Cortisol Perturbation in the Pathophysiology of Septicaemia, Complicated Pregnancy and Weight Loss/Obesity

Jui Ting Ho
MBBS, BSc (Med), FRACP

For the degree of Doctor of Philosophy
Department of Medicine
University of Adelaide

April 2007

Table of contents

Chapter 3

The hypothalamic-pituitary-adrenal axis in critical illness

Chapter 4

Nitric oxide as a potential physiologically relevant measure of cortisol action in tissues

Chapter 5

The maternal hypothalamic-pituitary-adrenal axis in pregnancy

Abstract

Cortisol, the principal glucocorticoid secreted from the adrenal glands, is essential for life. Healthy cortisol levels are maintained through negative feedback on the central nervous system (CNS) – pituitary stimulatory apparatus which regulates production of adrenocorticotropin (ACTH) and contains a light–entrained intrinsic CNS driven diurnal rhythm. Cortisol participates in a regulatory mechanism where inflammatory cytokines stimulate cortisol release and cortisol in turn suppresses cytokine release. The effects of cortisol in inflammatory states include elevating blood pressure and metabolic regulation. This thesis contains three exploratory studies examining circulating cortisolaemia using the best available methodologies (total and free cortisol and corticosteroid-binding globulin (CBG)) in clinical states characterized by immune activation/ inflammation and altered blood pressure. These clinical states include: (1) septic shock, (2) hypertensive disorders of pregnancy and (3) obesity-induced hypertension. Prior to the studies described here, little was know about cortisolaemia in these common pathological states.

Septic shock is a life threatening condition that complicates severe infection and is characterized by systemic inflammation and refractory hypotension. High plasma total cortisol levels and attenuated responses to synthetic ACTH stimulation are associated with increased mortality. The use of corticosteroids in septic shock has been highly controversial for decades, however recent trials have reported haemodynamic and survival benefits associated with the use of physiologic steroid replacement in patients with relative adrenal insufficiency (RAI) – currently defined as a total cortisol increment of 248 nmol/L or less following ACTH (250 μg) stimulation. However, CBG and albumin levels fall by around 50% with an increase in plasma free cortisol in critical illness. Hence, total cortisol may not reflect

the biologically active free (unbound) cortisol, suggesting that standard assays for plasma cortisol (which measure total plasma cortisol) underestimate HPA axis activity.

In this study, we have showed that plasma free cortisol is a better guide to circulating glucocorticoid activity in systemic infection than total cortisol. We have also validated the use of Coolens' method in estimating free cortisol in systemic infection, using plasma total cortisol and CBG measurements as plasma free cortisol is not performed in clinical laboratories. Free cortisol measurement allows better categorization of RAI and non-RAI groups with a free cortisol increment of 110 nmol/L as cut-off. Moreover, we have shown that survivors of RAI have normal adrenocortical function on follow-up testing suggesting a lack of functional adrenal reserve rather than adrenal damage during critical illness. Larger randomized controlled trials will be required to redefine RAI using free cortisol measurements and relate that to clinical outcomes and responses to corticosteroid therapy.

Nitric oxide (NO) is normally produced in the endothelium by the constitutive form of the NO synthase and this physiologic production is important for blood pressure regulation and blood flow distribution. Studies have shown that an overproduction of NO by the inducible form of NO synthase (iNOS) may contribute to the hypotension, cardiodepression and vascular hyporeactivity in septic shock. Clinical studies of non-selective inhibitors of the L-arginine nitric oxide pathway showed increased mortality from cardiovascular complications. However, glucocorticoids, which improve vasopressor sensitivity, may act by partially suppressing NO synthesis through selective direct inhibition of iNOS, and suppression of inflammatory cytokine synthesis. Hence, plasma nitrate/ nitrite (NOx) levels may provide a titratable end point to individualize glucocorticoid therapy in sepsis.

The NOx study in this thesis showed that cortisol (total and free), CBG and NOx correlated to illness severity. Free cortisol, and to a lesser extent total cortisol, but not NOx levels, predicted septic shock. NOx levels were characteristically stable within individuals but inter-individual differences were only partly accounted for by illness severity or renal dysfunction. NOx levels correlated weakly with cortisol, did not relate to the need for vasopressors and were not suppressed by hydrocortisone treatment. Thus, NOx is not a suitable target for glucocorticoid therapy in septic shock.

Pregnancy is the only sustained physiologic state of hypercortisolism in humans. A large body of data suggests that excessive foetal and prenatal glucocorticoid exposure leads to reduced birth weight and adverse health in offspring such as elevated blood pressure and insulin resistance. Pre-eclampsia and gamete donor pregnancies are associated with immune activation, elevated inflammatory cytokines as well as elevated blood pressure. Prior to the study described in this thesis however, there was no prospective data on maternal cortisolaemia in these complicated pregnancies.

My study has demonstrated for the first time that there was a substantial fall in plasma CBG levels in the last few weeks of gestation with a corresponding rise in free cortisol in normal pregnancy, a finding obscured for methodological reasons in past studies. This free cortisol elevation in late pregnancy may facilitate organ maturation in the foetus and perhaps prepare the mother for the metabolic demands of labour. In pre-eclampsia and gestational hypertension, plasma CBG, total and free cortisol levels were lower in late third trimester; and in IUGR, plasma CBG levels were suppressed from 28 weeks gestation until delivery but with no significant difference in plasma total and free cortisol. Women with assisted reproduction using donor gametes/ embryos had significantly lower plasma CBG, total and free cortisol levels even in those with normal pregnancy outcomes. Low CBG may be due to reduced

synthesis or enhanced inflammation-driven degradation. Low maternal cortisol may be due to a lack of placental corticotropin-releasing hormone, or reduced maternal ACTH, driving cortisol production. This unanticipated maternal hypocortisolism in complicated pregnancies may trigger precocious activation of the foetal HPA axis and could have implications for postnatal and adult health. Speculatively, since excess prenatal GCs increase HPA axis activity, we proposed that maternal hypocortisolism may predispose to the hypocortisolaemic state characterized by fatigue, pain and stress sensitivity, in offspring.

The third state of immune/ inflammatory activation associated with blood pressure dysregulation studied in this thesis is obesity. The epidemiologic relationship between obesity and hypertension is widely recognised. Central obesity in particular has been associated with exaggerated HPA responses to stimuli. Studies of severe dieting and starvation resulted in hypercortisolism and a significant decrease in CBG. The HPA axis and the renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathophysiology of obesity-induced hypertension. However, there is little data on the effect of moderate weight loss (30% caloric restriction) on adrenocortical function, and the relation of adrenal hormones to altered blood pressure with weight loss.

In this study, measures of HPA axis and RAAS and blood pressure monitoring were performed in twenty-five obese subjects before and after a 12-week diet program (6000kJ/day). Short-term, moderate weight loss (mean 8.5 kg) was associated with a small reduction in blood pressure (mean arterial pressure 6 mmHg) and significantly reduced levels of aldosterone and renin but not cortisol levels. These findings suggest that aldosterone may have an important role in the blood pressure reduction with weight loss via a renin mediated mechanism, perhaps involving renal sympathetic tone. In contrast to severe caloric restriction, HPA axis activation does not occur with moderate weight loss. This suggests a threshold

effect of weight loss on the HPA axis where greater caloric restriction is required for HPA stimulation, or a counterbalancing of central and direct adrenal effects on HPA axis function.

Overall, these three exploratory studies have provided novel data on HPA axis function in systemic infection, pregnancy and in diet-induced weight loss. Each study offers a basis for further studies of HPA axis function in these disorders.

Statement of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this copy of my thesis being made available in the University Library.

…….

Jui Ting Ho

April 2007

Acknowledgements

I dedicate this thesis to my darling husband, Aeneas. This would not be possible without your love, support and patience. Thanks also to my parents and siblings for their encouragement and understanding.

I am grateful to my supervisor, Associate Professor David Torpy for his tremendous support and enthusiasm during this research. You have challenged me in many ways to be better, both personally and professionally. Thank you for your guidance and encouragement. It has been a privilege working with you.

My sincere thanks go to the following individuals:

- Dr. Chris Bagley of the Hanson Institute and University of Adelaide for teaching me the basic skills of immunoassay techniques and computer programs of the laboratory.
- Dr, John Lewis of Canterbury Health Laboratories, Christchurch, New Zealand for sharing his free cortisol and CBG assays recipes, and his generous supply of SHBG standards and CBG monoclonal antibodies. I am also grateful for his time and effort in validating the ultrafiltration free cortisol assay (Chapter 3).
- Dr. Marianne Chapman and her research team of the Intensive Care Unit, Royal Adelaide Hospital for assistance in recruitment of volunteers in both sepsis and septic shock studies (Chapter 3 and 4).
- Professor Gus Dekker and his research midwives of the Women's and Children's Hospital for volunteer recruitment and sample collection for the pregnancy study (Chapter 5).

- Associate Professor Peter Clifton and Ms. Jennifer Keogh of CSIRO, Adelaide who performed and supervised the dietary management of the volunteers reported in Chapter 6.
- Professor Michael Horowitz of the Endocrine and Metabolic Unit, Royal Adelaide Hospital who provided helpful criticism on the septic shock and pregnancy manuscripts.
- Ms Lee Sampson of Pacific Laboratory, Queensland who performed the Renin and Aldosterone assays (Chapter 6).
- The researchers who shared the laboratory with me at the Hanson Institute, in particular, Sam, Leila and Ally, who taught me how to enjoy being a student again.

Finally, I am grateful for the financial support from Dawes' Scholarship and the University of Adelaide during my candidature; and the Royal Adelaide Hospital Clinical Research Grants for funding the studies.

Publications

Publications related to work presented in this thesis:

Ho JT, Al-Musalhi H, Chapman MJ, Quach T, Thomas PD, Bagley CJ, Lewis JG, Torpy DJ. Septic shock and sepsis: a comparison of total and free plasma cortisol levels. *Journal of Clinical Endocrinology and Metabolism* 2006 91(1):105-114

Ho JT, Lewis JG, O'Loughlin P, Bagley CJ, Romero R, Dekker GA, Torpy DJ. Reduced Maternal Corticosteroid-binding Globulin and Cortisol Levels in Pre-eclampsia and Gamete Recipient Pregnancies. *Clinical Endocrinology (Oxf)* 2007 Jun; 66(6):869-77

Ho JT, Keogh JB, Lewis JG, Clifton PM, Torpy DJ. Moderate weight loss reduces renin and aldosterone but does not influence basal or stimulated pituitary-adrenal axis function. *Hormone and Metabolic Research* 2007 (In Press)

Torpy DJ, **Ho JT**. Value of Free Cortisol Measurement in Systemic Infection. *Hormone and Metabolic Research* 2007 Jun; 39(6):439-44

Torpy DJ, **Ho JT**. Corticosteroid binding globulin gene polymorphisms: clinical implications and links to idiopathic chronic fatigue disorders. *Clinical Endocrinology (Oxf)* 2007 Aug; 67(2):161-7

Torpy DJ, **Ho JT**. Evaluation of adrenocortical function in adults. Australian Prescriber 2007

Submitted:

Ho JT, Lam S, Chapman MJ, O'Connor S, Edwards J, Ludbrook G, Lewis JG, Torpy DJ. Nitric oxide activity increases with sepsis severity but does not predict shock.

Dorin RI, Qualls CR, Pai HK, **Ho JT**, Lewis JG, Torpy DJ, Urban FK. Comparison of equilibrium estimates of free cortisol: Reversible changes in cortisol affinity for albumin in septic shock and relative adrenal insufficiency.

List of tables and figures

Tables

Figures

Abbreviations

ACTH	Adrenocorticotropic hormone
AngII	Angiotensin II
APACHE II	Acute Physiology and Chronic Health Evaluation
AVP	Arginine Vasopressin
BMI	Body mass index
BW	Body Weight
CBG	Corticosteroid-binding globulin
CNS	Central Nervous System
CRH	Corticotropin-releasing hormone
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
GC	Glucocorticoids
GH	Gestational hypertension
GR	Glucocorticoid receptor
GRE	Glucocorticoid-response element
HC	Healthy control
hGRα/ β	human Glucocorticoid receptor alpha/ bets
HPA	Hypothalamic-pituitary-Adrenal
HPLC	High performance liquid chromatography
ICU	Intensive care unit
ICV	Intracerebroventricular
IGF	Insulin like growth factor
IL	Interleukin
iNOS	inducible nitric oxide synthase
IUGR	Intrauterine growth restriction
MAP	Mean arterial pressure
MODS	Multiple organ dysfunction syndrome
MR	Mineralocorticoid receptor
MW	Molecular weight
NO	Nitric oxide

NOx	Nitrate/ nitrite
NOS	Nitric oxide synthase
POMC	Pro-opiomelanocortin
PRA	Plasma renin activity
RAAS	Renin-angiotensin-aldosterone system
RAI	Relative adrenal insufficiency
RM-ANOVA	Repeated measures analysis of variance
SEM	Standard error of the meas
SIRS	Systemic inflammatory response syndrome
S	Sepsis
SGA	Small for gestational age
SNS	Sympathetic nervous system
SS	Septic shock
Th1/Th2	T helper cell 1 and 2
TNF-α	Tumour necrosis factor–alpha
11β-HSD_1 & $_2$	11 beta hydroxysteroid dehydrogenase type 1 and 2