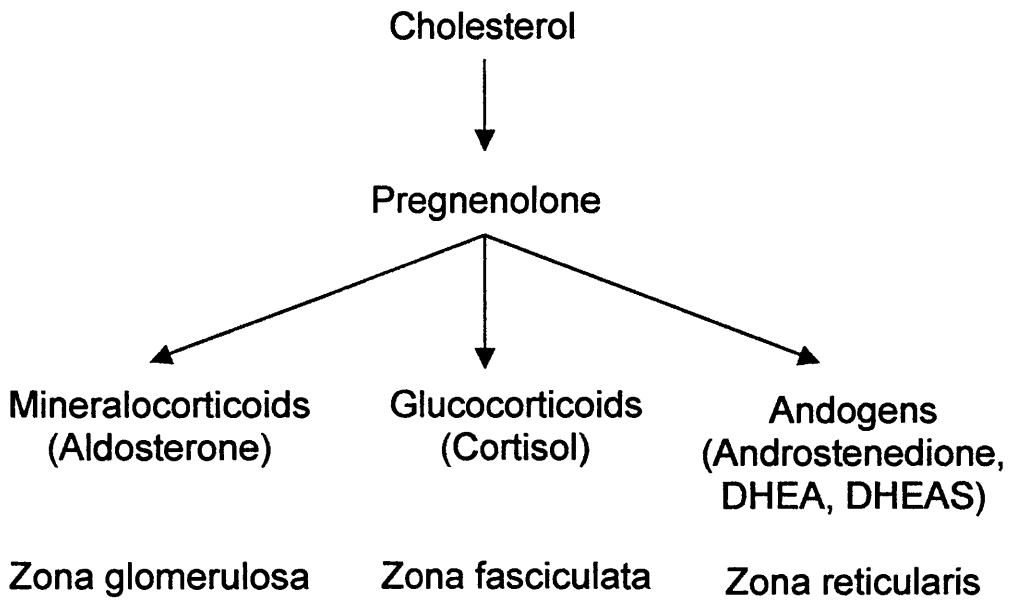
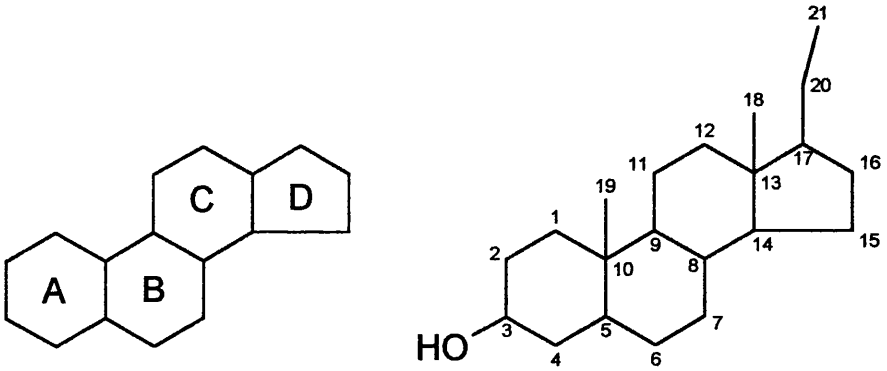


Fig 8.1 Hormones of the adrenal cortex and their site of production.

The adrenal gland utilizes cholesterol as a starting material in steroid hormone biosynthesis, which it is able to produce de novo, from acetate within the cell, from cholesterol ester stores in intracellular lipid droplets, however, it also derives most cholesterol from plasma low density lipoproteins (cholesterol of dietary origin). The biosynthesis of steroid hormones involves a battery of oxidative enzymes located in the mitochondria and the endoplasmic reticulum. The rate–limiting step in this process is the transport of free cholesterol from the cytoplasm into the mitochondria.

All adrenal steroids have in common a 17 carbon cyclopentanoperhydrophenanthrene nucleus with four rings labeled A-D (Fig 8.2). Steroid hormones as well as their precursors and metabolites differ in number and type of substituted groups, number and location of double bonds and the stereochemical configuration. Stimulation of the adrenal gland by

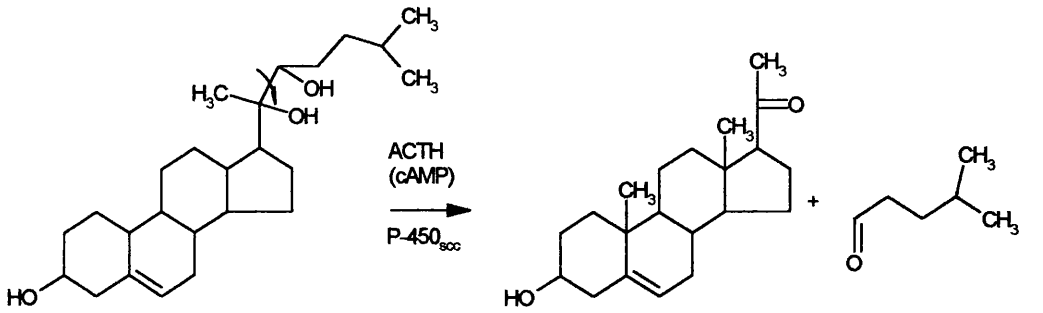
ACTH is known to activate the cAMP dependant esterase that initiates the formation of free cholesterol, its transport into the mitochondria where CYP11A1 converts cholesterol to pregnenalone. The biosynthetic pathways for both cortisol and aldosterone synthesis are shown below (Fig 8. 5 and Fig 8. 6).



Cyclopentanoperhydro-phenanthrene nucleus

Numbering of the carbon atoms. Carbons 3, 5, 8, 9, 10, 13, 14 and 17 are assymetric.

Fig 8.2 Structural features of steroid molecules.



Cholesterol

Pregnenolone and isocaproaldehyde

Figure 8.3 Cholesterol side-chain cleavage.

8.1.4 Cortisol

8.1.4.1 Cortisol synthesis

Cortisol synthesis depends on sequential hydroxylation of C_{17}, C_{21}, C_{11} by three hydroxylases. The first two reactions are rapid, whearas the C_{11}-hydroxylation is relatively slow. If the C_{21} is hydroxylated prior to C_{17}, the action of 17α-hydroxylase is impeded and the precursor is diverted towards the mineralocorticoid pathway depending on the cell type. 17α-hydroxylase is an endoplasmic reticulum enzyme that acts either upon progesterone or more commonly pregnenalone. 17α-hydroxyprogesterone is hydroxylated at C_{21} to form 11-deoxycortisol, which undergoes further hydroxylation at C_{11} to form cortisol, the most potent glucocorticoid in humans. The 21-hydroxylase is an endoplasmic reticulum enzyme, whereas 11β-hydroxylase is a mitochondrial enzyme. Steroidogenesis thus requires repeated shuttling of substrates into and out of the mitochondria of the zona fasciculata and zona reticularis cells (Fig. 8.4).

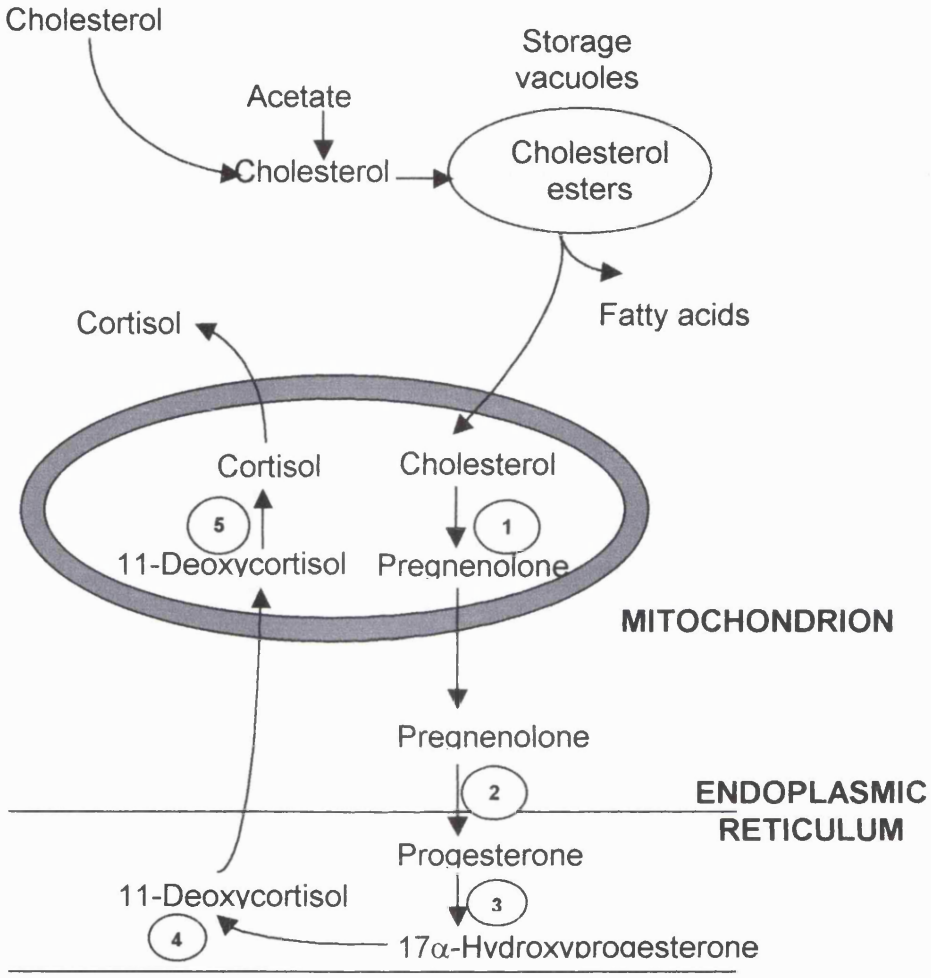


Figure 8.4 Subcellular compartmentalisation of glucocorticoid biosynthesis. Adrenal steroidogenesis involves the shuttling of precursors between mitochondria and the endoplasmic reticulum. The enzymes involved are (1) C20-22 lyase, (2) 3β-hydroxysteroid dehydrogenase and Δ5-Δ4 isomerase, (3) 17α-hydroxylase, (4) 21-hydroxylase, and (5) 11β-hydroxylase.

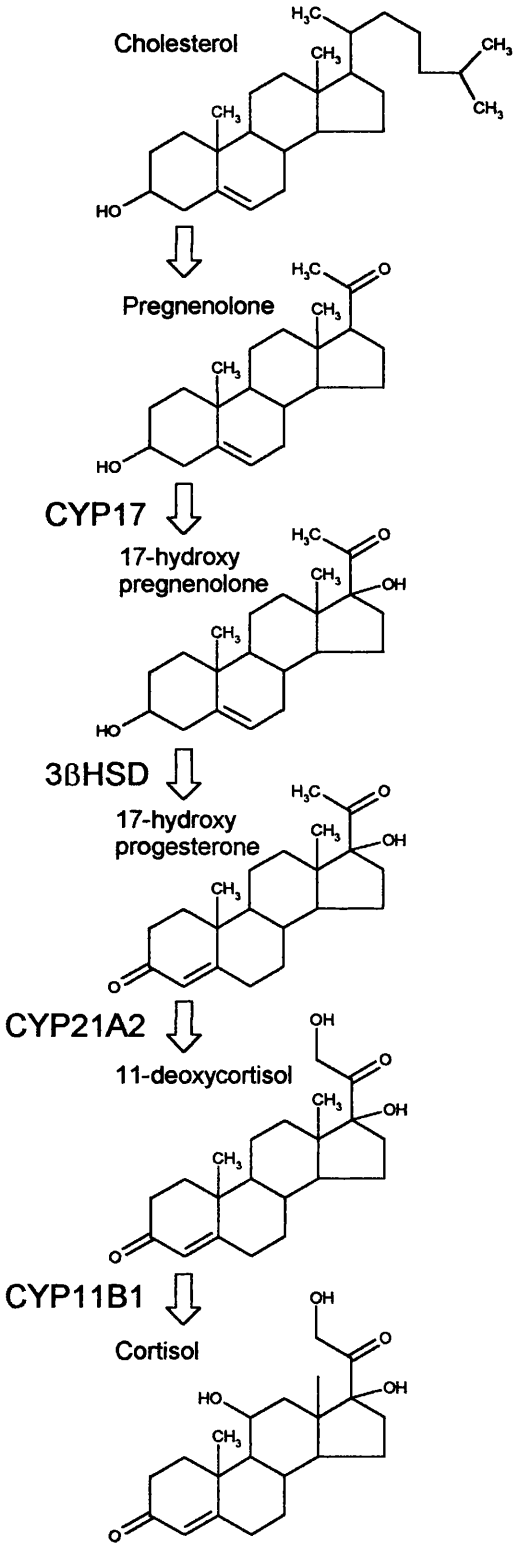


Figure 8.5 Biosynthesis of cortisol.

8.1.4.2 Regulation of cortisol synthesis, secretion and plasma transport

The secretion of cortisol is regulated by ACTH, which in turn is controlled by corticotrophin-releasing hormone (CRH). The three are linked by a classical negative feedback loop. Excessive plasma levels of free cortisol exert immediate and delayed negative feedback control (inhibition) on the anterior pituitary and hypothalamus. The immediate response is triggered by the action of cortisol on the hypothalamus, the delayed response is dependant on absolute cortisol levels and influences pro-opiomelanocortin (POMC) and its influence on mRNA production in the basophilic cells of the anterior pituitary.

Low levels of free cortisol enhance CRH release from the hypothalamus. CRH, a 41 amino acid polypeptide, reaches the basophilic cells of the anterior pituitary by the portal system and stimulates the production and release of ACTH from its precursor POMC molecule. In the adrenal cortex, ACTH activates the mitochondrial cytochrome P-450 side-chain cleavage enzyme and enhances the conversion of cholesterol to pregnenalone, which is the rate limiting step in steroidogenesis. The restoration of a normal level of free cortisol in turn results in a reduced release of CRH, decreased ACTH and consequently diminished cortisol production, thereby completing the feedback loop.

ACTH and cortisol secretion are controlled by neural input from a number of sites within the nervous system. The endogenous synthesis or circadian cycle is normally set to provide an increase in plasma cortisol to reach a peak shortly after waking, and a nadir in the late evening. This general profile is maintained by a series of episodic, pulsatile bursts of cortisol release, which are preceeded by pulsatile ACTH release. Cortisol release is also affected by physical and emotional stress. The neuronal input from the nuclei of the amygdala mediate the ACTH response to emotional stress, apprehension, fear and anxiety, whereas the fibres from the spinothalamic pathway and reticular formation mediate the ACTH response to pain. These

responses can override both the negative feedback system and the circadian rhythm.

Cortisol circulates in the plasma in free and protein-bound forms. The main plasma binding protein is an α-binding globulin called corticosteroid binding globulin (CBG) or transcortin. CBG is produced in the liver and its synthesis, like that of thyroid binding globulin is regulated by oestrogens. CBG binds to cortisol when it is within the normal range. Deoxycorticosterone and progesterone interact also with CBG with sufficient affinity to compete for cortisol binding. The binding avidity determines the biological half-lives of the of the various glucocorticoids. Cortisol binds tightly to CBG and has a half-life of 1.5-2 hours. The unbound fraction constitutes 8% of the total plasma cortisol and represents the biologically active fraction.

8.1.4.2 Metabolism and excretion of cortisol

Cortisol and its metaolites constitute about 80% of the 17-hydroxycorticoid in plasma. About half the cortisol circulates in the form of reduced dihydro and tetrahydrometabolites. Substantial amounts of the compounds are also modified in the liver by conjugation with glucoronide or to a lesser extent with sulphate residues. These modifications render the lipophyllic steroid molecules into water-soluble and excretable entities. In humans most of the conjugated steroids that enter the intestine by biliary excretion are reabsorbed by the enterohepatic circulation, about 70% are excreted in the urine, 20% leave in faeces and the rest exit through the skin.

8.1.3 Aldosterone

8.1.3.1 Aldosterone synthesis

Pregnenalone, that is imported into the mitochondria is converted into progesterone by the action of two endoplasmic reticulum (ER) enzymes, 3β-hydroxysteroid dehydrogenase (3β-OHSD) and Δ^5 -Δ^4 isomerase. Progesterone is hydroxylated at the C_{21} position to form 11-deoxycorticosterone (DOC) which is an active mineralocorticoid. The next hydroxylation at C_{11} produces corticosterone, which has glucocorticoid activity and is a weak mineralocorticoid. It has less than 5% potency of aldosterone. In the zona glomerulosa, which does not have the ER enzyme, 17α-hydroxylase, a mitochondrial 18-hydroxylase is present. The 18-hydroxylase converts the corticosterone to to 18-hydroxycorticosterone, which is then changed to aldosterone by the conversion of the 18-alcohol to an aldehyde. Aldosterone biosynthesis is shown in Fig 8.6. The final product, aldosterone, exists in two forms in equilibrium as shown in Fig. 8.7.

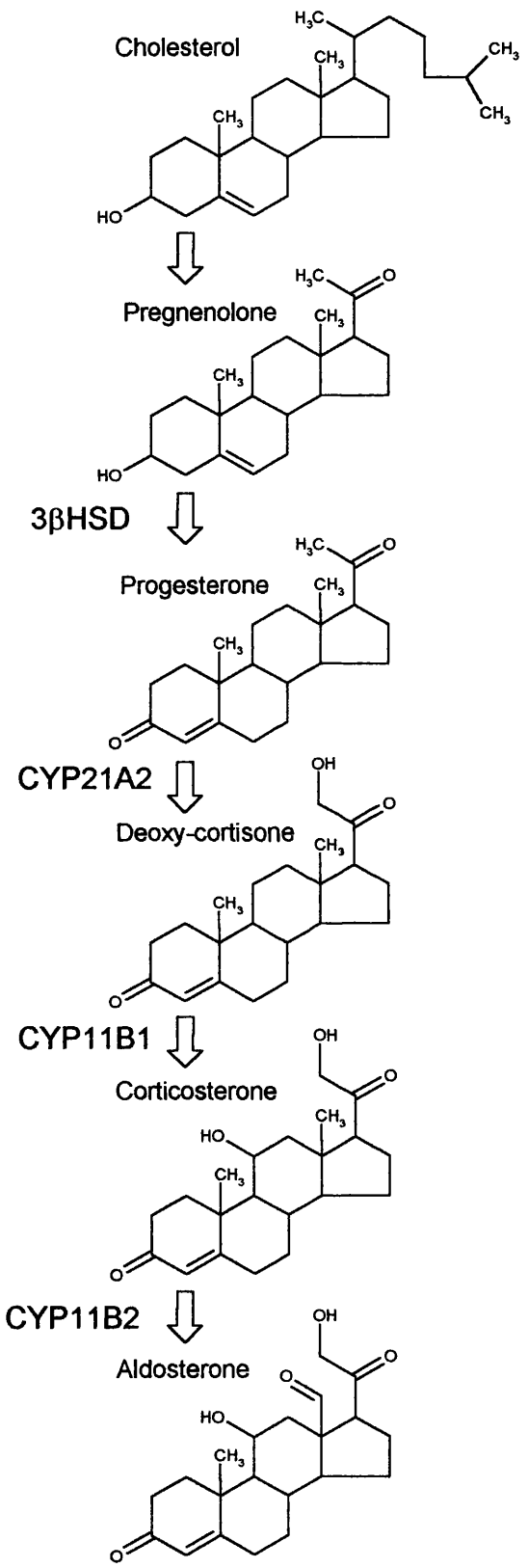
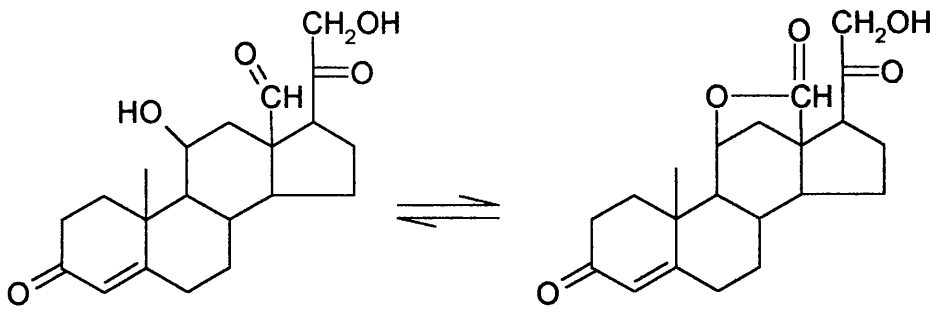


Fig 8.6 Aldosterone biosynthesis.



Aldehyde form Hemiacetal form

Figure 8.7 Equilibrium forms of aldosterone.

8.1.3.1.1 Regulation of aldosterone synthesis, secretion, plasma transport and excretion

Aldosterone, as stated is synthesized exclusively by the zona glomerulosa and its secretion is regulated by multiple factors. The renin-angiotensin system and potassium ions are the major regulators, whereas ACTH and other POMC peptides, sodium ions, vasopressin, dopamine, atrial natiuretic peptide (ANP), β- adrenergic agents, 5-HT and somatostatin are minor modulators.

The renin-angiotensin system is involved in the regulation of blood pressure and electrolyte metabolism. The primary hormone in this process is angiotensin II, an octapeptide made from angiotensinogen (Fig 8.8).

Angiotensinogen, an α_2- globulin is made in the liver and is a substrate for renin, an enzyme produced in the juxtaglomerular cells of the renal afferent arteriole. The secretion of renin is controlled by renal arterial blood pressure, sodium concentrations of tubular fluid sensed by the macula densa and renal sympathetic nervous activity. Factors that decrease renal blood flow, such as haemorrhage, hypovolaemia, dehydration, salt restriction, upright posture and renal artery narrowing increase renin levels. In contrast factors which increase blood pressure, such as high salt intake, peripheral

vasoconstrictors and supine posture decrease renin release. Hypokalaemia decreases and hyperkalaemia increases renin release.

Renin acts upon angiotensinogen to produce angiotensin I. Angiotensin converting enzyme (ACE) then removes two carboxyterminal amino acids to form angiotensin II. A small proportion of angiotensin II is then further cleaved by aminopeptidases to angiotensin III.

The effect of angiotensin II and III on the zona glomerulosa is initiated by binding to the G protein coupled receptors. The first mechanism of the intracellular signal transduction is activation of phospholipase C, which hydrolyses P1P2 to IP3, which then releases intracellular calcium ions. Angiotensin II stimulation leads to increased transfer of cholesterol to the inner mitochondrial membrane with increased conversion to pregnenalone and corticosterone to aldosterone.

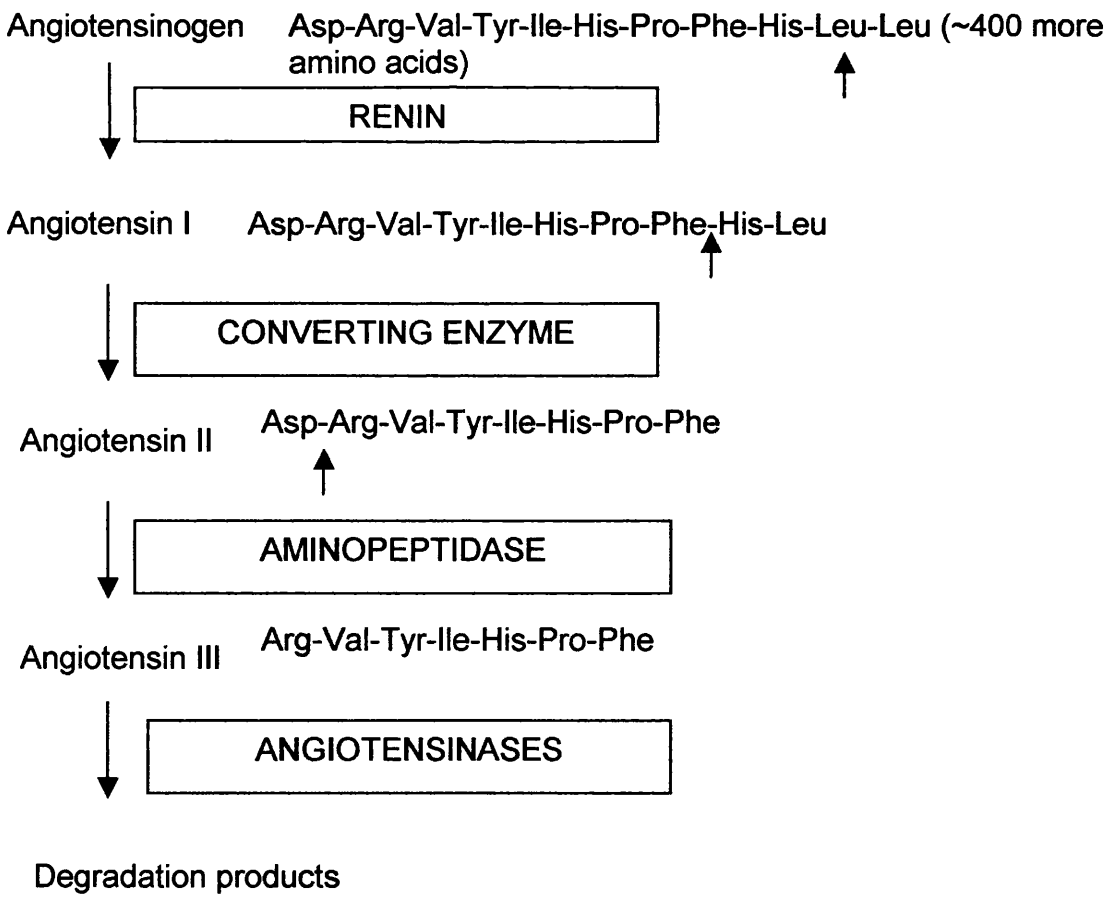


Figure 8.8 Formation and metabolism of angiotensins. Small arrows indicate cleavage sites.

8.1.3.2.1 Potassium

Potassium directly increases aldosterone secretion by the adrenal cortex, the aldosterone then acts to lower plasma potassium by stimulating its excretion by the kidney. High dietary potassium intake increases plasma aldosterone level and enhances the aldosterone response to subsequent potassium or angiotensin II infusion. The effects of potassium are independent of sodium or plasma level of angiotensin II. Potassium depolarizes the plasma membrane, which activates voltage–dependant calcium channels that permit the influx of extracellular calcium. The increased cytosolic calcium then then stimulates the same pathway of aldosterone biosynthesis as does angiotensin II.

8.1.3.2.2 Sodium

Sodium intake influences aldosterone secretion indirectly through renin and directly through effects on zona glomerulosa responsiveness to angiotensin II. High sodium increases vascular volume, which suppresses renin secretion and angiotensin II production and decreases the sensitivity of the aldosterone response to angiotensin II.

8.1.3.2.3 Pituitary factors

ACTH and possibly other POMC- derived peptides including α-MSH, βLPH and β-END influence aldosterone secretion, but their role is relatively minor. ACTH acts by binding to the glomerulosa cell surface melanocortin-2 receptor, activating adenylate cyclase and increasing intracellular cAMP. ACTH stimulates the same two early and late steps of aldosterone biosynthesis.

8.1.3.2.4 Inhibitory agents

Dopamine inhibits aldosterone secretion in humans possibly by binding to D2 receptors on glomerulosa cells. The effect is independent of the effects of ACTH, prolactin, electrolytes and the renin-angiotensin system. Atrial natiuretic peptide (ANP) directly inhibits aldosterone secretion and blocks the stimulatory effects of angiotensin II, potassium and ACTH, at least in part by interfereing with extracellular calcium influx.

8.1.3.3 Plasma transport and excretion of aldosterone

Aldosterone does not have a specific plasma transport protein, but it forms a very weak association with albumin. However, corticosterone and 11-deoxycorticosterone, other steroids with mineralocorticoid activity bind to corticosteroid binding globulin (CBG).
Aldosterone is very rapidly cleared from plasma by the liver, because it lacks a plasma carrier protein. The liver forms tetrahydroaldosterone-3-glucoronide which is excerted in the urine. There is no published data on aldosterone clearance rates in critical illness, let alone on differential clearance in sepsis and other forms of critical illness.

8.1.3.4 Effects of aldosterone

Aldosterone is crucial for sodium conservation in the kidney, salivary glands, sweat glands and colon. Aldosterone promotes active sodium transport and excretion of potassium in its major target tissues. It exerts its effects via the mineralocorticoid receptor (MR) and the resultant activation of specific amiloride sensitive sodium channels (ENaC) and the Na-K ATPase pump. It increases the number of active sodium channels and augments the action and number of the Na-K ATPase pump units in its target tissues, possibly by

regulation of genes coding for the subunits of the amiloride sensensitive sodium channels and Na-K ATPase pump, as well as of other proteins.

8.1.3.5 The mineralocorticoid receptor (MR)

MR is a member of the nuclear receptor superfamily (as are the glucocorticoid, progesterone and androgen receptors). The MR is found in the cytoplasm and nucleus and the sodium channels expressed in the apical membrane of the epithelial cells of the distal convoluted tubule as well as in the cells of other tissues involved with the conservation of salt.

The human MR (hMR) and the human glucocorticoid receptor (hGR) have almost identical DNA binding domains (94% in the amino acid homology) and very similar ligand binding domain (57%), but divergent in the terminal A/B domain. Expression of the two different hMR variants is under the control of the two different promoters. Both hMRα and hMRβ mRNA are expressed at approximately the same level in the mineralocorticoid target tissues.

MRs in their unliganded state are located in the cytoplasm as part of the hetero-oligomeric complexes containing heat shock proteins 90, 70 and 50. Upon binding with their ligand, the receptor-ligand complex dissociates from heat shock proteins, homo or heterodimerizes and translocates into the nucleus. Homo or heterodimers of the MR interact with hormone responsive elements (HRE) and/ or other transcription factors in the promoter regions of target genes, including the subunits of EnaC or other proteins related to this channel and sodium transport in general, and modulates the transcription rates of these genes.

Since plasma cortisol levels are several orders of magnitude higher than those of aldosterone, and since cortisol has high affinity for the MR, it would be expected that cortisol would overwhelm this receptor. A local enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) however converts the active cortisol to inactive cortisone and protects the MRs from the effect of cortisol. This enzyme forms what is known as part of the steroid shuttle.

8.1.4 Meningococcal infection

The Neisseria are gram-negative diplococci, which primarily colonize human mucosal surfaces. Besides several commensal species, the genus includes two human pathogens, Neisseria gonorrhoeae and meningitides, the latter of which is a major cause of bacterial meningitis and sepsis. These pathogens are highly adapted to survive within a single host population, humans. These microorganisms colonize mucosal surfaces reflecting the route of transmission, namely the nasopharynx after the airbourne spread of meningococci. Colonization in the majority of cases remains asymptomatic, however invasive disease may develop after breaching the epithelial or endothelial barrier, purulent inflammation and haematogenous spread resulting in severe and often life-threatening clinical conditions. Meningococci may disseminate from locally infected tissues into the blood stream, penetrate the blood-brain barrier to cause severe inflammation within the central nervous system (256;257).

Various bacterial components have been implicated in the pathogenesis of neisserial infection (256;258). Pili and opa proteins contribute to the attatchment and the invasion of different host cells and the outer membrane porin protein 1 was found to prevent the microbial activities of phagocytes (256) and to induce apoptosis in target cells (259). The pili of N. meningitides interact with CD46, a human cell surface protein involved in the regulation of complement activation. Human CD46 facilitates pilus-dependant interactions at the epithelial mucosa (260). Opa (opacity associated) proteins are variable outer membrane proteins of Neisseria that mediate tight interactions between these pathogens with human cells. The meningococci usually encode three or four opa proteins. The functional consequences of these interactions with regard to bacterial attatchment, invasion and responses elicited in particular host cells are reviewed recently (261).

Circumstantial evidence related to the unique association of Immunoglobulin A_1 (IgA_1) protease with human pathogenic bacterial sepsis suggests that this enzyme plays significant multiple roles in the pathogenesis and pathophysiology (262). IgA_1 proteases are produced by a variety of gram positive and gram negative human pathogens (263). The neisserial IgA_1 protease, a serine protease is a sequence specific endopeptidase which cleaves single peptide bond of distinct proline rich consensus sequences that are found in the hinge region of human IgA_1 (263;264). As intact IgA_1 protects against microorganisms affecting mucosal tissues by prevention of microbial adhereance, toxin neutralization and activation of Fc-α-receptor dependent phagocytosis the cleavage of IgA_1 has been postulated to impair protease – mediated effector function. However, most individuals have neutralizing antibodies against IgA_1 protease that may interfere with the function of the enzyme (265). Hence the role of IgA_1 protease in neisserial pathogenesis remains obscure.

Thomas Meyer and his group in 1999 (256) have reported a novel function for the neisserial IgA_1 proteases ie. the induction of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8 from peripheral blood mononuclear cells. The capacity of IgA_1 protease to elicit such cytokine responses in monocytes was enhanced in the presence of T-lymphocytes. IgA_1 protease did not induce the regulatory cytokine IL-10, which was however found in response to lipopolysaccharide and phytohemagglutinin. The above finding suggests that IgA_1 protease may play an important immuno-stimulatory function and may contribute substantially to the pathogenesis of neisserial infections by inducing large amounts of TNF-α and other pro-inflammatory cytokines, and may in particular represent a key virulence determinant of neisserial infection (266).

8.1.5 Meningococcal disease in childhood

Meningococcal disease is the major infectious cause of death in childhood in the U.K. with an overall mortality of 10%, rising to 50% in those who develop septic shock, purpura fulminans and multisystem failure (267).

Postmortem specimens of the adrenal glands of patients who die of meningococcaemia have been reported to show a range of abnormality from mild haemorrhage or thrombi on histological examination only, through degeneration of the adrenocortical cells to massive bilateral adrenal haemorrhages (268), a feature more commonly found in paediatric rather than adult post mortem specimens. Adrenal damage is said to be diffuse, there are no reports of zonal damage in the literature. The frequency of histological abnormalities in survivors is unknown.

The problem of functional adrenal insufficiency in septic shock has recently received much attention, where basal cortisol levels are substantially elevated, but the response to synacthen stimulation in meningococcal septicaemia is severely attenuated (159;160;269). The reported incidence of functional adrenocortical insufficiency varies depending on the test used (270), criteria used to define the condition (271-273) and the age of the population studied. Evidence for functional adrenal insufficiency in meningococcal infection has been provided by several studies, some dating back to 1978 where patients with petechiae had poor plasma cortisol rise to ACTH given im (269). A recent finding has shown 52% of children had inadequate plasma cortisol response to short synacthen provocation (159), whilst another report a lower incidence of 14% failing low dose synacthen (160).
The degree of functional adrenal insufficiency as defined by poor response to synacthen stimulation was also associated with increasing vasopressor requirement and duration of shock (159).

Defects in the mineralocorticoid axis have been described in a subset of

critically ill adults where hyperreninemic hypoaldosteronism has been associated with a high mortality rate (84). This subset showed little or no aldosterone rise to ACTH, a feature that was reversible on patient recovery.
Little information exists however on how the paediatric mineralocorticoid axis behaves in critical illness. The aim of this chapter was to define the adrenocortical hormone status at presentation to PICU, with particular attention to the meningococcal diagnostic category, who have poor perfusion, hypotension, oliguria due to loss of intravascular circulating volume and decreased cardiac function. Elevation in both mineralocorticoid and glucocorticoid secretion would be expected in these patients.

8.2 Patients and Methods

8.2.1 Patients

60 children (34M; 26F) admitted to the paediatric intensive care units (PICU) of Great Ormond Street Hospital for Children, London and St. Mary's Hospital London were recruited to the study. Patients were classified into two groups. Group A consisted of 31 children (15M; 16F) with meningococcal disease, mean age 4.4 years (range 0.5-14.4) with a predicted risk of mortality calculated from the PRISM score, mean value 32.3% range (0.5 – 99.3%). Group B consisted of 29 critically ill children without sepsis and included children post surgery (n=12)(mainly gastrointestinal and laryngological) and with respiratory (n=13) and neurological (n=4) diagnoses. Their mean age was 4.1 years, range (0.3-16.3), with a predicted risk of mortality 9.4%, range (0.2-83%). The groups were not significantly different for age.
Ethical approval for the study was granted by the ethics committee of Great Ormond Street Hospital for Children, NHS Trust and Institute of Child Health and informed written consent was obtained from the parents.
The groups were not significantly different for age.
All patients recruited to the study survived.

The severity of illness was also assessed by quantifying the inotrope requirement to maintain normotension. Details of the classification applied are given in Table 8.1.

Table 8.1. Vasopressor dose to maintain normotension.

Inotrope group	**Inotropes used** **Doses in µg/kg/min**
Group 0	No inotropes
Group 1	dopamine <10 ± dobutamine
Group 2	dopamine >10, adrenaline <0.5
Group 3	Adrenaline >0.5, noradrenaline

8.2.2 Methods

Blood samples were obtained from indwelling arterial lines on admission to PICU. Plasma was stored at -70°C until the samples were analysed. Plasma levels of cortisol, aldosterone and renin were determined as described in chapter 2 and used for the following analysis. Concurrent levels of potassium, sodium, volume of colloid received in the preceding 8 hours, level of inotropic support, age of patient and risk of mortality were collated.

8.3 Statistical analysis

All data of continuous variables were expressed as the mean +/- SEM. Students t test and ANOVA were used to compare between group differences. Correlation analysis was performed with least squares method and expressed as Paerson Product moment. Statistical significance was defined as $P < 0.05$.

For statistical analysis, the program DATADESK 6.1.1 for Windows was used for analysis of the data.

8.4 Results

8.4.1 Cortisol

Plasma levels of cortisol at presentation are shown in Fig 8.9. Cortisol levels were elevated in both groups, with the meningococcal group A, having higher levels than the non-meningococcal patients group B, (mean 799.51 ±75.9 mmol/l vs 703.4 ±78.6mmol/l, $p < 0.05$).

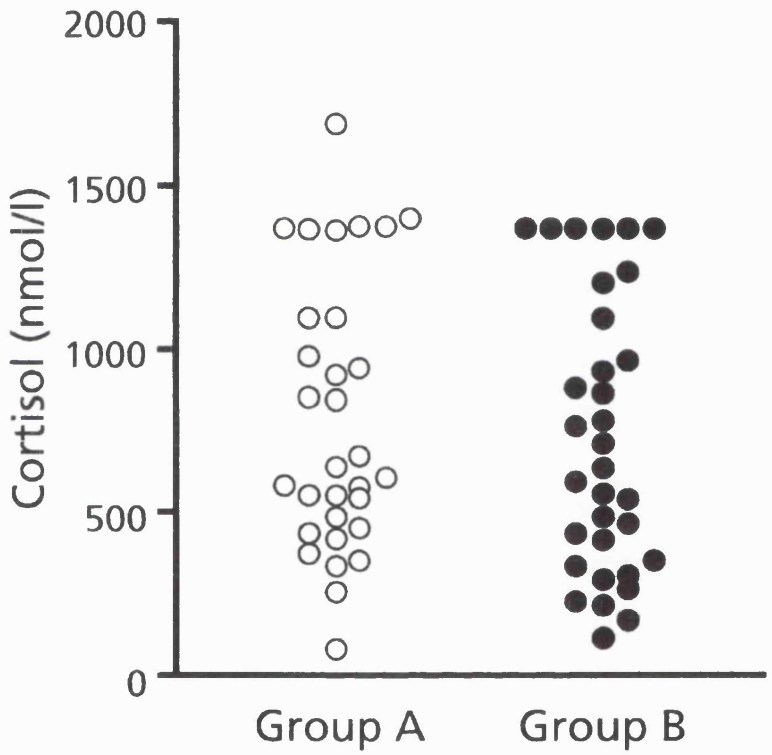


Fig 8.9 Cortisol concentrations in groups A and B at presentation.

o Group A – meningococcal patients

● Group B – patients with other diagnoses.

The plasma cortisol levels in the critically ill children over the period 0-48 hours are shown in Fig. 8.10. The cortisol levels 500nmol/l or above are considered as appropriate in healthy children on stimulation (154). A total of 235 cortisol measurements were obtained in the 48 hours on the patients

studied. 100 (42.5%) of measurements were above 500nmol/l. The maximal and minimal values of cortisol recorded were 1700nmol/l and 49nmol/l respectively. On admission the cortisol levels tended to be higher with 40 (66.6%) out of 60 being above500nmol/l. At 24 hours however the cortisol levels had fallen to 11 (25.6%) out of 43 measurements. Plasma cortisol levels at t=24 were significantly lower (p=0.0003) than on admission to PICU. Patients in whom intravenous steroids were given were excluded from the analysis from that point onwards.

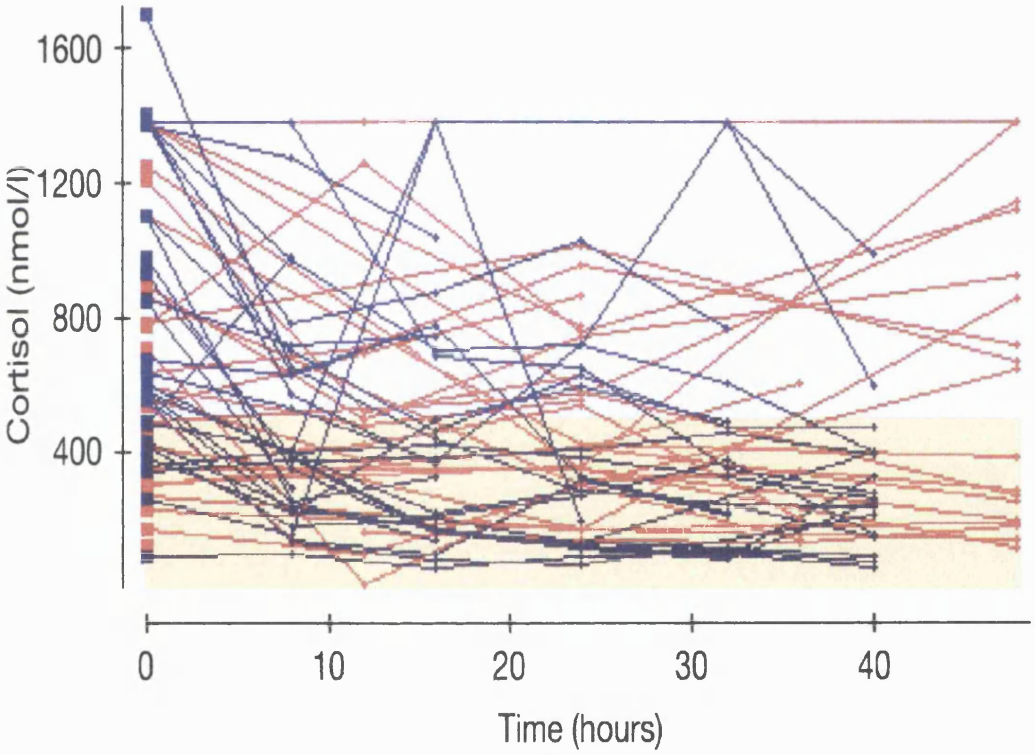


Fig. 8.10 Cortisol levels in critically ill children shown over the first 48 hours from admission to PICU. Each line represents the cortisol levels in one patient.

Critically ill children - other diagnoses (n=29)

Critically ill children - meningococcal sepsis (n=31).

Cortisol levels below 500nmol/l are considered as inappropriate in healthy children on stimulation (154).

The rate of fall of plasma cortisol over the first 24 hours from admission to PICU, independent of presenting cortisol levels (adjusted fror regression to the mean) was significantly different in the meningococcal sepsis category than in the other diagnostic groups, that is cortisol levels in patients with meningococcal disease fell faster than in other critically ill patients ($p=0.0044$).

8.4.1.1 Vasopressor requirement and plasma levels of cortisol and aldosterone.

High vasopressor requirement (to maintain normotension) on admission to PICU correlated with lower serum cortisol concentrations $p<0.05$ (Fig 8.11a). Patients with the highest plasma aldosterone concentrations on admission also had the lowest serum cortisol concentrations (Fig 8.11b). This was not related to the age distribution of the patients.

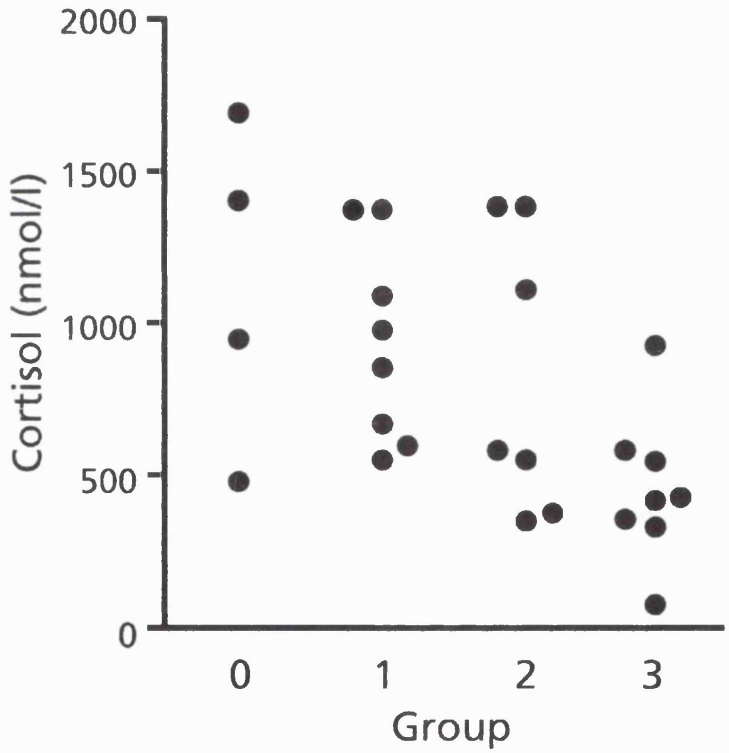


Fig. 8.11a Vasopressor requirement to maintain normotension and cortisol concentrations on admission to PICU in critically ill children with meningococcal sepsis. High vasopressor requirement correlated with low serum cortisol concentrations at presentation $p < 0.05$.

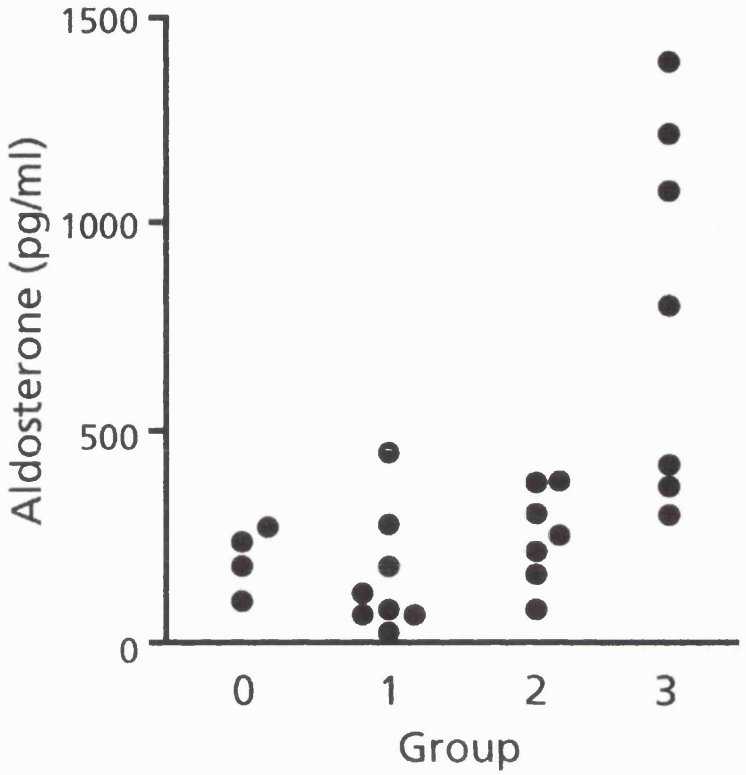


Fig. 8.11b Vasopressor requirement to maintain normotension and plasma aldosterone concentrations on admission to PICU in critically ill children with meningococcal sepsis. Higher aldosterone levels found only in patients requiring most inotropes ANOVA $p<0.05$.

8.4.2 Aldosterone

Plasma aldosterone concentrations at presentation were significantly lower in Group A (meningococcal category) compared to Group B (other critically ill children). Mean plasma aldosterone concentration in the meningococcal septicaemia category was 427.5 ±88.1pg/ml while that of other diagnoses was 1489.2 ±244pg/ml, ($p<0.0001$) (Fig. 8.12).

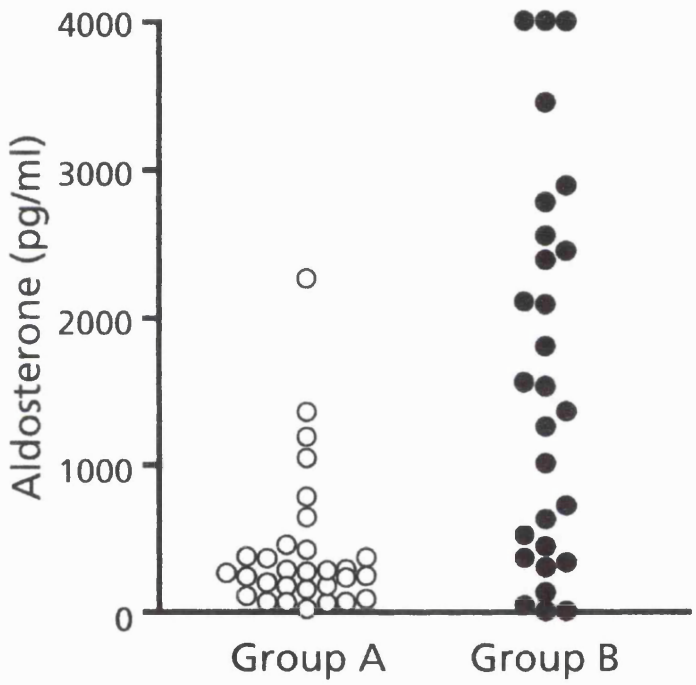


Fig 8.12 Plasma Aldosterone concentrations (pg/ml) at presentation.
Group A o meningococcal patients,
Group B • patients with other diagnoses.

Fig.8.13 shows that in 96.7% of meningococcal patients, plasma aldosterone concentrations fell within or below the range for healthy recumbent children (274). In contrast, patients in group B had significantly higher concentrations, with nearly 60% of values above the normal range. Fig 8.14 details the plasma aldosterone concentrations in the meningococcal cases plotted

against age. The presenting aldosterone level proved to be the highest within the time frame measured (the first 40 hours from admission to PICU) for the patients with meningococcal disease. Plasma aldosterone levels measured after admission to PICU were lower and fell with time (Fig 8.15a and b). In the non-meningococcal group this was not always the case.

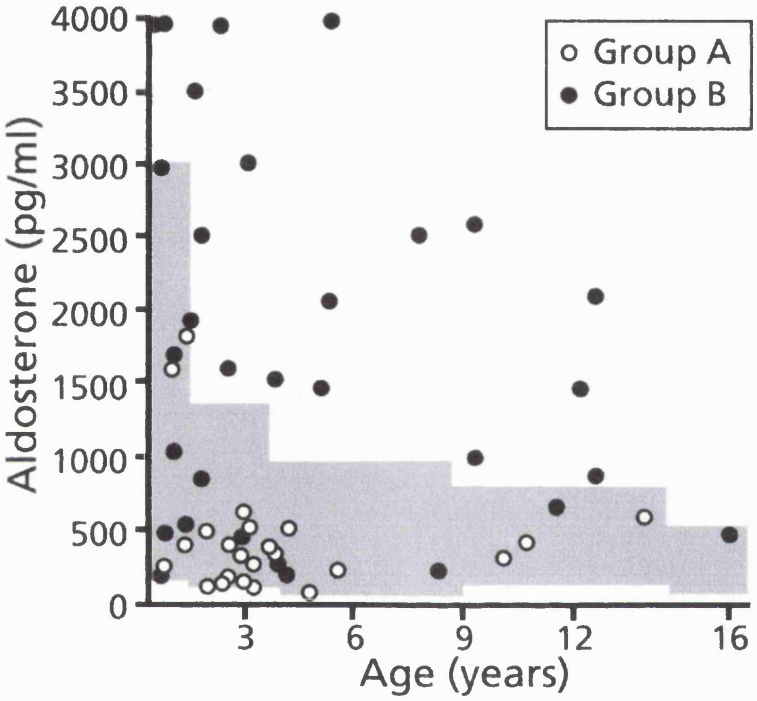


Fig 8.13. Plasma aldosterone concentrations at presentation to PICU compared to normal range for healthy recumbent children (shown in shaded zone).

o Group A meningococcal patients.

• Group B other diagnoses.

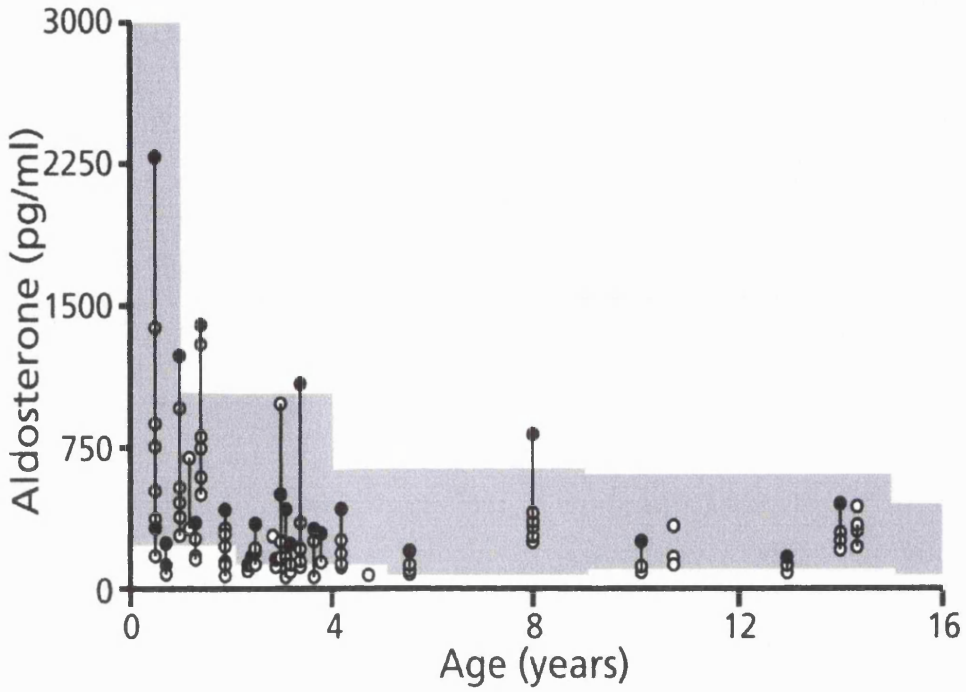


Fig 8.14 Plasma aldosterone concentrations in meningococcal patients plotted against age over the first 40 hours from admission to PICU. Shaded area shows normal range for healthy recumbent children.

- aldosterone level on admission to PICU

o shows plasma aldosterone values taken at 8 hourly intervals thereafter showing falling aldosterone concentrations. Serial samples on the same patient are connected by a line.

In Fig 8.15a the aldosterone levels obtained in the critically ill children are depicted against time (0-48 hours). The second graph (Fig 8.15b) shows the same data, but plotted against age, since aldosterone values are age

dependent. These are displayed against a shaded area, the cut-off corresponds to the reference range for the ages displayed (274). A total of 223 aldosterone values were obtained in the first 48 hours on the patients studied. The maximal value was 4000 pg/ml and the minimum was 10 pg/ml. On admission only 4 (7.14%) out of the 56 aldosterone values were below the reference range. 19 values (33.9%) were above the reference range of which only 2 (3.3%) were patients with meningococcal disease. Overall the aldosterone levels tended to fall, so that at 24 hours 6 (13.6%) out of the 44 values were below the reference range of which 5 (11.3%) were patients with meningococcal disease. 9 (20,4%) aldosterone values were above the reference range at 24 hours. All these patients were not within the meningococcal diagnostic category. The change in the plasma aldosterone level in the non-meningococcal group did not achieve signficance ($p= 0.1106$) between the two time points ($t=0$ and $t=24$). However in Group A, the meningococcal patients, the plasma aldosterone level fell significantly over the first 24 hours from admission ($p=0.0016$).

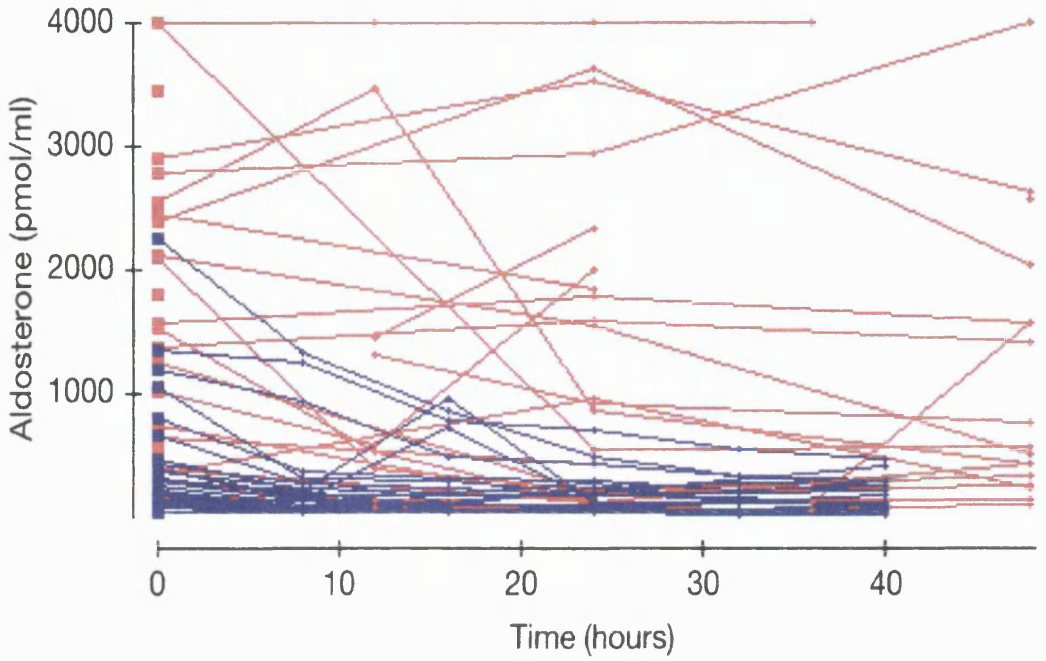


Fig 8.15a. Plasma aldosterone levels in critically ill children shown over the first 48 hours from admission to PICU plotted against time. Each line represents the aldosterone level in one patient.

Critically ill children - other diagnoses (n=29)

Critically ill children - meningococcal sepsis (n=31).

There was also a considerable difference between the mean values of aldosterone obtained in the meningococcal disease category at both time points and the other patients. The aldosterone values in the meningococcal disease group were significantly lower than the non-meningococcal group (t=0; p=0.0024, t=24; p=0.0004).

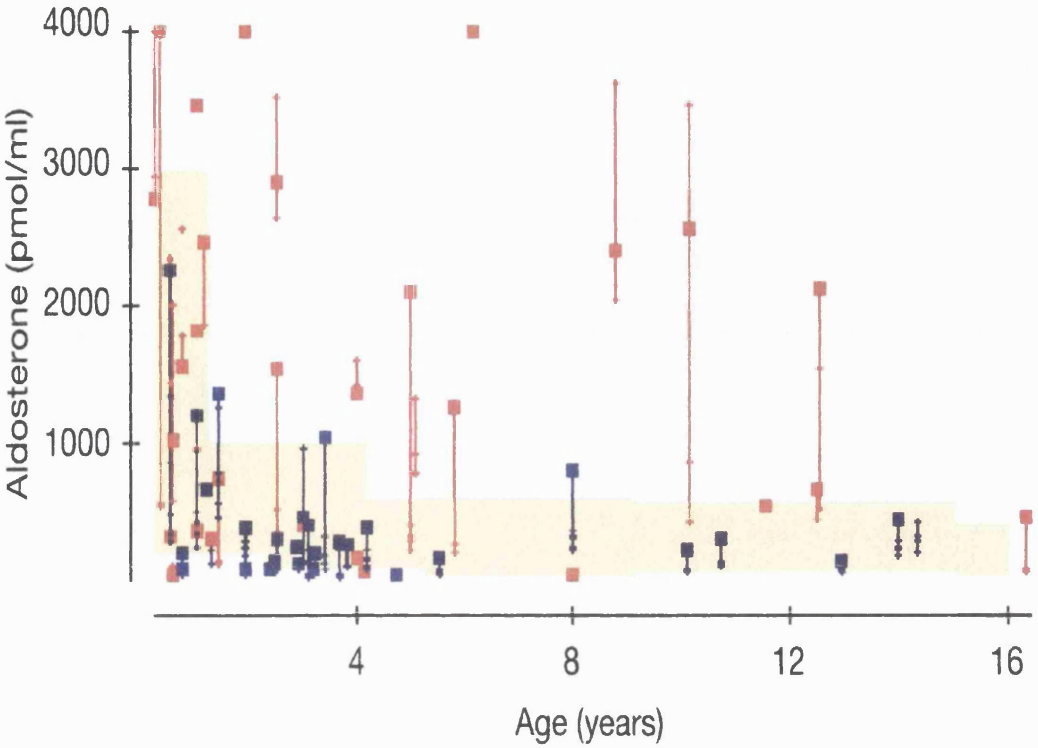


Fig 8.15b Plasma aldosterone levels in critically ill children shown over the first 48 hours from admission to PICU plotted against age. Each line represents the aldosterone level in one patient.

■ ■ Depicts aldosterone level on admission to PICU.

→ Critically ill children - other diagnoses (n=29)

→ Critically ill children - meningococcal sepsis (n=31).

▭ Shaded area depicts the normal range for healthy recumbent children.

There was no difference however in the rate of change of aldosterone (adjusted for regression to the mean) dependent on patient diagnosis (meningococcal group p=0.7968) or age (p=0.7147).

There was no linear correlation between plasma aldosterone values and severity as defined by PROM and the patients age.

8.4.2.1 Relationship between plasma aldosterone levels and other variables

There was no correlation between plasma aldosterone concentration and plasma concentrations of sodium, potassium, severity (as defined by PROM-predicted risk of mortality score) and volume of colloid infused over the previous 8 hours (reflecting intravascular volume deficit), in children suffering from meningococcal septicaemia (Group A), as shown in table 8.2.

Table 8.2 Correlations between plasma aldosterone level on admission to PICU in meningococcal sepsis patients and factors known to influence aldosterone synthesis and release.

	Log aldosterone pg/ml	
	r	**p value**
Sodium mmol/l	0.317	0.1220
Potassium mmol/l	-0.033	0.8706
Severity PROM	0.049	0.7989
Log volume of colloid infused mls/kg	-0.231	0.2887
Log PRA	0.304	0.2703

Plasma renin activity was measured in 15 patients with meningococcal disease. Values were either normal or high at presentation and in contrast to aldosterone, did not necessarily fall with time. As shown in Fig.8.16, renin levels were highest in younger patients. At presentation, there was no correlation between plasma aldosterone concentration and plasma renin activity.

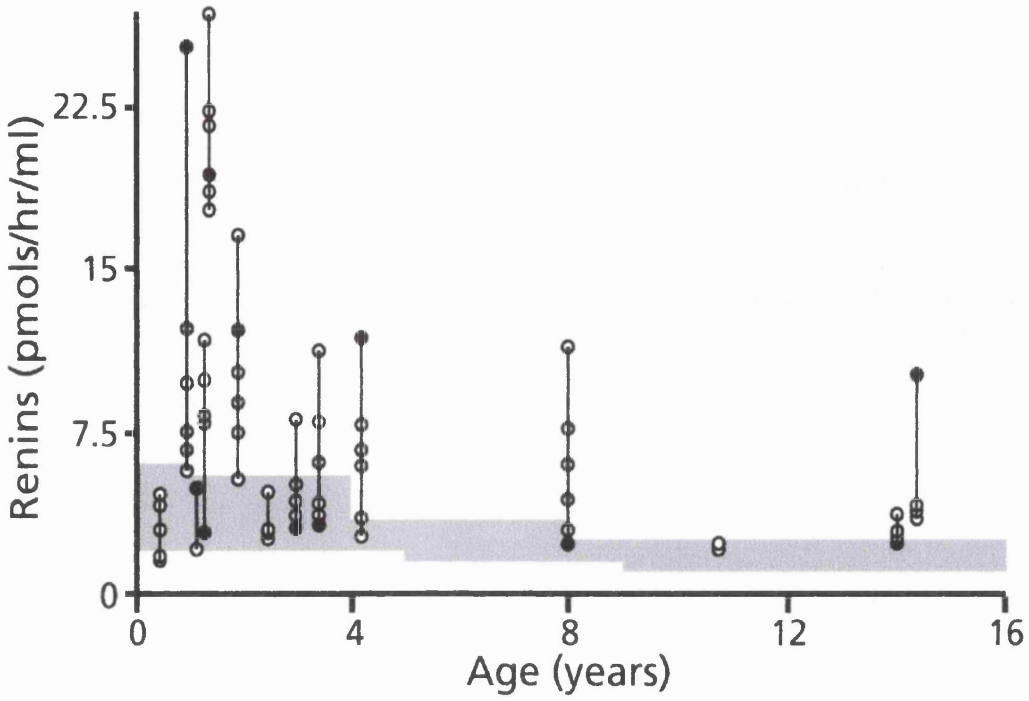


Fig 8.16 Renin values in 15 meningococcal patients – plotted against age.

- renin level on admission to PICU

o shows plasma renin values taken at 8 hourly intervals thereafter. The shaded area depicts the normal range for healthy recumbent children. Serial samples on the same patient are connected by a line.

The aldosterone (ALDO) / plasma renin activity ratio (PRA) may be used in support of the dissociation between these substances, as illustrated by Findling (275). On conversion of the present data to the same units as used by Findling et al, 80% (12 out of 15) of the ratios on admission to PICU and 93.5% (72 measurements out of a total of 77) of all the ALDO/PRA ratios were <2 in the present study. An ALDO/PRA ratio below 2 was considered inappropriately low by Findling et al (275). The ALDO/PRA ratios tended to fall over from the time of admission to PICU. This is illusrated in Fig 8.17.

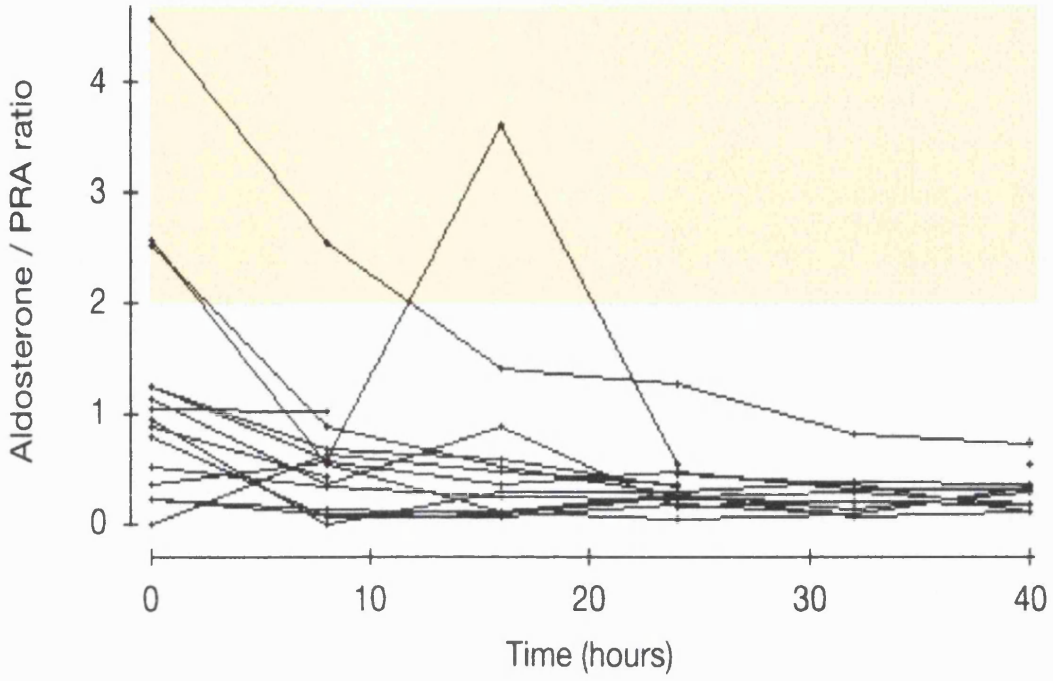


Fig 8.17 Aldosterone / Plasma renin activity ratio in 15 patients with meningococcal sepsis plotted against time (0-40 hours). The below the shaded area depict an ALDO/PRA ratio below 2 which was considered inappropriate by Findling et al (275). Serial samples on the same patient are connected by a line.

8.5 Discussion

8.5.1 Cortisol

Major acute stress, such as that experienced during critical illness is known to cause profound activation of the HPA axis (276). Absence of an appropriate stress-induced rise in cortisol results in rapid decompensation (73). An appropriate level of plasma cortisol in response to a stressor was taken as 500nmol/l based on Gonzalbez,-J;et al, 2000 findings in order to establish the reference values for responses to standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in normal subjects. Maximal plasma cortisol levels obtained in the 5th percentile in each test were 500nmol/l or above (154). It is therefore, rather surprising that, using the above mentioned definition, only 64.5% of cortisol levels on admission to PICU and only 24.5% at 24 hours were above the 500nmol/l level.

The duration of elevation of cortisol is considered to be dependant on the severity of the insult (277). Initially this is known to be driven through high ACTH levels (278), but within 48 hours the ACTH levels are known to fall, whilst cortisol remains elevated (278), presumably through a peripheral drive. It is therefore surprising that in the present study, children requiring ventilation, inotropic support and continuing intensive care exhibited relatively low and falling plasma cortisol levels within 24 hours of admission. In fact cortisol levels at t=24 were significantly lower ($p=0.0003$) than on admission to PICU.

Adrenal insufficiency is a common and underdiagnosed disorder that develops in critically ill patients (279). Most forms are acquired and resolve with treatment of the underlying disease. Hence the possibility of some degree of functional adrenocortical insufficiency cannot be ruled out in the children investigated in the present study.

Evidence for functional adrenal insufficiency in meningococcal infection

dates back to 1978 where patients with petechiae showed a poor plasma cortisol rise to intramuscular injection of ACTH (122). The reported incidence of functional adrenocortical insufficiency in critically ill children is variable, but a more recent finding has shown that 52% of children had inadequate plasma cortisol response to short synacthen provocation (159), whilst others report a lower incidence of 14% failing low dose synacthen (160).

The hypotension that is refractory to fluids and requires vasopressors is the most common presentation of adrenal insufficiency in the ICU. It is important to make the diagnosis of adrenal insufficiency, because current data in adults suggest that treatment with glucocorticoids improves outcome (161;279;280). Identification of paediatric adrenal insufficiency patients therefore becomes important. The issue whether this feature is more commonly found in the meningococcal diagnostic category could not be resolved by the data available. PICU presentation cortisol levels in the meningococcal diagnostic category were 799 ± 75 nmol/l and in the other diagnostic groups were 710 ± 75 nmol/l. The rate of fall of plasma cortisol over the first 24 hours from admission to PICU, independent of presenting cortisol levels was however significantly different in the meningococcal sepsis category, the plasma cortisol falling faster, than in the other diagnostic groups. Whether the observed differences are specific to meningococcal sepsis requires further investigation.

The data obtained in the present study does confirm that the normal circadian pattern of cortisol is disturbed in critical illness. The cortisol rise has been correlated with the extent of infarction in adult patients post myocardial infarction (281;282), the extent of surgery in infants (14) and children and the severity of bronchioloitis in infants (114). Within the intensive care scenario cortisol levels were found to correlate to severity as defined by the modified APACHE II score (the Modified Acute Physiological and Chronic Health Evaluation II score) and were highest in those patients requiring most inotropic support for maintenance of blood pressure (283). Other workers have found variable activation of the HPA axis, not relating to severity as assessed by ventilator dependency and inotrope requirement

(284). In the present study there was no correlation between plasma cortisol levels and severity as defined by the PROM score either on admission or at t=24. Plasma cortisol levels were elevated, as expected, in all critically ill children. However, on more detailed examination in children with meningococcal disease, high vasopressor requirement to maintain normotension, and therefore high illness severity on admission to PICU was found to correlate with lower cortisol levels $p<0.05$ (see Fig 8.11a). This may suggest a degree of clinical adrenocortical insufficiency in the critically ill children investigated in the present study. This feature is of importance since modulation of plasma cortisol levels in critical illness appears to influence patient outcome.

Recent trials in critically ill septic adults, where modest doses of hydrocortisone were administered in the setting of pressor-dependent septic shock resulted in a significant improvement in haemodynamics (161).

8.5.2 Aldosterone

The data presented here demonstrate that critically ill children with meningococcal disease present with lower plasma aldosterone levels, a feature not previously reported. The physiological mechanisms underlying the present findings are as yet unclear. The clinical significance may be of relevance to patient management and outcome.

Aldosterone synthesis and secretion is controlled by multifactorial processes, however the renin- angiotensin system (285;286) is of prime importance. In health, both renin activity and plasma aldosterone levels correlate (287). The data presented in this study indicate, that such a correlation is lost in critically ill children with meningococcal disease. Several factors are known to influence aldosterone release or its response to angiotensin II. These include the extent of intravascular depletion or hypoperfusion. The volume of colloid infused per kg in the previous 8 hours in order to maintain normotension reflects the extent of hypoperfusion (see Fig 5.3).

In order to delineate the present observation a number of parameters known to influence both renin and aldosterone production and release were examined. Hyperkalaemia is also known to enhance the aldosterone response to angiotensin II (288), as is hyponatraemia (289). Finally, starvation is known to induce a dissociation between renin activity and aldosterone secretion (290). In patients in the present study, the plasma levels of potassium, sodium and the volume of colloid infused in the first 8 hours did not correlate with plasma aldosterone levels on admission to PICU. The children were also not previously malnourished. Therefore, alternative mechanisms may exist to explain these findings.

Low plasma aldosterone levels dissociated from elevated plasma renin

activity in the context of critical illness have been previously observed and documented in adults. The concept of "hyperreninemic hypoaldosteronism" (HH), was first introduced by Zipser et al in 1981 (84), who recognised the poor prognostic significance of the HH patients when compared to critically ill non-HH adults. However, neither the subgroup of patients who developed HH were further characterised nor the correlation to the pathophysiological process or to the diagnosis of the patients was established.

In the present study, some critically ill children presented with lower plasma aldosterone levels. This is a feature demonstrated specifically by the meningococcal disease category. To quantify the degree of dissociation between appropriately elevated plasma renin activity and lower plasma aldosterone levels Findling et al introduced the comparative ratio of aldosterone / plasma renin activity (275). Although the data from Findling et al and the present study cannot be directly compared due to different nature of the methodologies, the conversion of the units for both variables (in the present study) in order to conduct a comparison, 80% of the ratios on admission to PICU were low and tended to fall over the next 40 hours (see Fig 8.17). This may suggest the basis of HH in the meningococcal patients in the present study. However, plasma renin activity for the non-meningococcal patients was not measured in the present study and therefore a comparison cannot be made.

Whether this phenomenon relates to diagnosis or the basic pathophysiological processes remains to be elucidated. There are also differences in the clinical management of the meningococcal patients that should be addressed. It is important to note that in the patients with meningococcal disease large volumes of fluid for resuscitation were required even prior to admission to PICU. It was not however possible to obtain preresuscitation samples to assess plasma aldosterone prior to volume infusion, since these patients were transferred to a central PICU from surrounding hospitals. Correction of deficit in intravascular circulating fluid volume would tend to lower both plasma aldosterone and renin. Renin activity was either elevated or normal in the meningococcal patients studied

in group A in the face of low plasma aldosterone and this dissociation persisted for the first 48 hours after admission, although the need for fluid and colloid resuscitation diminished off rapidly from the time of admission to PICU (see Fig.5.3). The high fluid volume resuscitation requirement in these patients would therefore cannot explain the present observations.

In order to explain the low plasma aldosterone levels in critically ill children with meningococcal disease, several potential underlying mechanisms are discussed below.

Zipser et al, reported that in the adult critically ill HH patients, there was little response of the mineralocorticoid axis to ACTH stimulation, thus implying a defect or block in the pathway (84). Until recently there has been no data on the response of the mineralocorticoid axis to ACTH stimulation in critically ill children. Some preliminary observations have been reported, where 51% of critically ill children with meningococcal disease were found to show a suboptimal aldosterone response (<40% increment) to ACTH stimulation (291). It must be emphasized that ACTH is not a major regulator of aldosterone synthesis or secretion.

The unexpectedly low plasma aldosterone levels in critically ill children with meningococcal disease may be the result of generalized endothelial damage owing to the invasive nature of N.meningitidis. This could affect the the endothelial membrane bound angiotensin converting enzyme II (ACE II), thus limiting the production of angiotensin II which in turn could lead to the lack of stimulation for aldosterone secretion. The lack of angiotensin II may also contribute to the aetiology of hypotension in meningococcaemia, thus having clinical management implications. However in the adults angiotensin converting enzyme II levels were found to be similar in both hyperreninemic hypoaldosteronic (HH) patients and unaffected non-HH critically ill individuals (84).

Lack of stimulation for aldosterone secretion may be owing to an increased proteolysis of the angiotensin I and II peptides by non-specific proteases,

which may contribute to the aetiology of hypotension. The non-specific 'protease' phenomenon is known to occur in sepsis, decreasing IGFBP-3 levels by the IGFBP-3 protease. However, in critically ill adults with HH the levels of angiotensin II when compared to non HH critically ill patients appear to be similar and angiotensin infusions did not increase aldosterone levels in adult HH patients (84).

Both cortisol and aldosterone have high binding affinity for the mineralocorticoid receptor (MR), cortisol being converted into the inactive metabolite cortisone by the enzyme 11 β-hydroxysteroid dehydrogenase type II, thus allowing aldosterone to act (292). Cortisol, when in high concentration has the potential to saturate the steroid shuttle or the enzyme 11-β dehydrogenase type II at the mineralocorticoid receptor. In this instance cortisol would then bind to the MR, obviating the need for aldosterone (293), and hence suppression of aldosterone would occur. In the present study, however, high plasma cortisol levels in group B (non-meningococcal patients) were not associated with low aldosterone levels. Cortisol concentrations found in the non-meningococcal group at the outset of critical illness were not therefore sufficient to saturate the steroid shuttle and assume mineralocorticoid function. The clinical state of patients in whom the steroid shuttle becomes saturated is one of hypertension and hypokalaemic alkalosis (294), which was not the clinical picture of the group A patients with meningococcal disease, who tend to exhibit low plasma potassium levels, even in the scenario of a metabolic acidosis. In patients with 11-β hydroxysteroid dehydrogenase saturation, where cortisol is acting as a mineralocorticoid, renin should be suppressed. Since elevated levels of renin occur in conjunction with the low aldosterone in both a subset of critically ill adult patients and in the children in Group A with meningococcal disease at presentation to PICU, the saturation of the steroid shuttle would probably not explain this phenomenon. Further analysis of urinary steroid metabolites would aid with this hypothesis.

Anatomical damage of the zona glomerulosa has been suggested as the mechanism underlying HH. However in cases of hypotension the area at

maximal risk of hypoxic damage is the corticomedullary junction and not the glomerulosa. Post mortem studies on adult HH patients do not reveal macro- or microscopic evidence of glomerulosa damage (275). However, this possibility should be explored further in children who have died from meningococcal sepsis.

An enzymatic block in the synthesis or secretion of aldosterone is another possible mechanism for the observed HH in meningococcal sepsis. Several studies have been conducted to elucidate this finding in adults. The aldosterone secretion rate has been evaluated in critically ill adults in response to ACTH stimulation with and without the dopamine receptor antagonist metaclopramide (295). Aldosterone rise in HH patients on synthetic ACTH stimulation was suboptimal and normalised if these patients were pre-treated with metaclopramide. Dopamine therefore provides tonic inhibition to the glomerulosa (296). The data from the present study show that children with meningococcal disease and maximum inotropes including dopamine (Fig.8.9) have higher aldosterone values than groups requiring less or no dopamine ($p<0.05$). There may however be a difference in response to exogenously administered dopamine and that, which is endogenously released by the adrenal medulla and neurons innervating the adrenal cortex may therefore play a part in the control of glomerulosa function.

The levels of atrial natriuretic peptide (ANP), known to directly inhibit aldosterone release, were also found to be no different in HH and non-HH critically ill adults (295) and are therefore probably not implicated in the pathophysiology. There are no reports to date on ANP levels in critically ill children and, again, this area requires further exploration.

Stern at al, (297) investigated basal and ACTH-stimulated secretion of corticosterone, in the zona fasciculata and 18- hydroxycorticosterone produced predominantly in the zona glomerulosa. The HH critically ill adults showed normal zona fasciculata response to stimulation but suboptimal zona glomerulosa response. These observations led them to suggest that

there may exist a generalised impairment of glomerulosa function in HH patients. Further work on the measurement of 18-hydroxycorticosterone/ aldosterone and corticosterone/ aldosterone ratio may yield information specifically on the activity of aldosterone synthase (298) and establish whether the activity of this enzyme is transiently influenced in patients affected by HH in the course of critical illness.

The adrenal cortex is not only under ACTH and neuronal control, but the immune system is also known to exert a degree of paracrine modulation, especially in chronic disease states (4). This is mediated by cytokines secreted either from macrophages resident in the adrenal cortex or by the adrenocortical cells themselves. Although there is to date no evidence that aldosterone release in man is controlled by cytokines, work on rat adrenal cells in vitro showed, that TNF was capable of blocking aldosterone release (299). The recognition that cytokines may be important in acute severe stress, such as meningococcaemia, where high cytokine levels exist, should be further investigated. It is important however to recognise that local intra-adrenal levels of cytokines, which may modulate aldosterone production or release, may not necessarily correspond to the circulating cytokine levels that may be easily measured. Further work is needed in this area to substantiate or disprove this theory.

One of the emerging and most important issues is the local tissue based or intra-adrenal, endothelial and neuronal cells control mechanism in steroidogenesis and aldosterone production. This pathway is apparently independent of central HPA axis. The interactions between local aldosterone production at the specific tissue sites versus TNF-α and IL-6 production in the case of meningococcal infection may be of central and primary cause of lowering the overall plasma levels of aldosterone. However to find direct evidence to support this hypothesis in critically ill children with meningococcal disease would neither be feasible nor ethical.

A number of in vitro and in vivo studies on the human and rat provide convincing evidence that TNF-α and IL-6 inhibit both basal and renin-angiotensin stimulated aldosterone synthesis (299;300). In brief, these studies point to the reduced expression of CYP11β2 mRNA in general irrespective of the effects of angiotensin II and ACTH. TNF-α reduced the angiotensin II and potassium induced aldosterone synthesis and the CYP11β2 mRNA levels. ACTH did not influence the expression of CYP11β2 mRNA levels (300).

The hypothesis that may explain inhibition of alsosterone synthesis and secretion in meningococcal sepsis may well be related to the immunomodulation of the zona glomerulosa by N. meningitides IgA_1 protease via increased production of TNF-α and reduced synthesis of aldosterone.

It is noteworthy however, that in the critically ill meningococcal disease children, the lowest plasma cortisols in the highest vasopressor requirement group were not mirrored by lowest aldosterone values, although generally plasma aldosterone levels were low. These are small samples and inference is therefore difficult, but it does suggest a discordance between the different zones of the adrenal cortex in the most severely ill meningococcal patients.

The clinical implications of functional adrenocortical insufficiency in the critically ill patients was addressed by Annane et al, 2000 where administration of stress doses of hydrocortisone and daily fludrocortisone in target populations of poor ACTH stimulation test responders resulted in a 30% relative reduction in mortality (280), again in critically ill adults. However, there was no data available in this abstract on plasma aldosterone levels. In the light of this data, and the results presented in this paper, it is necessary first to address the role mineralocorticoids play in the critically ill septic patient and whether clinical benefit relates solely to glucocorticoid supplementation, or a combination of both glucocorticoid and mineralocorticoid therapy.

8.6 Conclusions

Children with meningococcal disease may present with unexpectedly low aldosterone levels and probably inadequate cortisol levels.
Renin levels were high or normal, being appropriate for the clinical scenario of the patients, and showing little relation to the low aldosterone levels.

The dissociation between plasma renin and plasma aldosterone has been previously described in adults although the mechanism underlying this finding is not known.
It remains to be determined whether low plasma aldosterone may result from pathway disturbances solely in meningococcal sepsis, or is the end product of wider pathophysiological process.
Since HH in critically ill adults is associated with a high mortality rate (72) it is necessary in future to establish the prevalence of low plasma aldosterone in critically ill septic children, and relating this to patient outcome parameters including mortality. This feature of low aldosterone may be therefore of use as a prognostic indicator in PICU.
Further work needs to be done to explore the mechanism of this finding, damage to the aldosterone producing axis is postulated, which may in turn relate to the aetiology of hypotension in meningococcal sepsis.
The clinical and therapeutic implications of these findings require further research.

Chapter 9 – Discussion and conclusion.

9.1 Retrospective criticism on the plan and design of the present study

The present study, describing the hormonal and metabolic changes in critically ill children was one arm of a larger project aimed at examining glucose and protein turnover in critically ill children that had been set up and had undergone ethical approval under its current format. The retrospective analysis of the structure of the study identified several pitfalls as a result of the protocol set up.

The protocol did not include the collection of fluoride oxalate samples for the laboratory assessment of plasma glucose levels obtained at the time of other hormonal sampling. The description of the metabolic changes in critically ill children is therefore incomplete and the issue of the degree of hyperglycaemia or insulin resistance in relation to patient outcome could not be addressed.

Collection of samples for plasma renin activity and for catecholamine levels was not included in the protocol and added subsequently, post analysis of preliminary data on the last 15 meningococcal patients recruited to the study. There are consequently no plasma renin activity data in non-meningococcal sepsis patients and it was not possible to assess the whether any dissociatiation between plasma renin activity and plasma aldosterone had occurred in the non-meningococcal group, although plasma aldosterone levels were not depressed. Since the cohort of patients was small, no reliable conclusions can be derived regarding the influence of catecholamines on lipolysis and plasma NEFA levels.

PICU inpatient records were checked for blood pressure recordings, intravascular circulating volume readings (CVP), cardiac function indices and plasma sodium and potassium measurements. Unfortunately only some of

these coincided with hormonal sampling times. The patients were however normotensive at the time of blood sampling and the inotrope requirement at the time was noted.

Samples were collected for cytokine levels (TNF-α, IL-1, IL-2, IL-6, IL-8 and IL-10) in the Great Ormond Street cohort of patients and batched for analysis by ELISA. The second batch of samples were sent to the immunology laboratory for analysis, but due to laboratory error this assay failed. Cytokine levels were therefore only available on a small number of patients from Great Ormond Street PICU only. A relatively small number of samples combined with problems in the reliability of analysis, this information was considered insufficient to draw any conclusion regarding the cytokine load with the hormonal and metabolic milieu of these patients.

Blood sampling at 8-12 hourly intervals precluded analysis on the altered pulsatility of GH secretion that is expected during the course of critical illness. However, the blood volume that may be sampled at regular intervals from these critically ill children was limited and did not allow for 20 minute sampling.

Analysis of the longitudinal data was complicated by different sampling schedules at the two different hospitals. The protocol employed at Great Ormond Street Hospital PICU allowed for blood samples to be collected at 12 hourly intervals in children over 10kg in weight for up to 5 days, and once daily sampling in children less than 10kg in weight. The timing of the sampling was different in the meningococcal group recruited at St. Marys Hospital where samples were collected at 8 hourly intervals for the first 40 hours from admission to PICU. This resulted in only 2 time points, where the sampling in both cohorts coincided (t=0 and t=24), where cross-sectional data could be analysed. It was also not possible to use repeated measures ANOVA to analyse longitudinal time trends, as the different sample timings resulted in the computer model treating the non-sampled times as missing values and excluding all the data on the patient in question. Due to the

differing durations of sample collections in the two hospitals, only one data set had some hormone and metabolite values past 48 hours. It was therefore not possible to follow the correlations into the prolonged phase of critical illness.

There was no incidence of death among the critically ill children recruited in the present study. Based on the predicted mortality rate of 6.4% some 4 deaths could be expected in the total number of the children treated in the PICU in the present study. This discrepancy, no doubt fortunate per se, may be owing to two different reasons. Children with pre-existing immune, renal, hepatic and metabolic disease were excluded from the study at the outset and these have a higher mortality rate in PICU than previously well children with an acute decompensation requiring PICU support. The second reason relates to the early mortality in meningococcal sepsis. The meningococcal sample of patients is unrepresentative of children with meningococcal sepsis, since one third of the fatalities occur within 6 hours (256) and may have died at the referring hospital prior to stabilization and transfer to the tertiary PICU and therefore entry into the present study. In brief, the population of meningococcal sepsis patients reported in the present study does not represent the true 'meningococcal population' but naturally selected survivors. The actual information on the initial fatality incidence among the meningococcal sepsis patients from the GPs and referring hospitals was not made available. Hence under the present circumstance, the previously reported metabolic and endocrine derangements typical to survivor versus non-survivor meningococcal patients cannot be addressed in the present study.

The issue of collecting samples at the referring hospital was addressed, at the resuscitation process, however processing and freezing of the samples could not be standardized or maintained and was therefore not performed. The exact metabolic and endocrine derangement at the time of surgery could be defined, as the timing of surgery was noted in the patients. However, the evolution of the medical illnesses made the process of pinpointing of the onset of illness more difficult. In these cases the stress

response may have been more advanced in its evolution than had been recognised.

9.1.1 Pyruvate instability

It is apparent from the data presented in chapter 6 that pyruvate undergoes considerable degradation over the storage period even when the samples were stored at -70°C. In fact, the majority of samples analysed for pyruvate levels had been stored for periods of up to 24 weeks. This may explain the discrepancy in the pyruvate levels reported in this work and also the difficulty in interpretation of lactate /pyruvate ratios. Prolonged storage of biological samples prior to laboratory analysis resulting in their degradation therfore raises an important issue regarding the interpretation of the data. Although the results obtained for other metabolites and hormones appeared appropriate, their stability, for completeness should also be addressed.

9.2 Assessment of the scientific and clinical achievements of the present study

Knowledge on the stress response and the pathophysiological sequelae to critical illness in childhood is sparse compared to that in adults. Although the initial design of this investigation was rather elborate and ambitious the work reported in this thesis is discussed in this section and the extent to which the original aims of the project have been met are reviewed.

The original aims of the study were:

- to describe the magnitude and nature of the metabolic and endocrine response to critical illness in childhood,
- to correlate this with patient severity and age,
- to describe whether patterns of metabolic and endocrine response are diagnosis specific both on admission to PICU and in the first few days post onset of critical illness.

The study demonstrated that critically ill children did show evidence of metabolic and endocrine stress on admission to PICU. Levels of lactate, NEFA, ketones, cortisol, GH, insulin, IGFBP-1 and glucagon being elevated and IGF-1, IGFBP-3, TSH, total T_4 being depressed in response to the stress of critical illness. However, only some of these changes were consistent in magnitude and duration to the metabolic and endocrine responses described in previous studies conducted in subgroups of stressed children. The magnitude and duration of the ME changes were described in chapter 6. Hormonal and metabolic interrelations and influences were also explored in relation to several axes, namely the GH axis and lipid metabolism in chapter 7 and the HPA axis in chapter 8. Correlations with illness severity and differences in the metabolic and endocrine responses between different diagnostic categories were explored in chapter 5.

The NEFA levels observed in these children were only mildly elevated and comparable to the degree of elevation documented in critically ill head injured children (126) and older children undergoing major surgery (127). The elevated insulin levels appeared to exert little effect on lipolysis on admission, however, by 24 hours a negative correlation was noted. It was only by 24 hours that any correlation was detected between NEFA and ketones. The process of resuscitation may distort the initial relationships between hormones and metabolites, that only begin to appear once patients become more clinically stable.

The present study also noted a marked ketosis in the critically ill children on admission to PICU, that improved over 24 hours despite the continuing critically ill status of the subjects. This supports previous observations that the overall lipid turnover measurements do not correlate with the duration of critical illness (184).

The effect of individual hormones on different aspects of lipid metabolism (lipolysis and β–oxidation) is a complex field to address in the context of critical illness, since the patients are subject to changing levels of hormones and metabolites as well as altered responsiveness throughout the course of illness.

9.2.1 Severity of illness and metabolic and endocrine responses

One of the aims of the present study was to correlate the degree of metabolic and endocrine derangement in critically ill children with patient severity and age. MEM is known to be related to severity scoring systems such as surgical severity score (14), illness severity score (114), GCS (Glasgow Coma Score) in head injury (229) and surface area of burn in children (122) at presentation.

In the present study, however, there was little correlation between indices of metabolic and endocrine stress and PROM, a measure of illness severity used in PICU. However, the arterial blood lactate levels did appear to reflect severity to a greater extent, especially in specific diagnostic groups. In the

light of the above results it is possible to propose that the metabolic and endocrine milieu of critically ill children among the different diagnostic groups may reflect different aspects of patient severity and outcome which are as yet not addressed by PROM scoring.

Lactate levels themselves, although known to be of prognostic significance may rise in response to hypoperfusion, increased Na^+-K^+ ATPase pump activity or reduced clearance and the relative contribution of each of these underlying mechanisms may influence its specificity as an indicator of prognosis.

Future studies should address other metabolic, endocrine and immunological markers that are known to show a higher sensitivity and specificity than lactate for the severity of systemic inflammation in critically ill patients (301). These include cytokines, notably IL-6, eicosanoids, C-reactive protein and calcitonin precursors to name but a few. Generalised activation of CALC-1 gene transcription and translation throughout the body is thought to be stimulus related, rather than tissue specific and is known to correlate significantly with patient outcome (302;303). Such hormone/ cytokine activity may prove a useful tool in assessment of patient prognosis. The relevance of calcitonin precursors, IGFBP-1 levels, extent of hyperglycaemia etc. to illness severity and patient outcome in the paediatric critical illness scenario remains a goal for the future.

It may be useful to create a single measure that integrates morbidity and mortality and may include certain metabolic and endocrine parameters that may enhance the prognostication and illness severity assessment, but also may lead to new therapeutic approaches.

9.2.2 Differences in metabolic and endocrine milieu between diagnostic categories

Significant differences were found between the diagnostic categories and the MEM of critically ill children. Plasma lactate levels were higher and plasma aldosterone levels significantly lower in the meningococcal sepsis

category. Therefore the endocrine and metabolic stress response not only related to severity of illness but diagnostic category or pathophysiological process. Multiple Organ Dysfunction Scores as a descriptor of patient outcome have also been used in the intensive care scenario and a case may be made for endocrine organ failure.

The definition of organ failure is that of altered organ function in an acutely ill individual so that homeostasis cannot be maintained without intervention (304).

9.2.2.1 Adrenocortical insufficiency

There is an increasing body of evidence for the existence of functional adrenocortical insufficiency (159;160;273), and the morbidity associated with it. Intervention is necessary through increasing requirement for inotropes, and / or the replacement of physiological doses of hydrocortisone (161). In the present study the faster rate of fall of plasma cortisol levels and the lower presenting levels of plasma cortisol in the patients requiring greater degrees of inotropic support (in the meningococcal disease category) may be classed as exhibiting a degree of failure of the inner zones of the adrenal cortex. The prevelance of functional adrenocortical insufficiency needs to be established in the critically ill paediatric population, the definition used to diagnose this condition as well as the test used should be standardized. The need or otherwise for hydrocortisone supplementation in the critically ill child may then be established and its impact on patient outcome may be studied. This area requires further input in the future.

9.2.2.2 Hyperreninaemic hypoaldosteronism

A separate entity of hyperreninaemic hypoaldosteronism, initially described in critically ill adults and known to carry a much higher mortality over and above that seen in critical care (305) may also be described as effectively a manifestation of endocrine organ failure. A dissociation between lower plasma aldosterone levels and appropriately elevated renin activity was demonstrated in the present study for the first time in critically ill children.

The novelty of this observation relates not only to the fact that this entity has been described in children, but also that it appears to be limited to the children with meningococcal sepsis.
Further research is required in this field in order to determine the prevalence of HH in critically ill children and whether it has an impact on patient morbidity and mortality. Several underlying mechanisms for lower plasma aldosterone levels in the meningococcal sepsis category have been discussed in chapter 8. The lower plasma aldosterone levels may be related to increased aldosterone clearance in this patient subgroup, although there is no literature to date on differential rates of aldosterone clearance in differing disease processes or critical illness. Another avenue to explore relates to the specific inhibition of aldosterone synthase enzyme, possibly by intra-adrenal production of cytokines e.g. TNFα. This may be determined by a chromatographic system for the simultaneous measurement of plasma 18-hydroxy-11-deoxycorticosterone and 18-hydroxycorticosterone by radioimmunoassay (298). Other possible underlying mechanisms such as saturation of the steroid shuttle at the mineralocorticoid receptor can be investigated by analysis of urinary steroid metabolites.

The low plasma aldosterone may therefore be related to failure of the zona glomerulosa, a discrete endocrine organ. It is noteworthy that supplementation of critically ill adults with both hydrocortisone and fludrocortisone decreases mortality by 30% (280). If indeed HH carries a higher mortality in children, identification of such individuals and supplementation of this axis may be required to improve patient outcome.

9.2.2.3 GH axis and aldosterone

A further novel observation made in the present study was the negative correlation between plasma aldosterone and IGF-1 and IGFBP-3 and a positive correlation between IGFBP-1 in critically ill children. The underlying mechanism is unknown but IGF-1 is known to influence mRNA levels of the AT-1 receptor in bovine adrenocortical cells (255). This would however lead

to a positive and not negative correlation between IGF-1 and aldosterone. The underlying mechanism must therefore be different and will require elucidation in the future. It still remains to be established whether the present finding has clinical significance.

9.2.2.4 Control of hyperglycaemia and outcome in critically ill children

Other key areas for future research in this field of critical care include the determination of the magnitude of hyperglycaemia in the paediatric intensive care population, its manipulation or modulation and its relationship with outcome. The impact of intensive insulin therapy for rigorous blood glucose control will require RCT studies possibly involving several PICUs to achieve sufficient power to the study in order to address whether a similar benefit may be obtained from this tight glucose control regimen in the paediatric intensive care population as has been achieved in the adult cohort (142).

Further studies aiming to elucidate the mechanisms underlying the metabolic and endocrine responses to critical illness in childhood are now required to highlight therapeutic interventions which may aid management and improve outcome.

List of publications arising from the study

Proceedings of XXIInd International Congress of Paediatrics Aug1998. " The stress reponse to critical illness in childhood - what is the role of growth hormone?" E. J. Lichtarowicz-Krynska, N. Klein, A. Aynsley-Green.

Care of the Critically Ill, 1999, vol 15.4, p145. " Unexpectedly low aldosterone levels in children with meningococcal disease on admission to PICU." E. J. Lichtarowicz-Krynska, O.Bodamer, F.Feillet, C.Camacho-Hubner, J.Britto, A. Aynsley-Green.

Care of the Critically Ill, 1999, vol 15.4, p146. " Endocrine and Metabolic Parameters ar presentation in critically ill children-lack of correlation with clinical severity scores." E. J. Lichtarowicz-Krynska, O.Bodamer, F.Feillet, C.Camacho-Hubner, J.Britto, A. Aynsley-Green.

Hormone Research, 1999, vol 51, S2, p83. "The Metabolic and Endocrine Milieu of Critically Ill Children at presentation- lack of correlation with clinical severity scores." E. J. Lichtarowicz-Krynska, O.Bodamer, F.Feillet, C.Camacho-Hubner, J.Britto, A. Aynsley-Green.

Hormone Research, 1999, vol 51, S2, p83. "Circulating Aldosterone Levels are unexpectedly low in children with meningococcal disease on admission to PICU." E. J. Lichtarowicz-Krynska, O.Bodamer, F.Feillet, C.Camacho-Hubner, J.Britto, A. Aynsley-Green.

Paediatric Research; (Suppl) 47:59A:343, 2000. "Endocrine and metabolic parameters (EMP) at presentation in critically ill children- lack of correlation with clinical severity scores." E.J.Lichtarowicz-Krynska, O.Bodamer, C.Camacho-Hubner, J.Britto, M.Levin, A. Aynsley-Green.

Paediatric Research; (Suppl) 47:59A:344, 2000. "Circulating Aldosterone levels are surprisingly low in children with meningococcal disease (MD) – the first 48 hours in PICU." E.J.Lichtarowicz-Krynska, O.Bodamer, C.Camacho-Hubner, J.Britto, M.Levin, A. Aynsley-Green.

Hormone Research, 2000, vol 53, S2, p76. "Aldosterone (A) and cortisol (C) responses to low dose ACTH stimulation (LDST) in critically ill children with meningococcal disease (MD)." E.J.Lichtarowicz-Krynska, M.P.Bone, M.J.Diver, A. Aynsley-Green, P.E.Clayton.

Arch Dis Child; 85:26-28, 2001 "Congenital adrenal hyperplasia: management during critical illness" Charmandari E, Lichtarowicz-Krynska EJ, Hindmarsh PC, Johnston A, Aynsley-Green A, Brook CGD

Growth hormone and IGF Research 13(5) 282-286, 2003 "Growth hormone enhances proinflammatory cytokine production by monocytes in whole blood". Uronen-Hansson H, Allen ML, Lichtarowicz-Krynska E, Aynsley-Green A, Cole TJ, Höidén-Guthenberg I, Fryklund L,Klein N.

Submitted for publication 2003, "Circulating aldosterone levels are unexpectedly low in children with acute meningococcal disease: Aldosterone levels in PICU" Lichtarowicz-Krynska EJ, Cole TJ, Camacho-Hübner C, Britto J, Levin M, Klein N, Aynsley-Green A. (published J. Clin. Endocrinol. Metab., 89: 1410 – 1414 Mar 2004).

Reference List

1. Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur.J.Pharmacol. 2003;463(1-3):235-72.

2. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. J.Clin.Endocrinol.Metab. 1998;83(6):1827-34.

3. Moore FA. Posttraumatic complications and changes in blood lymphocyte populations after multiple trauma. Crit Care Med 1999;27(4):674-5.

4. Bornstein SR, Chrousos GP. Clinical review 104: Adrenocorticotropin (ACTH)- and non-ACTH- mediated regulation of the adrenal cortex: neural and immune inputs. J.Clin.Endocrinol.Metab. 1999;84(5):1729-36.

5. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. J.Clin.Endocrinol.Metab. 1998;83(6):1827-34.

6. Hennemann G, Krenning.E.P. Thyroid hormones. In: Jenkins RC, Ross RJM, editors. The endocrine response to acute illness. 1 ed. Basel: Karger; 1999. p. 87-109.

7. British Paediatric Association. The Care of Critically Ill Children - Report of the Multidisciplinary Working Party on Paediatric Intensive Care convened by the British Paediatric Association. 1993. London.

8. Hunter J. Treatise on Blood, Inflammation and Gunshot wounds. London: Nichol, London; 1794. p. 459-64.

9. Selye H. Stress in health and disease. London: Butterworth; 1976.

10. Jenkins RC, Ross RJM. The Endocrine Response to Acute Illness - Introduction. In: Jenkins RC, Ross RJM, editors. The Endocrine Response to Acute Illness. 1 ed. Basel: Karger; 1999. p. 1-3.

11. Thompson P, Jr., Burman KD, Lukes YG, McNeil JS, Jackson BD, Latham KR et al. Uremia decreases nuclear 3,5,3'-triiodothyronine receptors in rats. Endocrinology 1980;107(4):1081-4.

12. Scheinhorn DJ, Chao DC, Stearn-Hassenpflug M. Liberation from prolonged mechanical ventilation. Crit Care Clin. 2002;18(3):569-95.

13. Chernow B. Variables affecting outcome in critically ill patients. Chest 1999;115(5 Suppl):71S-6S.

14. Anand KJ, Aynsley Green A. Measuring the severity of surgical stress in newborn infants. J.Pediatr.Surg. 1988;23(4):297-305.

15. Ludwigs UG, Baehrendtz S, Wanecek M, Matell G. Mechanical ventilation in medical and neurological diseases: 11 years of experience. J Intern.Med 1991;229(2):117-24.

16. Van den Berghe G, de Zegher F. Anterior pituitary function during critical illness and dopamine treatment. Crit.Care Med. 1996;24(9):1580-90.

17. Frayn KN. Hormonal control of metabolism in trauma and sepsis. Clin.Endocrinol.Oxf. 1986;24(5):577-99.

18. Hume DM, Egdahl RH. The importance of the brain in the endocrine response to injury. Annals of Surgery 1959;150:697-712.

19. Engquist A, Brandt MR, Fernandes A, Kehlet H. The blocking effect of epidural analgesia on the adrenocortical and hyperglycemic responses to surgery. Acta Anaesthesiol.Scand. 1977;21(4):330-5.

20. Kelhet H, Brandt MR, Prange Hanson A, Alberti KGMM. The effect of epidural analgesia on metabolic profiles during and after surgery. British Journal Of Surgery 1979;66:543-6.

21. Bernard C. Chiens rendus diabetiques. Compterendu de la Societe de Biologie 1849;1:60-3.

22. George JM, Reier CE, Lanese RR, Rower M. Morphine anesthesia blocks cortisol and growth hormone response to surgical stress in humans. J.Clin.Endocrinol.Metab. 1974;38(5):736-41.

23. Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur.J Pharmacol. 2003;463(1-3):235-72.

24. Jessop DS. Stimulatory and inhibitory regulators of the hypothalamo-pituitary-adrenocorticalaxis. Baillieres.Best.Pract.Res.Clin.Endocrinol.Metab. 1999;13(4):491-501.

25. de Kloet ER. Steroids, stability and stress. Front Neuroendocrinol. 1995;16(4):416-25.

26. Gesing A, Bilang-Bleuel A, Droste SK, Linthorst AC, Holsboer F, Reul JM. Psychological stress increases hippocampal mineralocorticoid receptor levels: involvement of corticotropin-releasing hormone. J.Neurosci. 2001;21(13):4822-9.

27. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. N.Engl.J.Med. 1980;303(8):436-44.

28. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr.Rev. 2000;21(1):55-89.

29. Paris JM, Lorens SA, Van de Kar LD, Urban JH, Richardson-Morton KD, Bethea CL. A comparison of acute stress paradigms: hormonal responses and hypothalamic serotonin. Physiol Behav. 1987;39(1):33-43.

30. Stuart AG, Ward Platt MP. The ontogeny of the metabolic and endocrine stress response to elective surgery. In: Aynsley-Green A, Ward-Platt MP, Lloyd-Thomas AR, editors. Stress and pain in infancy and childhood. London: Bailliere Tindall; 1995. p. 529-46.

31. Wolfe RR, Durkot MJ, Allsop JR, Burke JF. Glucose metabolism in severely burned patients. Metabolism 1979;28(10):1031-9.

32. Ross R, Miell J, Freeman E, Jones J, Matthews D, Preece M et al. Critically ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of insulin-like growth factor-I. Clin.Endocrinol.Oxf. 1991;35(1):47-54.

33. Timmins AC, Cotterill AM, Hughes SC, Holly JM, Ross RJ, Blum W et al. Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding proteins, and induction of an insulin-like growth factor binding protein 3 protease. Crit.Care Med. 1996;24(9):1460-6.

34. Rothwell PM, Lawler PG. Prediction of outcome in intensive care patients using endocrine parameters. Crit Care Med. 1995;23(1):78-83.

35. Kaptein EM, Grieb DA, Spencer CA, Wheeler WS, Nicoloff JT. Thyroxine metabolism in the low thyroxine state of critical nonthyroidal illnesses. J.Clin.Endocrinol.Metab 1981;53(4):764-71.

36. Dahn MS, Lange P. Hormonal changes and their influence on metabolism and nutrition in the critically ill. Intensive Care Med. 1982;8(5):209-13.

37. Frayn KN. Substrate turnover after injury. Br.Med.Bull. 1985;41(3):232-9.

38. Arnold J, Campbell IT, Samuels TA, Devlin JC, Green CJ, Hipkin LJ et al. Increased whole body protein breakdown predominates over increased whole body protein synthesis in multiple organ failure [published erratum appears in Clin Sci (Colch) 1993 Dec;85(6): following xxv]. Clin.Sci.Colch. 1993;84(6):655-61.

39. Birkhahn RH, Long CL, Fitkin D, Jeevanandam M, Blakemore WS. Whole-body protein metabolism due to trauma in man as estimated by L-[15N]alanine. Am.J.Physiol. 1981;241(1):E64-71.

40. Birkhahn RH, Long CL, Fitkin D, Geiger JW, Blakemore WS. Effects of major skeletal trauma on whole body protein turnover in man measured by L-[1,14C]-leucine. Surgery 1980;88(2):294-300.

41. Moore FA. Posttraumatic complications and changes in blood lymphocyte populations after multiple trauma. Crit Care Med 1999;27(4):674-5.

42. Gore DC, Jahoor F, Hibbert J, DeMaria EJ. Except for alanine, muscle protein catabolism is not influenced by alterations in glucose metabolism during sepsis. Arch.Surg. 1995;130(11):1171-6.

43. Lang CH, Dobrescu C. Gram-negative infection increases noninsulin-mediated glucose disposal. Endocrinology 1991;128(2):645-53.

44. Gump FE, Long CL, Geiger JW, Kinney JM. The significance of altered gluconeogenesis in surgical catabolism. J.Trauma. 1975;15(8):704.

45. Frayn KN, Little RA, Stoner HB, Galasko CS. Metabolic control in non-septic patients with musculoskeletal injuries. Injury. 1984;16(2):73-9.

46. Black PR, Brooks DC, Bessey PQ, Wolfe RR, Wilmore DW. Mechanisms of insulin resistance following injury. Ann.Surg. 1982;196(4):420-35.

47. Heller SR, Robinson RTC. Glucose metabolism. In: Jenkins RC, Ross RJM, editors. The Endocrine Response to Acute Illness. 1 ed. Basel: Karger; 1999. p. 4-26.

48. Wolfe RR, Shaw JHF. Glucose and FFA kinetics in sepsis: Role of glucagon and sympathetic nervous system activity. Am J Physiol 1985;248:E236-E243.

49. Meszaros K, Lang CH, Bagby GJ, Spitzer JJ. Tumour necrosis factor increases in vivo glucose utilization of macrophage rich tissues. Biochem Biophys Res Commun 1987(149):1-6.

50. Siegel JH, Cerra FB, Coleman B, Giovannini I, Shetye M, Border JR et al. Physiological and metabolic correlations in human sepsis. Invited commentary. Surgery 1979;86(2):163-93.

51. Wolfe RR, Herndon DN, Jahoor F, Miyoshi H, Wolfe M. Effect of severe burn injury on substrate cycling by glucose and fatty acids. N.Engl.J.Med. 1987;317(7):403-8.

52. Jeevanandam M, Young DH, Schiller WR. Nutritional impact on the energy cost of fat fuel mobilization in polytrauma victims. J.Trauma. 1990;30(2):147-54.

53. Miyoshi H, Shulman GI, Peters EJ, Wolfe MH, Elahi D, Wolfe RR. Hormonal control of substrate cycling in humans. J.Clin.Invest. 1988;81(5):1545-55.

54. Wolfe RR. Carbohydrate metabolism in the critically ill patient. Implications for nutritional support. Crit.Care Clin. 1987;3(1):11-24.

55. Owen OE, Patel MS, Boden G. Substrate utilization of the human brain under normal and pathological conditions. Verh.Dtsch.Ges.Inn.Med 1981;87:1444-52.

56. Stoner HB, Frayn KN, Barton RN, Threlfall CJ, Little RA. The relationships between plasma substrates and hormones and the severity of injury in 277 recently injured patients. Clin.Sci. 1979;56(6):563-73.

57. Dahn MS, Lange P. Hormonal changes and their influence on metabolism and nutrition in the critically ill. Intensive Care Med 1982;8(5):209-13.

58. Baracos V, Rodemann HP, Dinarello CA, Goldberg AL. Stimulation of muscle protein degradation and prostaglandin E2 release by leukocytic pyrogen (interleukin-1). A mechanism for the increased degradation of muscle proteins during fever. N.Engl.J.Med. 1983;308(10):553-8.

59. Oliff A. The role of tumor necrosis factor (cachectin) in cachexia. Cell 1988;54(2):141-2.

60. Watters JM, Bessey PQ, Dinarello CA, Wolff SM, Wilmore DW. Both inflammatory and endocrine mediators stimulate host responses to sepsis. Arch.Surg. 1986;121(2):179-90.

61. Watters JM, Bessey PQ, Dinarello CA, Wolff SM, Wilmore DW. Both inflammatory and endocrine mediators stimulate host responses to sepsis. Arch.Surg. 1986;121(2):179-90.

62. Anand KJ. The stress response to surgical trauma: from physiological basis to therapeutic implications. Prog.Food Nutr.Sci. 1986;10(1-2):67-132.

63. Wilmore DW. Catabolic illness. Strategies for enhancing recovery. N.Engl.J.Med. 1991;325(10):695-702.

64. Goins WA, Reynolds HN, Nyanjom D, Dunham CM. Outcome following prolonged intensive care unit stay in multiple trauma patients. Crit.Care Med. 1991;19(3):339-45.

65. Hakanson E, Rutberg H, Jorfeldt L, Wiklund L. Endocrine and metabolic responses after standardized moderate surgical trauma: influence of age and sex. Clin.Physiol 1984;4(6):461-73.

66. Anker SD. Catecholamine levels and treatment in chronic heart failure. Eur.Heart J. 1998;19 Suppl F:F56-F61.

67. Clutter WE, Bier DM, Shah SD, Cryer PE. Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. J Clin.Invest 1980;66(1):94-101.

68. Douglas RG, Shaw JH. Metabolic response to sepsis and trauma. Br.J.Surg. 1989;76(2):115-22.

69. Catanese VM, Kahn CR. Secondary forms of diabetes mellitus. In: Becker KL, editor. Principles and practice of endocrinology and metabolism. 3 ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1327-36.

70. Jahoor F, Herndon DN, Wolfe RR. Role of insulin and glucagon in the response of glucose and alanine kinetics in burn-injured patients. J.Clin.Invest. 1986;78(3):807-14.

71. Bessey PQ, Watters JM, Aoki TT, Wilmore DW. Combined hormonal infusion simulates the metabolic response to injury. Ann.Surg. 1984;200(3):264-81.

72. Frayn KN, Little RA, Maycock PF, Stoner HB. The relationship of plasma catecholamines to acute metabolic and hormonal responses to injury in man. Circ.Shock 1985;16(3):229-40.

73. Newell-Price J, Trainer P. The pituitary adrenal axis. In: Jenkins RC, Ross RJM, editors. The Endocrine Response to Acute Illness. Basel: Karger; 1999. p. 110-30.

74. Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 1987;238(4826):522-4.

75. Uehara A, Gottschall PE, Dahl RR, Arimura A. Interleukin-1 stimulates ACTH release by an indirect action which requires endogenous corticotropin releasing factor. Endocrinology 1987;121(4):1580-2.

76. Rivier C, Vale W. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature 1983;305(5932):325-7.

77. Bailey RR, Abernethy MH, Beaven DW. Adrenocortical response to the stress of an acute myocardial infarction. Lancet 1967;1(7497):970-3.

78. Chernow B, Alexander HR, Smallridge RC, Thompson WR, Cook D, Beardsley D et al. Hormonal responses to graded surgical stress. Arch.Intern.Med. 1987;147(7):1273-8.

79. Jurney TH, Cockrell JL, Jr., Lindberg JS, Lamiell JM, Wade CE. Spectrum of serum cortisol response to ACTH in ICU patients. Correlation with degree of illness and mortality. Chest 1987;92(2):292-5.

80. Naito Y, Fukata J, Tamai S, Seo N, Nakai Y, Mori K et al. Biphasic changes in hypothalamo-pituitary-adrenal function during the early recovery period after major abdominal surgery. J.Clin.Endocrinol.Metab. 1991;73(1):111-7.

81. Aun F, McIntosh TK, Lee A, Egdahl RH. The effects of surgery on the circadian rhythms of cortisol. Int.Surg. 1984;69(2):101-5.

82. Escher G, Galli I, Vishwanath BS, Frey BM, Frey FJ. Tumor necrosis factor alpha and interleukin 1beta enhance the cortisone/cortisol shuttle. J.Exp.Med. 1997;186(2):189-98.

83. Vermes I, Beishuizen A, Hampsink RM, Haanen C. Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. J.Clin.Endocrinol.Metab. 1995;80(4):1238-42.

84. Zipser RD, Davenport MW, Martin KL, Tuck ML, Warner NE, Swinney RR et al. Hyperreninemic hypoaldosteronism in the critically ill: a new entity. J.Clin.Endocrinol.Metab. 1981;53(4):867-73.

85. Parker LN, Levin ER, Lifrak ET. Evidence for adrenocortical adaptation to severe illness. J.Clin.Endocrinol.Metab. 1985;60(5):947-52.

86. Hindmarsh PC, Fall CH, Pringle PJ, Osmond C, Brook CG. Peak and trough growth hormone concentrations have different associations with the insulin-like growth factor axis, body composition, and metabolic parameters. J.Clin.Endocrinol.Metab. 1997;82(7):2172-6.

87. Van den BG. Growth hormone secretagogues in critical illness. Horm.Res. 1999;51 Suppl 3:21-8.

88. Maiter D, Walker JL, Adam E, Moatsstaats B, Mulumba N, Ketelslegers JM et al. Differential regulation by growth hormone (GH) of insulin-like growth factor I and GH receptor/binding protein gene expression in rat liver. Endocrinology 1992;130(6):3257-64.

89. Botfield C, Hinds CJ. Growth hormone and IGF-1 as anabolic therapy. In: Jenkins RC, Ross RJM, editors. The endocrine response to acute illness. Basel: Karger; 1999. p. 176-200.

90. Van den Berghe G, de Zegher F, Baxter RC, Veldhuis JD, Wouters P, Schetz M et al. Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. J.Clin.Endocrinol.Metab. 1998;83(2):309-19.

91. Van den Berghe G, de Zegher F, Lauwers P. Dopamine suppresses pituitary function in infants and children. Crit.Care Med. 1994;22(11):1747-53.

92. Van den Berghe G, de Zegher F, Veldhuis JD, Wouters P, Awouters M, Verbruggen W et al. The somatotropic axis in critical illness: effect of continuous growth hormone (GH)-releasing hormone and GH-releasing peptide-2 infusion. J.Clin.Endocrinol.Metab. 1997;82(2):590-9.

93. Slag MF, Morley JE, Elson MK, Labrosse KR, Crowson TW, Nuttall FQ et al. Free thyroxine levels in critically ill patients. A comparison of currently available assays. JAMA 1981;246(23):2702-6.

94. Ziegler MG, Morrissey EC, Marshall LF. Catecholamine and thyroid hormones in traumatic injury. Crit.Care Med. 1990;18(3):253-8.

95. Kaptein EM, Feinstein EI, Nicoloff JT, Massry SG. Serum reverse triiodothyronine and thyroxine kinetics in patients with chronic renal failure. J.Clin.Endocrinol.Metab. 1983;57(1):181-9.

96. Kaptein EM, Robbinson WJ, Grieb DH, Nicholoff JT. Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute non-thyroidal illness. J Clin Invest 1981;69:526-35.

97. Carr FE, Seelig S, Mariash CN, Schwartz HL, Oppenheimer JH. Starvation and hypothyroidism exert an overlapping influence on rat hepatic messenger RNA activity profiles. J.Clin.Invest. 1983;72(1):154-63.

98. Docter R, Krenning EP, de Jong M, Hennemann G. The sick euthyroid syndrome: changes in thyroid hormone serum parameters and hormone metabolism. Clin.Endocrinol.Oxf. 1993;39(5):499-518.

99. Mendel CM, Laughton CW, McMahon FA, Cavalieri RR. Inability to detect an inhibitor of thyroxine-serum protein binding in sera from patients with nonthyroid illness. Metabolism 1991;40(5):491-502.

100. Reilly CP, Wellby ML. Slow thyroxine binding globulin in the pathogenesis of increased dialysable fraction of thyroxine in nonthyroidal illnesses. J.Clin.Endocrinol.Metab. 1983;57(1):15-8.

101. Bacci V, Schussler GC, Kaplan TB. The relationship between serum triiodothyronine and thyrotropin during systemic illness. J.Clin.Endocrinol.Metab. 1982;54(6):1229-35.

102. St Germain DL, Galton VA. Comparative study of pituitary-thyroid hormone economy in fasting and hypothyroid rats. J.Clin.Invest. 1985;75(2):679-88.

103. Van den Berghe G, de Zegher F. Anterior pituitary function during critical illness and dopamine treatment. Crit.Care Med. 1996;24(9):1580-90.

104. Elenkov IJ, Chrousos GP. Stress, cytokine patterns and susceptibility to disease. Baillieres.Best.Pract.Res.Clin.Endocrinol.Metab. 1999;13(4):583-95.

105. Blackwell TS, Christman JW. Sepsis and cytokines: current status. Br.J.Anaesth. 1996;77(1):110-7.

106. van der Poll T, Buller HR, ten Cate T, Wortel CH, Bauer KA, van Derventer SJ et al. Activation of coagulation after administration of tumour necrosis factor to normal subjects. N Engl J Med 1990;322(23):1622-7.

107. Sherman ML, Spriggs DR, Arthur KA, Imamura K, Frei E3, Kufe DW. Recombinant human tumor necrosis factor administered as a five-day continuous infusion in cancer patients: phase I toxicity and effects on lipid metabolism. J.Clin.Oncol. 1988;6(2):344-50.

108. Selby P, Hobbs S, Viner C, Jackson E, Jones A, Newell D et al. Tumour necrosis factor in man: clinical and biological observations. Br.J.Cancer 1987;56(6):803-8.

109. Chapman PB, Lester TJ, Casper ES, Gabrilove JL, Wong GY, Kempin SJ et al. Clinical pharmacology of recombinant human tumour necrosis factor in patients with advanced cancer. J Clin Oncol 1987;5(12):1942-51.

110. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA et al. Detection of circulating tumour necrosis factor after endotoxin administration. N Engl J Med 1988;318(23):1481-6.

111. Abraham E, Wunderink R, Silverman H, Perl TM, Nasraway S, Levy H et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. JAMA 1995;273(12):934-41.

112. Abraham E, Anzueto A, Gutierrez G, Tessler S, San Pedro G, Wunderink R et al. Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. Lancet 1998;351(9107):929-33.

113. Charmandari E, Kino T, Souvatzoglou E, Chrousos GP. Pediatric stress: hormonal mediators and human development. Horm.Res. 2003;59(4):161-79.

114. Deshpande S, Platt MP, Aynsley Green A. Patterns of the metabolic and endocrine stress response to surgery and medical illness in infancy and childhood. Crit.Care Med. 1993;21(9 Suppl):S359-61.

115. Giannakoulopoulos X, Sepulveda W, Kourtis P, Glover V, Fisk NM. Fetal plasma cortisol and beta-endorphin response to intrauterine needling [see comments]. Lancet 1994;344(8915):77-81.

116. Greenough A, Nicolaides KH, Lagercrantz H. Human fetal sympathoadrenal responsiveness. Early Hum.Dev. 1990;23(1):9-13.

117. Anand KS. Relationships between stress responses and clinical outcome in newborns, infants, and children. Crit.Care Med. 1993;21(9 Suppl):S358-9.

118. Wilmore DW. Hormonal responses and their effect on metabolism. Surg.Clin.North Am. 1976;56(5):999-1018.

119. Powis MR, Smith K, Rennie M, Halliday D, Pierro A. Effect of major abdominal operations on energy and protein metabolism in infants and children. J.Pediatr.Surg. 1998;33(1):49-53.

120. Low JF, Herndon DN, Barrow RE. Effect of growth hormone on growth delay in burned children: a 3-year follow-up study. Lancet 1999;354(9192):1789.

121. Ward Platt MP, Tarbit MJ, Aynsley Green A. The effects of anesthesia and surgery on metabolic homeostasis in infancy and childhood. J.Pediatr.Surg. 1990;25(5):472-8.

122. Smith A, Barclay C, Quaba A, Sedowofia K, Stephen R, Thompson M et al. The bigger the burn, the greater the stress. Burns. 1997;23(4):291-4.

123. Matthews DS, Aynsley Green A, Matthews JN, Bullock RE, Cooper BG, Eyre JA. The effect of severe head injury on whole body energy expenditure and its possible hormonal mediators in children. Pediatr.Res. 1995;37(4 Pt 1):409-17.

124. Wilmore DW, Mason AD, Jr., pruitt BA, Jr. Impaired glucose flow in burned patients with gram-negative sepsis. Surg.Gynecol.Obstet. 1976;143(5):720-4.

125. Miller SI, Wallace RJ, Jr., Musher DM, Septimus EJ, Kohl S, Baughn RE. Hypoglycemia as a manifestation of sepsis. Am.J.Med. 1980;68(5):649-54.

126. Lichtarowicz-Krynska EJ, Bodamer O, Feillet F, Camacho-Hubner C, Britto J, Aynsley-Green A. The Metabolic and Endocrine Milieu of Critically Ill Children at presentation- lack of correlation with clinical severity scores. Hormone Research 1999;51(S2):83.

127. van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, Frolich M. Anti-inflammatory cytokine profile and mortality in febrile patients. Lancet 1998;351(9107):950-3.

128. Westendorp RG, Langermans JA, Huizinga TW, Verweij CL, Sturk A. Genetic influence on cytokine production in meningococcal disease. Lancet 1997;349(9069):1912-3.

129. Harding D, Baines PB, Brull D, Vassiliou V, Ellis I, Hart A et al. Severity of meningococcal disease in children and angiotensin-converting enzyme insertion/deletion polymorphism. Am J Respir Crit Care Med 2002;165:1103-6.

130. Cheadle WG, Hershman MJ, Wellhausen SR, Polk HC, Jr. HLA-DR antigen expression on peripheral blood monocytes correlates with surgical infection. Am.J.Surg. 1991;161(6):639-45.

131. Ziegler TR, Gatzen C, Wilmore DW. Strategies for attenuating protein-catabolic responses in the critically ill. Annu.Rev.Med. 1994;45:459-80.

132. Chwals WJ. Overfeeding the critically ill child: fact or fantasy? New Horiz. 1994;2(2):147-55.

133. Anand KJ, Sippell WG, Aynsley Green A. Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response [published erratum appears in Lancet 1987 Jan 24;1(8526):234]. Lancet 1987;1(8524):62-6.

134. Anand KJ, Sippell WG, Schofield NM, Aynsley Green A. Does halothane anaesthesia decrease the metabolic and endocrine stress responses of newborn infants undergoing operation? Br.Med.J.Clin.Res.Ed. 1988;296(6623):668-72.

135. Ponting GA, Halliday D, Teale JD, Sim AJ. Postoperative positive nitrogen balance with intravenous hyponutrition and growth hormone. Lancet 1988;1(8583):438-40.

136. Voerman HJ, van Schijndel RJ, Groeneveld AB, de Boer H, Nauta JP, van der Veen EA et al. Effects of recombinant human growth hormone in patients with severe sepsis. Ann.Surg. 1992;216(6):648-55.

137. Knox JB, Wilmore DW, Demling RH, Sarraf P, Santos AA. Use of growth hormone for postoperative respiratory failure. Am.J.Surg. 1996;171(6):576-80.

138. Pichard C, Kyle U, Chevrolet JC, Jolliet P, Slosman D, Mensi N et al. Lack of effects of recombinant growth hormone on muscle function in patients requiring prolonged mechanical ventilation: a prospective, randomized, controlled study. Crit.Care Med. 1996;24(3):403-13.

139. Herndon DN, Barrow RE, Kunkel KR, Broemeling L, Rutan RL. Effects of recombinant human growth hormone on donor-site healing in severely burned children. .Ann.Surg. 1990;212(4):424-9.

140. Takala J, Ruokonen E, Webster NR, Nielsen MS, Zandstra DF, Vundelinckx G et al. Increased mortality associated with growth hormone treatment in critically ill adults [see comments]. N.Engl.J.Med. 1999;341(11):785-92.

141. Van den Berghe G, de Zegher F, Bowers CY, Wouters P, Muller P, Soetens F et al. Pituitary responsiveness to GH-releasing hormone, GH-releasing peptide-2 and thyrotrophin-releasing hormone in critical illness. Clin.Endocrinol.Oxf. 1996;45(3):341-51.

142. Van den BG, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M et al. Intensive insulin therapy in the critically ill patients. N.Engl.J.Med. 2001;345(19):1359-67.

143. Bouloux P, Perrett D, Besser GM. Methodological considerations in the determination of plasma catecholamines by high-performance liquid chromatography with electrochemical detection. Ann.Clin.Biochem. 1985;22 (Pt 2):194-203.

144. Menard J, Catt KJ. Measurement of renin activity, concentration and substrate in rat plasma by radioimmunoassay of angiotensin I. Endocrinology 1972;90(2):422-30.

145. Lemeshow S, Le Gall JR. Modeling the severity of illness of ICU patients. A systems update. JAMA 1994;272(13):1049-55.

146. Pollack MM, Ruttimann UE, Getson PR. Pediatric risk of mortality (PRISM) score. Crit Care Med 1988;16(11):1110-6.

147. Pollack MM, Ruttiman UE, Getson PR. Pediatric risk of mortality (PRISM) score. Critical Care Medicine 1988;16(11):1110-6.

148. Ruttimann UE, Albert A, Pollack MM, Glass NL. Dynamic assessment of severity of illness in pediatric intensive care. Crit Care Med. 1986;14(3):215-21.

149. Balakrishnan G, Aitchison T, Hallworth D, Morton NS. Prospective evaluation of the Paediatric Risk of Mortality (PRISM) score. Arch.Dis.Child 1992;67(2):196-200.

150. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research [see comments]. BMJ. 1990;300(6719):230-5.

151. Shann F, Pearson G, Slater A, Wilkinson K. Paediatric index of mortality (PIM): a mortality prediction model for children in intensive care [see comments]. Intensive.Care Med. 1997;23(2):201-7.

152. Castellanos Ortega A, Delgado Rodriguez M. Comparison of the performance of two general and three specific scoring systems for meningococcal septic shock in children. Crit.Care Med. 2000;28(8):2967-73.

153. Thomson AP, Sills JA, Hart CA. Validation of the Glasgow Meningococcal Septicemia Prognostic Score: a 10-year retrospective survey [see comments]. Crit.Care Med. 1991;19(1):26-30.

154. Gonzalbez J, Villabona C, Ramon J, Navarro MA, Gimenez O, Ricart W et al. Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary- adrenal axis in normal subjects. Clin.Endocrinol.(Oxf) 2000;53(2):199-204.

155. Butte NF, Moon JK, Wong WW, Hopkinson JM, Smith EO. Energy requirements from infancy to adulthood. Am.J.Clin.Nutr. 1995;62(5 Suppl):1047S-52S.

156. Bitar A, Vermorel M, Fellmann N, Coudert J. Twenty-four-hour energy expenditure and its components in prepubertal children as determined by whole-body indirect calorimetry and compared with young adults. Am.J.Clin.Nutr. 1995;62(2):308-15.

157. Algren JT, Lal S, Cutliff SA, Richman BJ. Predictors of outcome in acute meningococcal infection in children. Crit.Care Med. 1993;21(3):447-52.

158. Marshall JC. Charting the course of critical illness: prognostication and outcome description in the intensive care unit [editorial; comment]. Crit.Care Med. 1999;27(4):676-8.

159. Hatherill M, Tibby SM, Hilliard T, Turner C, Murdoch IA. Adrenal insufficiency in septic shock. Arch.Dis.Child 1999;80(1):51-5.

160. Bone MP, Diver MJ, Selby A, Sharples A, Addison GM, Clayton PE. Assessment of adrenal function in meningococcal disease. Arch.Dis.Child. 1999;80(suppl. 1):A15.

161. Bollaert PE, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A. Reversal of late septic shock with supraphysiologic doses of hydrocortisone [see comments]. Crit.Care Med. 1998;26(4):645-50.

162. McNelis J, Marini CP, Jurkiewicz A, Szomstein S, Simms HH, Ritter G et al. Prolonged lactate clearance is associated with increased mortality in the surgical intensive care unit. Am.J.Surg. 2001;182(5):481-5.

163. Gore DC, Jahoor F, Hibbert JM, DeMaria EJ. Lactic acidosis during sepsis is related to increased pyruvate production, not deficits in tissue oxygen availability. Ann.Surg. 1996;224(1):97-102.

164. Luchette FA, Jenkins WA, Friend LA, Su C, Fischer JE, James JH. Hypoxia is not the sole cause of lactate production during shock. J.Trauma 2002;52(3):415-9.

165. James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. Lancet 1999;354(9177):505-8.

166. Vanek VW, Seballos RM, Chong D, Bourguet CC. Serum potassium concentrations in trauma patients. South.Med.J. 1994;87(1):41-6.

167. Levraut J, Ichai C, Petit I, Ciebiera JP, Perus O, Grimaud D. Low exogenous lactate clearance as an early predictor of mortality in normolactatemic critically ill septic patients. Crit Care Med. 2003;31(3):705-10.

168. Husain FA, Martin MJ, Mullenix PS, Steele SR, Elliott DC. Serum lactate and base deficit as predictors of mortality and morbidity. Am.J Surg. 2003;185(5):485-91.

169. Kobayashi S, Gando S, Morimoto Y, Nanzaki S, Kemmotsu O. Serial measurement of arterial lactate concentrations as a prognostic indicator in relation to the incidence of disseminated intravascular coagulation in patients with systemic inflammatory response syndrome. Surg.Today 2001;31(10):853-9.

170. McNelis J, Marini CP, Jurkiewicz A, Szomstein S, Simms HH, Ritter G et al. Prolonged lactate clearance is associated with increased mortality in the surgical intensive care unit. Am.J Surg. 2001;182(5):481-5.

171. Hatherill M, McIntyre AG, Wattie M, Murdoch IA. Early hyperlactataemia in critically ill children. Intensive Care Med. 2000;26(3):314-8.

172. Baxter RC. Changes in the IGF-IGFBP axis in critical illness. Best.Pract.Res.Clin.Endocrinol.Metab 2001;15(4):421-34.

173. de Groof F, Joosten KF, Janssen JA, de Kleijn ED, Hazelzet JA, Hop WC et al. Acute stress response in children with meningococcal sepsis: important differences in the growth hormone/insulin-like growth factor I axis between nonsurvivors and survivors. J.Clin.Endocrinol.Metab 2002;87(7):3118-24.

174. Soltesz G, Aynsley-Green A. Approach to the diagnosis of hypoglycaemmia in infants and children. In: Ranke MB, editor. Functional endocrinologic diagnostics in children and adolescents. 1 ed. Mannheim: J.J.Verlag; 1992. p. 168-83.

175. Garcia SC, Ruperez LM, Lopez-Herce CJ, Vigil ED, Garrido CG. [Prognostic value of the pediatric index of mortality (PIM) score and lactate values in critically-ill children]. An.Esp.Pediatr. 2002;57(5):394-400.

176. Kobayashi S, Gando S, Morimoto Y, Nanzaki S, Kemmotsu O. Serial measurement of arterial lactate concentrations as a prognostic indicator in relation to the incidence of disseminated intravascular coagulation in patients with systemic inflammatory response syndrome. Surg.Today 2001;31(10):853-9.

177. Mizock BA. Alterations in carbohydrate metabolism during stress: a review of the literature. Am.J Med. 1995;98(1):75-84.

178. Miles JM. Lipid fuel metabolism in health and disease. Curr.Opin.Gen.Surg. 1993:78-84.

179. Carpentier YA, Nordenstrom J, Robin A, Kinney JM. Glycerol turnover and kinetics of exogenous fat in surgical patients. Acta Chir Scand.Suppl 1981;507:226-37.

180. Matthews DS, Aynsley Green A, Eyre JA. Modified hormonal effects on fat metabolism after severe head injury in children. Pediatr.Res. 1996;39(6):1012-9.

181. Zierler KL, Rabinowitz D. Effect of very small concentrations of insulin on forearm metabolism: Persistance of its action on potassium and free fatty acids without its effect on glucose. J.Clin.Invest 1964;43:950.

182. Martinez-Riquelme AE, Allison SP. Insulin revisited. Clin.Nutr. 2003;22(1):7-15.

183. Anand KJ, Brown MJ, Causon RC, Christofides ND, Bloom SR, Aynsley Green A. Can the human neonate mount an endocrine and metabolic response to surgery? J.Pediatr.Surg. 1985;20(1):41-8.

184. Cogo PE, Carnielli VP, Rosso F, Cesarone A, Giordano G, Faggian D et al. Protein turnover, lipolysis, and endogenous hormonal secretion in critically ill children. Crit Care Med. 2002;30(1):65-70.

185. Sewell AC, Bohles HJ. Acylcarnitines in intermediary metabolism. Eur.J.Pediatr. 1995;154(11):871-7.

186. Nanduri V, Davies H, Jones J, Hindmarsh PC, Preece M, Brook CG et al. Diagnosis of growth hormone deficiency: an assay dependant phenomenon? 1996; 10th International Congress of Endocrinology.; 1996.

187. Mitchell H, Dattani M-T, Nanduri V, Hindmarsh P-C, Preece M-A, Brook C-G. Failure of IGF-I and IGFBP-3 to diagnose growth hormone insufficiency. Arch-Dis-Child. 1999;80 (5):443-7.

188. Bentham J, Rodriguez Arnao J, Ross RJ. Acquired growth hormone resistance in patients with hypercatabolism. Horm.Res. 1993;40(1-3):87-91.

189. Van den BG. Growth hormone secretagogues in critical illness. Horm.Res. 1999;51 Suppl 3:21-8.

190. Gelato MC. The growth hormone/insulin-like growth factor axis in critical illness. J.Pediatr.Endocrinol.Metab 2000;13 Suppl 2:1023-9.

191. Frost VJ, Helle SI, Lonning PE, van der Stappen JW, Holly JM. Effects of treatment with megestrol acetate, aminoglutethimide, or formestane on insulin-like growth factor (IGF) I and II, IGF-binding proteins (IGFBPs), and IGFBP-3 protease status in patients with advanced breast cancer. J.Clin.Endocrinol.Metab 1996;81(6):2216-21.

192. Wojnar MM, Fan J, Frost RA, Gelato MC, Lang CH. Alterations in the insulin-like growth factor system in trauma patients. Am.J.Physiol 1995;268(4 Pt 2):R970-R977.

193. Carter-Su C, Smit LS. Signaling via JAK tyrosine kinases: growth hormone receptor as a model system. Recent Prog.Horm.Res. 1998;53:61-82.

194. Jeay S, Sonenshein GE, Postel-Vinay MC, Kelly PA, Baixeras E. Growth hormone can act as a cytokine controlling survival and proliferation of immune cells: new insights into signaling pathways. Mol.Cell Endocrinol 2002;188(1-2):1-7.

195. Elsasser TH, Caperna TJ, Rumsey TS. Endotoxin administration decreases plasma insulin-like growth factor (IGF)-I and IGF-binding protein-2 in Angus x Hereford steers independent of changes in nutritional intake. J Endocrinol 1995;144(1):109-17.

196. Ghigo E, Arvat L, Gianotti L, Maccario M, Camanni F. The regulation of growth hormone secretion. In: Jenkins RC, Ross RJM, editors. The Endocrine Response to Acute Illness. 1 ed. Basel: Krager; 1999. p. 152-75.

197. Gump FE, Long CL, Geiger JW, Kinney JM. The significance of altered gluconeogenesis in surgical catabolism. J.Trauma 1975;15(8):704.

198. Wolfe R-R, Durkot M-J, Allsop J-R, Burke J-F. Glucose metabolism in severely burned patients. Metabolism 1979;28(10):1031-9.

199. Potau N, Ibanez L, Rique S, Carrascosa A. Pubertal changes in insulin secretion and peripheral insulin sensitivity. Horm.Res. 1997;48(5):219-26.

200. Hawdon JM. Perinatal stress. In: Aynsley-Green A, Ward-Platt MP, Lloyd-Thomas AR, editors. Stress and pain in infancy and childhood. 1 ed. London: Bailliere Tindall; 1995. p. 511-28.

201. Bereket A, Lang CH, Blethen SL, Fan J, Frost RA, Wilson TA. Insulin-like growth factor binding protein-3 proteolysis in children with insulin-dependent diabetes mellitus: a possible role for insulin in the regulation of IGFBP-3 protease activity. J.Clin.Endocrinol.Metab 1995;80(8):2282-8.

202. Bereket A, Wilson TA, Blethen SL, Sakurai Y, Herndon DN, Wolfe RR et al. Regulation of the acid-labile subunit of the insulin-like growth factor ternary complex in patients with insulin-dependent diabetes mellitus and severe burns. Clin.Endocrinol.(Oxf) 1996;44(5):525-32.

203. Bereket A, Lang CH, Blethen SL, Gelato MC, Fan J, Frost RA et al. Effect of insulin on the insulin-like growth factor system in children with new-onset insulin-dependent diabetes mellitus. J.Clin.Endocrinol.Metab 1995;80(4):1312-7.

204. Lang CH, Fan J, Frost RA, Gelato MC, Sakurai Y, Herndon DN et al. Regulation of the insulin-like growth factor system by insulin in burn patients. J Clin.Endocrinol Metab 1996;81(7):2474-80.

205. Ooi GT, Tseng LY, Tran MQ, Rechler MM. Insulin rapidly decreases insulin-like growth factor-binding protein-1 gene transcription in streptozotocin-diabetic rats. Mol.Endocrinol 1992;6(12):2219-28.

206. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr.Rev. 1995;16(1):3-34.

207. Lewitt MS, Baxter RC. Inhibitors of glucose uptake stimulate the production of insulin-like growth factor-binding protein (IGFBP-1) by human fetal liver. Endocrinology 1990;126(3):1527-33.

208. Delhanty PJ, Baxter RC. The regulation of acid-labile subunit gene expression and secretion by cyclic adenosine 3',5'-monophosphate. Endocrinology 1998;139(1):260-5.

209. Moran A, Jacobs DR, Jr., Steinberger J, Cohen P, Hong CP, Prineas R et al. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. J Clin.Endocrinol Metab 2002;87(10):4817-20.

210. Beckett PR, Wong WW, Copeland KC. Developmental changes in the relationship between IGF-I and body composition during puberty. Growth Horm.IGF Res. 1998;8(4):283-8.

211. Girgis R, Abrams SA, Castracane VD, Gunn SK, Ellis KJ, Copeland KC. Ethnic differences in androgens, IGF-I and body fat in healthy prepubertal girls. J.Pediatr.Endocrinol.Metab 2000;13(5):497-503.

212. Rolih CA, Ober KP. The endocrine response to critical illness. Med.Clin.North Am. 1995;79(1):211-24.

213. Russell RC, Walker CJ, Bloom SR. Hyperglucagonaemia in the surgical patient. Br.Med.J 1975;1(5948):10-2.

214. Giddings AE, O'Connor KJ, Rowlands BJ, Mangnall D, Clark RG. The relationship of plasma glucagon to the hyperglycaemia and hyperinsulinaemia of surgical operation. Br.J Surg. 1976;63(8):612-6.

215. Uzel N, Neyzi O. Thyroid function in critically ill infants with infections. Pediatr.Infect.Dis. 1986;5(5):516-9.

216. Kaptein EM, Kletzky OA, Spencer CA, Nicoloff JT. Effects of prolonged dopamine infusion on anterior pituitary function in normal males. J.Clin.Endocrinol.Metab 1980;51(3):488-91.

217. Uzel N, Neyzi O. Thyroid function in critically ill infants with infections. Pediatr.Infect.Dis. 1986;5(5):516-9.

218. Molitch ME, Hou SH. Neuroendocrine alterations in systemic disease. Clin.Endocrinol.Metab 1983;12(3):825-51.

219. Nylen ES, Alarifi AA. Humoral markers of severity and prognosis of critical illness. Best.Pract.Res.Clin.Endocrinol.Metab 2001;15(4):553-73.

220. Van den Berghe G, de Zegher F, Lauwers P. Dopamine and the sick euthyroid syndrome in critical illness. Clin.Endocrinol.Oxf. 1994;41(6):731-7.

221. Van den Berghe G, de Zegher F, Veldhuis JD, Wouters P, Gouwy S, Stockman W et al. Thyrotrophin and prolactin release in prolonged critical illness: dynamics of spontaneous secretion and effects of growth hormone- secretagogues. Clin.Endocrinol.Oxf. 1997;47(5):599-612.

222. Sumita S, Ujike Y, Namiki A, Watanabe H, Kawamata M, Watanabe A et al. Suppression of the thyrotropin response to thyrotropin-releasing hormone and its association with severity of critical illness. Crit Care Med. 1994;22(10):1603-9.

223. Fliers E, Alkemade A, Wiersinga WM. The hypothalamic-pituitary-thyroid axis in critical illness. Best.Pract.Res.Clin.Endocrinol.Metab 2001;15(4):453-64.

224. Kabadi UM. Thyrotropin dysregulation during a non-thyroidal illness: transient hypothalamic hypothyroidism? J.Endocrinol.Invest 2001;24(3):178-82.

225. Smith A, Barclay C, Quaba A, Sedowofia K, Stephen R, Thompson M et al. The bigger the burn, the greater the stress. Burns. 1997;23(4):291-4.

226. Ghigo E, Arvat E, Gianotti L, Ramunni J, DiVito L, Maccagno B et al. Human aging and the GH-IGF-I axis. J Pediatr.Endocrinol Metab 1996;9 Suppl 3:271-8.

227. Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. Endocr.Rev. 1993;14(1):20-39.

228. Meek SE, Nair KS, Jensen MD. Insulin regulation of regional free fatty acid metabolism. Diabetes 1999;48(1):10-4.

229. Matthews DS, Aynsley Green A, Eyre JA. Modified hormonal effects on fat metabolism after severe head injury in children. Pediatr.Res. 1996;39(6):1012-9.

230. Gravholt CH, Moller N, Jensen MD, Christiansen JS, Schmitz O. Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. J.Clin.Endocrinol.Metab 2001;86(5):2085-9.

231. Ottosson M, Lonnroth P, Bjorntorp P, Eden S. Effects of cortisol and growth hormone on lipolysis in human adipose tissue. J.Clin.Endocrinol.Metab 2000;85(2):799-803.

232. Owen OE, Cahill GF, Jr. Metabolic effects of exogenous glucocorticoids in fasted man. J.Clin.Invest 1973;52(10):2596-605.

233. Divertie GD, Jensen MD, Miles JM. Stimulation of lipolysis in humans by physiological hypercortisolemia. Diabetes 1991;40(10):1228-32.

234. Rebuffe-Scrive M, Krotkiewski M, Elfverson J, Bjorntorp P. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J.Clin.Endocrinol.Metab 1988;67(6):1122-8.

235. Rebuffe-Scrive M, Bronnegard M, Nilsson A, Eldh J, Gustafsson JA, Bjorntorp P. Steroid hormone receptors in human adipose tissues. J.Clin.Endocrinol.Metab 1990;71(5):1215-9.

236. Yang S, Bjorntorp P, Liu X, Eden S. Growth hormone treatment of hypophysectomized rats increases catecholamine-induced lipolysis and the number of beta-adrenergic receptors in adipocytes: no differences in the effects of growth hormone on different fat depots. Obes.Res. 1996;4(5):471-8.

237. Johannsson G, Marin P, Lonn L, Ottosson M, Stenlof K, Bjorntorp P et al. Growth hormone treatment of abdominally obese men reduces abdominal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure. J Clin.Endocrinol Metab 1997;82(3):727-34.

238. Snyder DK, Clemmons DR, Underwood LE. Treatment of obese, diet-restricted subjects with growth hormone for 11 weeks: effects on anabolism, lipolysis, and body composition. J.Clin.Endocrinol.Metab 1988;67(1):54-61.

239. Okuda Y, Pena J, Chou J, Field JB. Acute effects of growth hormone on metabolism of pancreatic hormones, glucose and ketone bodies. Diabetes Res.Clin.Pract. 2001;53(1):1-8.

240. Cahill GF, Jr. Starvation in man. N.Engl.J.Med. 1970;282(12):668-75.

241. Bartlett K, Aynsley-Green A, Leonard JV, TurnbullD.M. Inheritd disorders of mitochondrial beta-oxidation. In: Schaub J, Van Hoof F, Vis HL, editors. Inborn errors of metabolism. New York: Raven; 1991. p. 19-33.

242. Ceda, G.P. IGFs in the feedback control of Gh secretion: Hypothalamic and /or pituitary action? J Endocrinol Invest 1995;18:734-7.

243. Melmed S. Insulin suppresses growth hormone secretion by rat pituitary cells. J Clin.Invest 1984;73(5):1425-33.

244. Ghigo E, Bartolotta E, Imperiale E, Bellone J, Cardinale G, Aimaretti G et al. Glucagon stimulates GH secretion after intramuscular but not intravenous administration. Evidence against the assumption that glucagon per se has a GH-releasing activity. J Endocrinol Invest 1994;17(11):849-54.

245. Valcavi R, Zini M, Portioli I. Thyroid hormones and growth hormone secretion. J Endocrinol Invest 1992;15(4):313-30.

246. Giustina A, Wehrenberg WB. Influence of thyroid hormones on the regulation of growth hormone secretion. Eur.J Endocrinol 1995;133(6):646-53.

247. Dieguez C, Mallo F, Senaris R, Pineda J, Martul P, Leal-Cerro A et al. Role of glucocorticoids in the neuroregulation of growth hormone secretion. J Pediatr.Endocrinol Metab 1996;9 Suppl 3:255-60.

248. Wajchenberg BL, Liberman B, Giannella ND, Morozimato MY, Semer M, Bracco LO et al. Growth hormone axis in cushing's syndrome. Horm.Res. 1996;45(1-2):99-107.

249. Valcavi R. Oral glucose tolerance test: an inhibitory or a stimulatory input to growth hormone secretion? J.Endocrinol.Invest 1996;19(4):253-5.

250. Kerrigan JR, Rogol AD. The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. Endocr.Rev. 1992;13(2):281-98.

251. Lemmey AB, Glassford J, Flick-Smith HC, Holly JM, Pell JM. Differential regulation of tissue insulin-like growth factor-binding protein (IGFBP)-3, IGF-I and IGF type 1 receptor mRNA levels, and serum IGF-I and IGFBP concentrations by growth hormone and IGF-I. J.Endocrinol. 1997;154(2):319-28.

252. Baxter RC, Martin JL. Binding proteins for the insulin-like growth factors: structure, regulation and function. Prog.Growth Factor Res. 1989;1(1):49-68.

253. Baxter RC. The insulin-like growth factor (IGF)-IGF-binding protein axis in critical illness. Growth hormone and IGF Research 1999;9:67-9.

254. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr.Rev. 1995;16(1):3-34.

255. Dell GC, Morley SD, Mullins JJ, Williams BC, Walker SW. Multiple signal transduction systems regulate angiotensin II type 1 (AT1) receptor mRNA expression in bovine adrenocortical cells. Endocr.Res. 1996;22(4):363-8.

256. Meyer TF, Pohlner J, van Putten JP. Biology of the pathogenic Neisseriae. Curr.Top.Microbiol.Immunol. 1994;192:283-317.

257. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N.Engl.J Med. 2001;344(18):1378-88.

258. Wolff K, Stern A. Identification and characterization of specific sequences encoding pathogenicity associated proteins in the genome of commensal Neisseria species. FEMS Microbiol.Lett. 1995;125(2-3):255-63.

259. Muller A, Gunther D, Dux F, Naumann M, Meyer TF, Rudel T. Neisserial porin (PorB) causes rapid calcium influx in target cells and induces apoptosis by the activation of cysteine proteases. EMBO J 1999;18(2):339-52.

260. Johansson L, Rytkonen A, Bergman P, Albiger B, Kallstrom H, Hokfelt T et al. CD46 in meningococcal disease. Science 2003;301(5631):373-5.

261. Hauck CR, Meyer TF. 'Small' talk: Opa proteins as mediators of Neisseria-host-cell communication. Curr.Opin.Microbiol. 2003;6(1):43-9.

262. Mulks MH, Plaut AG. IgA protease production as a characteristic distinguishing pathogenic from harmless neisseriaceae. N.Engl.J Med. 1978;299(18):973-6.

263. Plaut AG, Gilbert JV, Artenstein MS, Capra JD. Neisseria gonorrhoeae and neisseria meningitidis: extracellular enzyme cleaves human immunoglobulin A. Science 1975;190(4219):1103-5.

264. Pohlner J, Klauser T, Kuttler E, Halter R. Sequence-specific cleavage of protein fusions using a recombinant Neisseria type 2 IgA protease. Biotechnology (N.Y.) 1992;10(7):799-804.

265. Brooks GF, Lammel CJ, Blake MS, Kusecek B, Achtman M. Antibodies against IgA1 protease are stimulated both by clinical disease and asymptomatic carriage of serogroup A Neisseria meningitidis. J Infect.Dis. 1992;166(6):1316-21.

266. Lorenzen DR, Dux F, Wolk U, Tsirpouchtsidis A, Haas G, Meyer TF. Immunoglobulin A1 protease, an exoenzyme of pathogenic Neisseriae, is a potent inducer of proinflammatory cytokines. J Exp.Med. 1999;190(8):1049-58.

267. Pollard AJ, Britto J, Nadel S, DeMunter C, Habibi P, Levin M. Emergency management of meningococcal disease. Arch.Dis.Child 1999;80(3):290-6.

268. Migeon CJ, Kenny FM, Hung W, Voorhess ML. Study of adrenal function in children with meningitis. Pediatrics 1967;40(2):163-83.

269. Wajchenberg B, Leme CE, Tambascia M, Boulos M, Okada H, Cesar FP et al. The adrenal response to exogenous adrenocorticotrophin in patients with infections due to Neisseria meningitidis. J.Infect.Dis. 1978;138(3):387-91.

270. Agwu JC, Spoudeas H, Hindmarsh PC, Pringle PJ, Brook CG. Tests of adrenal insufficiency. Arch.Dis.Child 1999;80(4):330-3.

271. Soni A, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T et al. Adrenal insufficiency occurring during septic shock: incidence, outcome, and relationship to peripheral cytokine levels. Am.J.Med. 1995;98(3):266-71.

272. Bouachour G, Tirot P, Gouello JP, Mathieu E, Vincent JF, Alquier P. Adrenocortical function during septic shock [see comments]. Intensive.Care Med. 1995;21(1):57-62.

273. Briegel J, Schelling G, Haller M, Mraz W, Forst H, Peter K. A comparison of the adrenocortical response during septic shock and after complete recovery [see comments]. Intensive.Care Med. 1996;22(9):894-9.

274. Dillon M-J, Ryness J-M. Plasma renin activity and aldosterone concentration in children. Br-Med-J. 1975;Nov 8(4(5992)):316-9.

275. Findling JW, Waters VO, Raff H. The dissociation of renin and aldosterone during critical illness. J.Clin.Endocrinol.Metab. 1987;64(3):592-5.

276. Gill GV, Prudhoe K, Cook DB, Latner AL. Effect of surgical trauma on plasma concentrations of cyclic AMP and cortisol. Br.J Surg. 1975;62(6):441-3.

277. Chernow B, Alexander HR, Smallridge RC, Thompson WR, Cook D, Beardsley D et al. Hormonal responses to graded surgical stress. Arch.Intern.Med. 1987;147(7):1273-8.

278. Naito Y, Fukata J, Tamai S, Seo N, Nakai Y, Mori K et al. Biphasic changes in hypothalamo-pituitary-adrenal function during the early recovery period after major abdominal surgery. J Clin.Endocrinol Metab 1991;73(1):111-7.

279. Zaloga GP, Marik P. Hypothalamic-pituitary-adrenal insufficiency. Crit Care Clin. 2001;17(1):25-41.

280. Annane D. Effects of the combination of hydrocortisone (HC) - fludrocortisone (FC) on mortality in septic shock. Crit Care Med 2000;28(12 (Suppl.)):A46.

281. Bailey RR, Abernethy MH, Beaven DW. Adrenocartical response to the stress of an acute myocardial infarction. Lancet 1967;1(7497):970-3.

282. Chopra MP, Thadani U, Aber CP, Portal RW, Parkes J. Plasma cortisol, urinary 17-hydroxycorticoids, and urinary vanilyl mandelic acid after acute myocardial infarction. Br.Heart J 1972;34(10):992-7.

283. Span LF, Hermus AR, Bartelink AK, Hoitsma AJ, Gimbrere JS, Smals AG et al. Adrenocortical function: an indicator of severity of disease and survival in chronic critically ill patients. Intensive Care Med. 1992;18(2):93-6.

284. Drucker D, Shandling M. Variable adrenocortical function in acute medical illness. Crit Care Med. 1985;13(6):477-9.

285. Laragh JH, Angers M, Kelly WG, Liberman S. Hypotensive agents and pressor substances. JAMA 1960;174(234).

286. Genest J, Biron P, Koiw E, Nowaczynski W, Chretein M, Boucle R. Adrenocortical hormones in human hypertension and the relation to angiotensin. Circ Res 1961;9(775).

287. Williams GH, Hollenberg NK, Brown C, Mersey JH. Adrenal responses to pharmacological interruption of the renin- angiotensin system in sodium-restricted normal man. J.Clin.Endocrinol.Metab. 1978;47(4):725-31.

288. Himathongkam T, Dluhy RG, Williams GH. Potassim-aldosterone-renin interrelationships. J.Clin.Endocrinol.Metab. 1975;41(1):153-9.

289. Boyd GW, Adamson AR, Arnold M, James VH, Peart WS. The role of angiotensin II in the control of aldosterone in man. Clin.Sci. 1972;42(1):91-104.

290. Boulter PR, Spark RF, Arky RA. Dissociation of the renin-aldosterone system and refractoriness to the sodium-retaining action of mineralocorticoid during starvation in man. J.Clin.Endocrinol.Metab. 1974;38(2):248-54.

291. Lichtarowicz-Krynska EJ, Bone MP, Diver MJ, Aynsley-Green A, Clayton PE. Aldosterone and cortisol responses to low dose ACTH stimulation (LDST) in critically ill children with meningococcal disease (MD) Horm Res,53,S2,76 2000. Horm Res 2000;53(S2):26 (Abstract).

292. Stewart PM, Whorwood CB, Walker BR. Steroid hormones and hypertension: the cortisol-cortisone shuttle. Steroids 1993;58(12):614-20.

293. Stewart P-M, Walker B-R, Holder G, O'Halloran D, Shackleton C-H. 11 beta-Hydroxysteroid dehydrogenase activity in Cushing's syndrome: explaining the mineralocorticoid excess state of the ectopic adrenocorticotropin syndrome. J.Clin.Endocrinol.Metab. 1995;80(12):3617-20.

294. Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr.Rev. 1998;19(5):647-72.

295. Raff H, Findling JW. Aldosterone control in critically ill patients: ACTH, metoclopramide, and atrial natriuretic peptide. Crit.Care Med. 1990;18(9):915-20.

296. Sowers JR, Beck FW, Stern N. Glucocorticoid suppression enhances the 18-hydroxycorticosterone and aldosterone response to metoclopramide in man. Life Sci. 1983;33(25):2489-95.

297. Stern N, Beck FW, Sowers JR, Tuck M, Hsueh WA, Zipser RD. Plasma corticosteroids in hyperreninemic hypoaldosteronism: evidence for diffuse impairment of the zona glomerulosa. J.Clin.Endocrinol.Metab. 1983;57(1):217-20.

298. Riepe FG, Krone N, Peter M, Sippell WG, Partsch CJ. Chromatographic system for the simultaneous measurement of plasma 18-hydroxy-11-deoxycorticosterone and 18-hydroxycorticosterone by radioimmunoassay: reference data for neonates and infants and its application in aldosterone-synthase deficiency. Journal of Chromatography 2003;785(B):293-301.

299. Natarajan R, Ploszaj S, Horton R, Nadler J. Tumor necrosis factor and interleukin-1 are potent inhibitors of angiotensin-II-induced aldosterone synthesis. Endocrinology 1989;125(6):3084-9.

300. Takeda Y, Miyamori I, Yoneda T, Hatakeyama H, Inaba S, Furukawa K et al. Regulation of aldosterone synthase in human vascular endothelial cells by angiotensin II and adrenocorticotropin. J Clin.Endocrinol Metab 1996;81(8):2797-800.

301. Alarifi AA, Van den Berghe G, Snider RH, Becker KL, Muller B, Nylen ES. Endocrine markers and mediators in critical illness. In: Becker KL, editor. Principles and practice of endocrinology and metabolism. 3 ed. Philadelphia: Lippincott Williams &Wilkins; 2001. p. 2077-87.

302. Whang KT, Steinwald PM, White JC, Nylen ES, Snider RH, Simon GL et al. Serum calcitonin precursors in sepsis and systemic inflammation. J Clin.Endocrinol Metab 1998;83(9):3296-301.

303. Nylen ES, Whang KT, Snider RH, Jr., Steinwald PM, White JC, Becker KL. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Crit Care Med. 1998;26(6):1001-6.

304. Jacobs S, Zuleika M, Mphansa T. The Multiple Organ Dysfunction Score as a descriptor of patient outcome in septic shock compared with two other scoring systems. Crit Care Med. 1999;27(4):741-4.

305. Findling JW, Waters VO, Raff H. The dissociation of renin and aldosterone during critical illness. J.Clin.Endocrinol.Metab. 1987;64(3):592-5.

Appendix 1

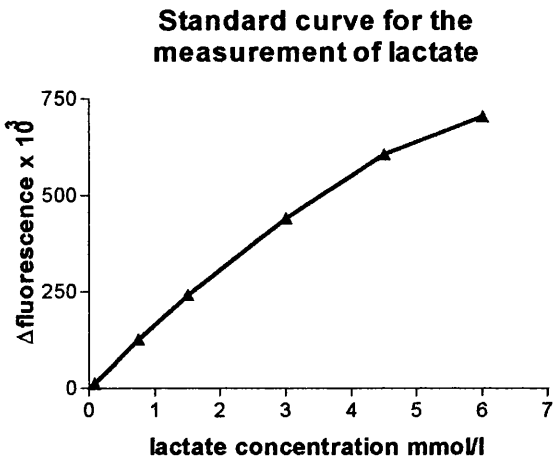
Standard curves for assays

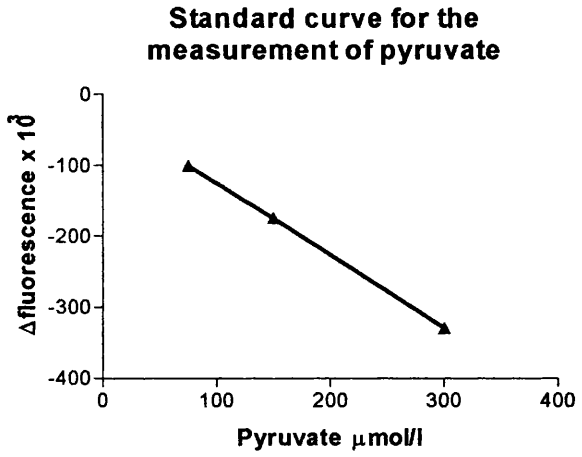
Standard curves are given for all the metabolites that were analysed in the present study and 3 hormonal assays each an example of different methodology. The GH standard curve is given as an example of an immunoradiometric assay (IRMA), the cortisol standard curve is given as an example of a solid phase radioimmunoassay and the IGFBP-3 is given as an example of an ELISA methodology.

Fig. 10.1 Standard curves for assays

a) Lactate
b) Pyruvate
c) Acetoacetate
d) NEFA
e) β-hydroxybutyrate
f) GH
g) Cortisol
h) IGFBP-3

Fig 10.1 a), b) and c)





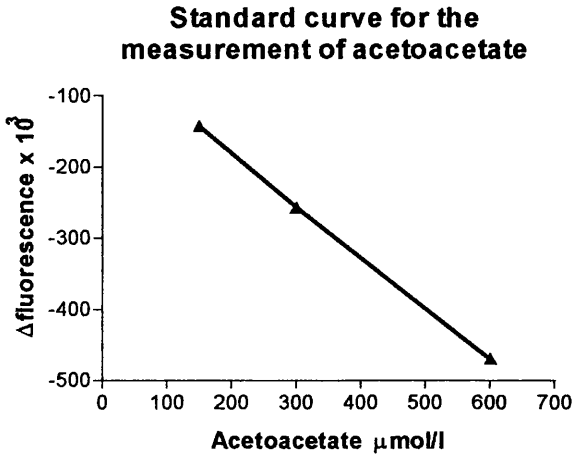
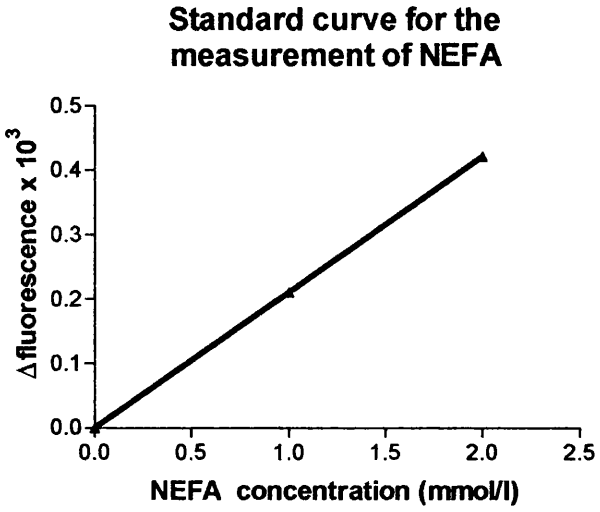


Fig 10.1 d) and e)



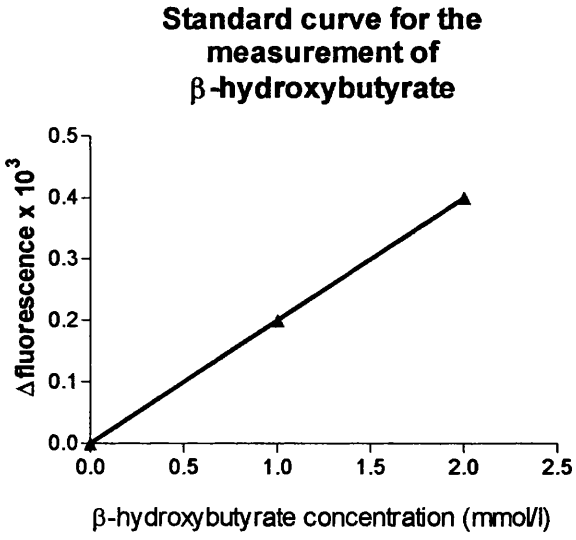


Fig 10.1f Standard curve for GH.

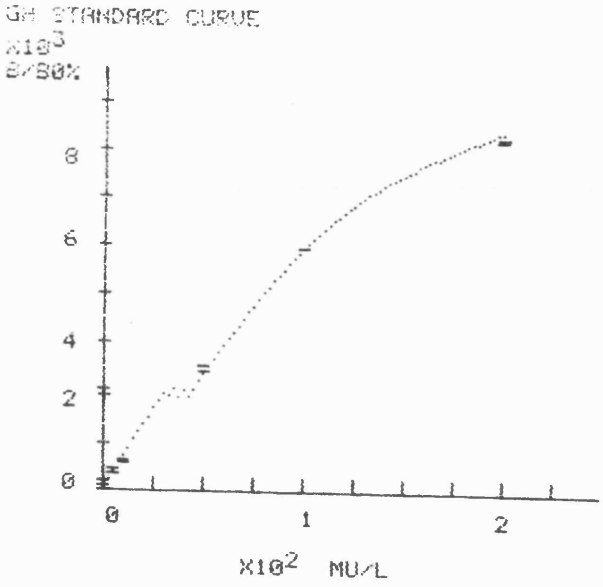


Fig 10.1g. Standard curve for cortisol.

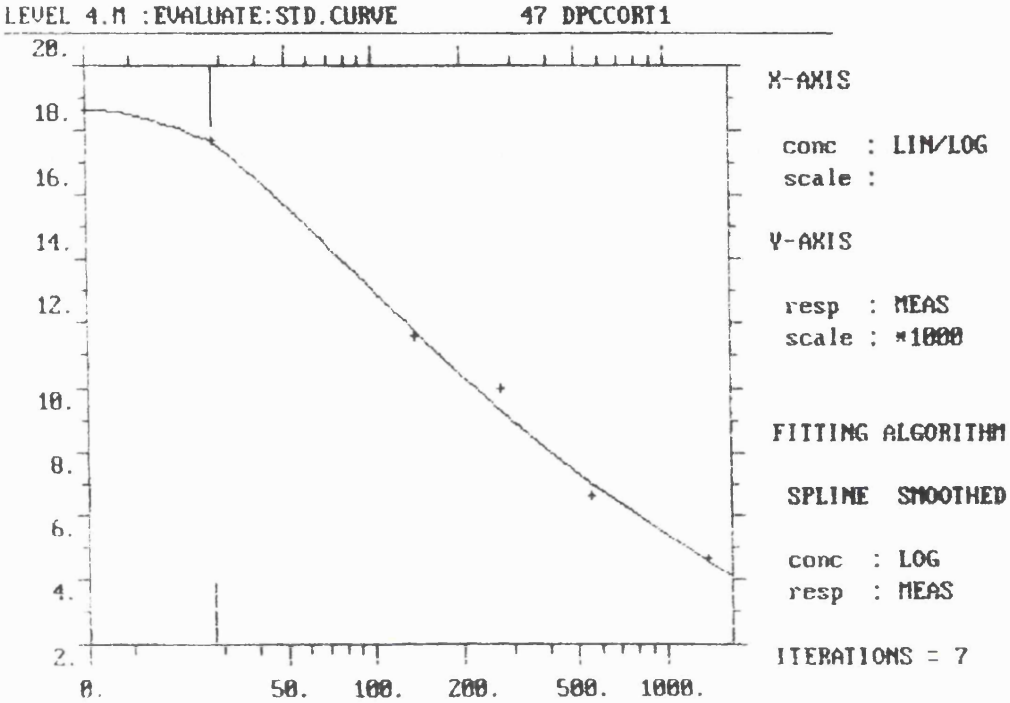


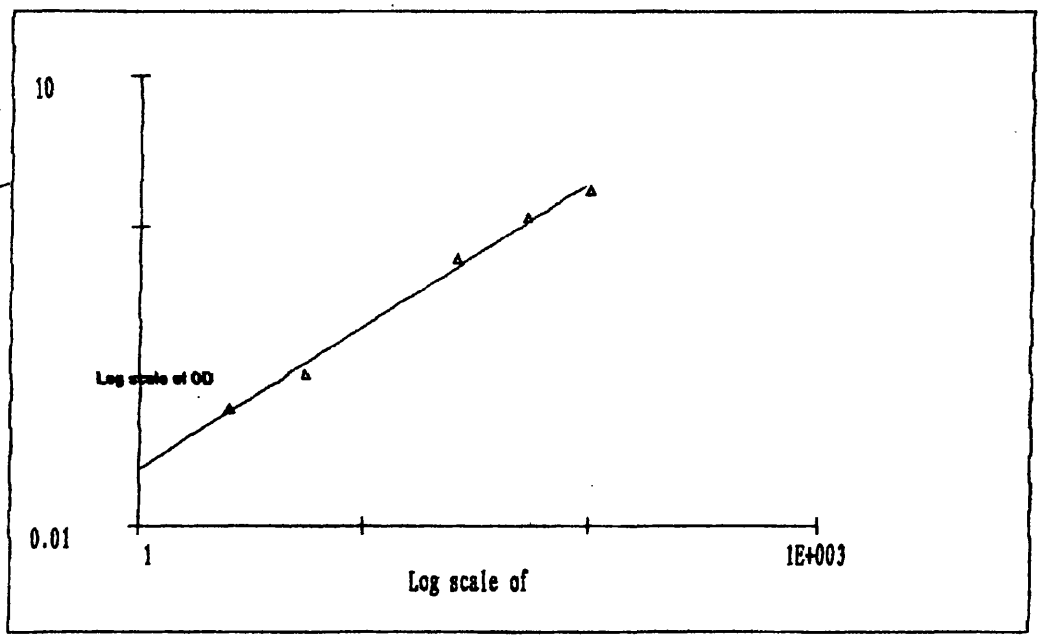
Fig 10.1h Standard curve for IGFBP-3.

Curve Fit: Log-Log

Corr. Coeff: 0.996

$\log(y) = A + B * \log(x)$

A = -1.62 B = 0.958



Appendix 2

Patient information sheets

Patient Information

Better understanding of illness in children on intensive care unit

The aim of the study?

The aim of this study is to compare the severity of illness with the biochemical and hormonal changes in critically ill children.

Why is the study being done?

The biochemical changes which occur in critically ill children are little understood. Therefore we are doing this study to gain a better understanding of the mechanism of the underlying conditions.

How is the study being done?

The study will be done in two parts.

First in addition to the blood samples, which will be taken routinely, we will take blood samples from indweliing cannulas which are already in place. These samples will be used to measure stress hormones, and other chemical compounds every 12 hours for the first 2 days, and then every second day thereafter.

Secondly we will infuse leucine, phenylalanine (aminoacids,which are used to form protein) and glucose (sugar), which have been chemically slightly changed. The body cannot tell the difference, but we can in our laboratory. These infusions will last 4 hours and be repeated every second day. Before and at the end of each infusion we will take additional blood samples (5

blood samples altogether). From these blood tests we can calculate the amount of glucose (sugar) produced by the liver and the total amount of protein produced and broken down by the body. During the infusion we will connect the ventilator of your child to a machine (indirect calorimeter), without affecting the
ventilation or care of your child. This machine allows us to measure the amount of energy, which is used by your child. The assessment of the optimal nutrition for your child, however is already part of the routine clinical management

What are the risks and discomforts?

There are no additional risks or discomforts. For the blood sampling and the infusions we will use cannulas, which have been inserted already, when your child was admitted. The total amount of blood being taken within the first day will not exceed 12 mls, then not 8 mls every second day. The total amount of blood taken will be reduced in smaller children, so it will not affect your child's condition. However these children will need blood transfusions for their routine care.

What are the potential benefits?

From the results of these studies we will better understand the underlying mechanism of critical illness in children. This will result in the development of new therapies to improve survival of these children. The measurement of the energy used by your child will benefit your child immediately by changing the nutrition towards the needs.

Who will access to the case/research records?

Only medical staff working on the ward and the researcher will have access to the case or research records. Research results will be presented without mentioning names.

What are the arrangements for compensation?

This project has been approved by an independent research ethics committee who
believe that it is of minimal risk to you. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or manufacturer involved.

Do I have to take part in this study?

No, participation is completely voluntary. Your decision will not affect the treatment of your child.

Who do I speak to if problems arise?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact The chairman of the Research Ethics Committee, by post via The Research and Development Office, The Institute of Child Health, 30 Guildford Street, London WCIN IEH, or if urgent, by telephone on 0171-242-9789 ex 2620, and the Committee administration will put you in contact with him.

Researcher who will have contact with the family

Principle Research Fellow Dr. 0. Bodamer.

Details of how to contact the Researcher

Message Pager: 01426316403 or through hospital switchboard. Tel. 0171-242 9789 ex. 2614 (an swerphone). Medical Unit, institute of Child Health, 30 Guildford Street,London WCIN IEH.

Consent forms

Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee

Consent Form for PARENTS OR GUARDIANS of Children Participating in Research Studies

95MD55 Catabolism in critically ill children: Can the use of growth hormone
improve the outcome?

NOTES FOR PARENTS OR GUARDIANS:

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.

2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.

3. If you decide, now or at any other stage, that you do not wish your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or ftiture treatment.

4. You will be given an information sheet which describes the research project. This information sheet is for you to keep and refer to. Please read carefully.

5. If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via The Research and Development Office, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH or if urgent, by telephone on 071 242 9789 ex 2620 and the committee administration will put you in contact with him.

CONSENT

I/We _____, being the arent(s)/guardian(s) of

_____agree that the Research Project named above has been explained to me to my/our satisfaction, and I/We give permission for our child to take part in this study. I/We have read both the notes written above and the Information Sheet provided, and understand what the research study involves.

SIGNED (Parent (s)/Guardian (s))

_____

SIGNED (Researcher)

_____

Study protocol sheets

Study sheet

(Dr. Ewa Lichtarowicz, Dr. Olaf Bodamer ext 2614 or LRP)

name: d.o.b.: hospitalno:

weight: diagnosis:

stable isotope studies:

date: tracer: blood volume:

metabolites/hormones:

date: time: blood volume:

blood volume taken:
(= % of total blood volume)

Indirect calorimetry:

date: result:

Checklist for patients enrolled in PICU study -2/97 Bo

date: name: hospitalno:

1. Consent form signed by parents and researcher
2. Copies of consent form in notes and patient file
3. Original consent form in study book
4. Study sheet in notes and copy in study book
5. Fill in cover sheet (i.e. diagnosis, PMH...)
6. Copy of cover sheet in patient file
7. Original cover sheet in study book
8. Fill in schedule (samples, blood volume...)
9. Copy of schedule in patient file
10. Original schedule in study book
11. Record weight
12. Record height (approx.)
13. Record impedance
14. Record skinfold measurements
15. Record indirect calorimetry within 6 h
16. Fill in nutritional assessment sheet day 1
17. Leucine and glucose kinetics day I
18. Bicarbonate kinetics day I (if appropriate)
19. Metabolites hormones according to schedule
20. Immune samples according to schedule

Study:_____Investigator:_____

_____

PICU- Catabolism in Critically ill patient

Name: Hospitalno:

Dob: Gender: Ethnic: National:

Address: Tel:

Diagnosis 1: Diagnosis 2:

Diagnosis 3: Diagnosis 4:

Diagnosis 5: Diagnosis 6:

Past medical history:

Asmission: Discharge:

Time or illness before admission:

Time in ICU before admission:

Admission

Weight: Height: Head: BMI:

LBM : Impedance:

Skinfold 1: Skinfold 2: Skinfold 3:

Skinfold 4: Skinfold 5:

date:

Nutritional status in critically ill children

name:	hospitalno:	studynolday:
dob:	diagnosis:	
weight:	height:	body impedance:

Indirect calorimetry:	from	to
VCO2 (ml/min):		$V0_2$ (ml/min):
RQ:		REE (kcal/24h):
F_i0_2 (%):		VE (l/min):
rel. glucose (g/h):	rel. fat (g/h):	rel. calorie (Kcal/h):
rel. amino acids (g/h):		

Ventilator:	respiratory rate:	
mode:	F_iO_2(%):	
PIP:	pH:	
Peep:	$p0_2$:	pCO_2:

Intake:	
total glucose (g/24h):	total aminoacids (g/24h):
total fat (g/24h):	total fluid (ml/24h):
total calories (kcal/24h):	

Urinary ouput:

checklist for stable isotope studies

date: _____ study:_____studyno:_____

name: _____ DOB: _____ diagnosis:_____

bodyweight: _____ lean body mass: _____

stable isotopes:

prime (doses):			infusion doses(_____ hours):		
1-^{13}C leucine	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
1-^{13}C bicarb.	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
ring-D_5 phe	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
$^2H_2$-tyrosine	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
$^2H_2$-Htyrosine	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
(6,6)-D_2-glu.	_____mg/kg	_____ml	_____mg/kg/h	_____ml	
D_5-glycerol	_____mg/kg	_____ml	_____mg/kg/h	_____ml	

weight _____ ml saline bag:_____ g or syringe (empty)_____g

+ 1-^{13} C leucine: _____ g
+ ring-D_5 phe. _____ g
$^2H_2$-tyrosine _____ g
+ (6,6)-D_2-glu _____ g+ _____ ml saline _____g
+ D_5-glycerol _____ g after _____ g

difference infusion bag _____g

weight infusion set before _____g after _____ g

difference infusion set _____g

O h breath samples (4x)	blood sample (heparin)	perchioracid
h breath samples (2x)	blood sample (heparin)	perchloracid
h breath samples (2x)	blood sample (heparin)	perchloracid
h breath samples (2x)	blood sample (heparin)	perchioracid
h breath samples (2x)	blood sample (heparin)	perchioracid
h breath samples {2x)	blood sample (heparin)	perchioracid
h breath samples (2x)	blood sample (heparin)	perchioracid
h breath samples (2i)	blood sample (heparin)	perchloracid

infusion rate_____ml/h start _____ end_____ length _____min

indirect calorimetry start _____ end_____ length_____min

studyno: date:

name: d.o.b: hospitalno:

permissive blood volume (5% of total):

time									
perchloric (500µl)									
heparin(3-4ml)									
citrate (2ml)									
aa, albmin (1ml)									
total volume									

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