Role of hypothalamic pituitary adrenal axis in prenatal programming of adult disease.

Sanita Grover, BSc (Hons)

School of Paediatrics and Reproductive Health

Discipline of Obstetrics and Gynaecology

Faculty of Health Science

The University of Adelaide

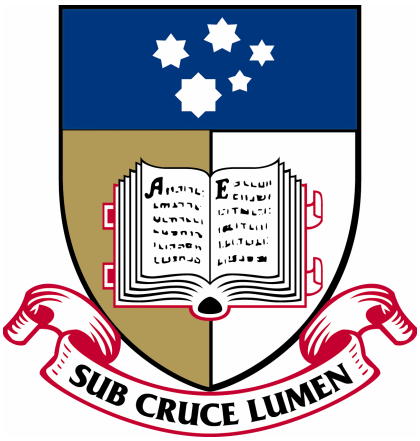
South Australia

Australia

Thesis submitted for the total fulfilment for the degree of

Doctor of Philosophy (PhD)

March 2008



To my gorgeous children Matrim and Sabriel and my husband Mark.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ACKNOWLEDGEMENTS

I would like to thank:

My supervisor, Professor Julie Anne Owens for her never ending fountain of support, knowledge and patience, for her excellent guidance and teaching, for providing me with many opportunities and assistance in attending scientific meetings, and in preparing scientific publications. I truly want to thank you for accepting me into your lab, giving me a chance as well as the opportunities that you have. I can't thank you enough.

I would also like to thank my second supervisor Dr Cathie Coulter for support, advice and endless chats.

I would like to thank Melissa Walker for assistance with post mortems, surgeries, experiments, growth measurements, mating, maintenance of animals and lab work and giving me very useful advice, Vicky Staikopoulos, Natasha Lloyd for assistance with post mortems, surgeries, experiments, growth measurements, mating, maintenance of animals and lab work, Miles DeBalsio with assistance with lab work and for giving me useful advice, Laura O'Carroll, Cathie Coulter, Karen Kind, and Pat Grant for endless advice and assistance with writing, without you all I would have never achieve all that I have.

I would like to especially thank Melissa Walker for teaching me everything there is to know about guinea pigs. Karen Kind for her help in writing thesis, abstracts and putting together talks. Thankyou so much for the endless support and patience over the years.

I would also like to thank the people who are or were part of the Research Centre for the Physiology of Early Development and also Department of Obstetrics and Gynaecology who have helped in many different ways and who were invaluable for the continuity of my research.

I would like to thank Professor Julie Anne Owens, the Department of Physiology, and the Department of Obstetrics and Gynaecology for financial assistance and the opportunity to work in their very professional establishments.

I would like to give a warm thanks to my husband Mark Grover who has put up with me throughout this PhD, you have stuck by me through all of it, thank you for all your support and always telling me to make the right decisions for me. I would like to thank my gorgeous children Matrim and Sabriel for always reminding me why I was doing this and teaching me the importance of saving my work every three minutes. I would like to also thank the rest of my family and friends for their support and interest in my work even though you had no idea what it was about, and for lifting my spirits during those crucial moments.

I believe that all of the people above have bettered me both professionally, and personally. Without you all I wouldn't be where I am today.

Thank you.

STATEMENT OF ORIGINALITY AND AUTHENTICITY

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying if accepted for the award of the degree.

Signed,

Sanita Grover,

Date:

TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

AC	Abdominal circumference
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
BW	Birth Weight
11ß HSD	11ß Hydroxy Steroid Dehydrogenase
CBG	Corticosteroid Binding Globulin
CNS	Central Nervous system
%CV	Coefficient of Variation
CRH	Corticotropin Releasing Hormone
CRL	Crown-rump length
ELISA	Enzyme-linked Immuno-Sorbent Assay
FFA	Free fatty acid
GAUC	Glucose Area Under the Curve
GLUT 1	Glucose transporter protein 1
GLUT4	Glucose transporter protein 4
GR	Glucocorticoid Receptor
HL	Head Length
HPAA	Hypothalamo-pituitary adrenal axis
HPGA	Hypothalamic Pituitary Gonadal Axis
HW	Head Width
i.m.	Intramuscular
IVGTT	Intravenous glucose tolerance test
mRNA	Messenger ribose nucleic acid

µl	Microliter
mmol	Millimolar
MR	Mineralocorticoid Receptor
NIDDM	Non-insulin dependent diabetes mellitus
Nmol	Nanomolar
PEPCK	Phosphoenolpyruvate carboxykinase
Pg	Picograms
PM	Post mortem
Pmol	pecomolar
PVN	Paraventricular Nucleolus
SEM	Standard error of the mean
SNS	Sympathetic Nervous System

ABSTRACT

Low birth weight is associated with an increased risk of impaired glucose tolerance and type 2 diabetes and with signs of increased hypothalamic pituitary adrenal axis activity in later life (1, 2). Low birth usually weight reflects a reduction in fetal growth, which largely depends on an adequate supply of nutrients and oxygen. Variations in supply modify the metabolic and neuroendocrine characteristics of the fetus, which in turn modulate the pattern of functional development as well as growth (3). An adverse fetal environment, evident as low birth weight, is therefore proposed to alter functional development with long term effects for the function and risk of disease in the individual later in life (4, 5). Increased HPAA impairs metabolic homeostasis and could therefore mediate effect of prenatal challenge on later metabolic control (6). It was therefore hypothesised that restriction of fetal growth, increases circulating cortisol and/or alters sensitivity to cortisol, which increases fasting blood glucose, and impairs glucose tolerance in the young adult. Large litter size in the guinea pig is characterised by reduced placental and fetal growth, reduced size at birth and insulin resistance in offspring in later life, providing a suitable model to test this hypothesis.

Spontaneous restriction of fetal growth in the guinea pig, evident as small size at birth, was associated with increased salivary cortisol, in both sexes but at different stages of postnatal life. In males, salivary cortisol was increased with small size at birth in early and adult life, but reduced later with ageing. In females however, salivary cortisol was increased in juveniles and in aged adults, possibly reflecting the impact of the oestrus cycle on cortisol production in mature cycling females. Altered activity of the HPGA, which can influence that of the HPAA, has also been reported to be programmed by prenatal restriction. In the guinea pig, salivary testosterone in males increased with age and small size at birth in juveniles, young and aged adults. In females, salivary progesterone increased with age up to 300 days, and decreased with size at birth in the young guinea pig. Although testosterone inhibits HPAA activity, in

males, mean salivary cortisol correlated positively with mean salivary testosterone at 100 and 300 days of age. In contrast, progesterone may enhance HPAA activity, and consistent with this, in females, mean salivary progesterone correlated with mean salivary cortisol at 400 days of age. Therefore, salivary testosterone in the male and salivary progesterone in the female guinea pig changes with maturation and has previously reported in this or other species, but small size at birth increases salivary testosterone in males with modest effects in early life in females. This together with the unexpected positive associations of salivary cortisol with testosterone in males, suggests that programming of the HPAA makes little contribution to that of the HPAA as indicated by salivary cortisol.

Here we show that low birth weight is associated with increased fasting blood glucose and impaired glucose tolerance in both male and female young adult guinea pigs aged 100 days. Fasting and mean (during IVGTT) plasma cortisol was reduced in low birth weight female adult guinea pigs, and is not vary with size at birth at this age in males. This suggests that circulating cortisol does not contribute to the impaired glycaemia associated with small size at birth in the guinea pig. Glucose tolerance was increasingly impaired in males but not females, as mean plasma cortisol increased. This is consistent with cortisol impairing glycaemia in the guinea pig as in other species, in males at least.

To assess the role of cortisol in prentally programmed impairment of glycaemia directly, metyrapone or vehicle containing 24% ethanol was administered to young adult guinea pigs for 3 days. Treatment with the latter impaired fasting blood glucose and glucose tolerance in females and the latter in males compared to a previous IVGTT and this was exacerbated in low birth weight females. Metyrapone prevented this impairment of fasting glycaemia and glucose tolerance in the low birth weight adult female guinea pig and in the male guinea pig regardless of birth weight class. Neither vehicle or metyrapone altered plasma cortisol, before or during a second IVGTT. Limited numbers of animals, particularly females, limited this study however and additional investigation is required. Nevertheless

this shows for the first time that inhibition of glucocorticoid synthesis in the guinea pig improves glucose control. Furthermore this suggests that the low birth weight guinea pig may be more sensitive to cortisol, have increased cortisol synthesis or reduced inactivation of cortisol in peripheral tissues, leading to increased local cortisol action.

In conclusion, alterations in peripheral HPAA activity in the guinea pig due to restricted fetal growth may contribute to their prenatally programmed development of impaired glucose tolerance as young adults, but the extent of that contribution may vary with age and gender.