

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

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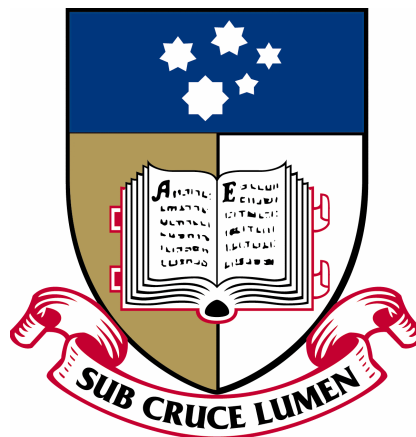
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To my gorgeous children Matrim and Sabriel and my husband Mark.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES	X
ACKNOWLEDGEMENTS	xvii
STATEMENT OF ORIGINALITY AND AUTHENTICITY	xix
TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES	xx
ABSTRACT	xxii
Chapter 1.....	1
Literature Review	1
1.1 Introduction	2
1.2 Prenatal environment and fetal growth	4
1.3 Glycaemia and glucose tolerance	5
1.3.1 <i>Insulin</i>	6
1.3.1.1 Synthesis	6
1.3.1.2 Secretion	7
1.3.2 Glucose uptake	7
1.4 Type 2 diabetes mellitus	9
1.5 Type 2 diabetes and early life influences	10
1.5.1 Impact of birth weight and fetal growth	11
1.5.2 Animal studies – impact of birth weight / fetal growth	11
1.5.2.1 Rat	11
1.5.2.2 Pig	12
1.5.2.3 Sheep	13
1.5.2.4 Guinea pig	13
1.6 Hypothalamic Pituitary Adrenal Axis (HPAA)	14
1.6.1 HPAA and its elements	14

1.6.2	Glucocorticoid action	16
1.6.2.1	Glucocorticoid action on pancreas.....	19
1.6.2.2	Glucocorticoid actions on skeletal muscle	19
1.6.2.3	Glucocorticoid action on liver.....	20
1.6.2.4	Glucocorticoid action on adipose tissue	20
1.7	HPAA and early life influences.....	21
1.8	HPAA in humans.....	22
1.9	HPAA in non human species	23
1.9.1	Rat.....	25
1.9.2	Sheep.....	25
1.9.3	Pig.....	25
1.9.4	Guinea pig.....	26
1.10	Prenatal programming of Hypothalamic Pituitary Gonadal axis (HPGA).....	27
1.11	Role of HPAA in prenatal programming for T2DM	28
	General hypothesis.....	29
	Specific hypothesis	29
	Hypothesis 1	29
	Hypothesis 2	29
	Hypothesis 3	29
	Hypothesis 4	29
	Specific Aims	29
	Aim 1	29
	Aim 2	30
	Aim 3	30
	Aim 4	30

Significance.....	30
Chapter 2.....	31
Materials and Methods.....	31
2.1 Mating and housing.....	32
2.2 Saliva collection	39
2.3 Insertion of indwelling vascular catheters	45
2.4 Intravenous glucose tolerance test (IVGTT)	45
2.5 Treatment with metyrapone	46
2.6 Postmortem	47
2.7 Blood, plasma and saliva hormone and metabolite assays.....	47
2.7.1 Cortisol	47
2.7.1.1 Validation of salivary cortisol radioimmunoassay	47
2.7.1.2 Plasma cortisol	48
2.7.2 Blood glucose	49
2.7.3 Plasma Free Fatty Acids.....	49
2.7.4 Salivary testosterone	50
2.7.5 Salivary progesterone.....	51
2.8 Statistical Analysis	52
Chapter 3.....	54
Effect of fetal growth restriction on salivary cortisol in the guinea pig throughout postnatal life.	54
3.1 INTRODUCTION	55
3.2 MATERIALS AND METHODS	59
3.2.1 Animals.....	59
3.2.2 Experiments in offspring:	59
3.2.3 Analysis of Salivary Cortisol:	60

3.2.4	Calculations:.....	60
3.2.5	Statistical Analysis:.....	60
3.3	RESULTS	62
3.3.1	Effects of sex, age and time of day on salivary cortisol concentration.....	62
3.3.2	Effect of size at birth tertile on salivary cortisol concentration.....	63
3.3.3	Effects of birth parameters on salivary cortisol concentration.....	64
3.4	DISCUSSION.....	65
Chapter 4.....		88
Relationship of salivary progesterone, testosterone and cortisol and size at birth in the guinea pig throughout postnatal life.....		88
4.1	INTRODUCTION	89
4.2	MATERIALS AND METHODS	92
4.2.1	Animals.....	92
4.2.2	Saliva collection.....	92
4.2.3	Hormone analysis.....	93
4.2.3.1	Salivary Cortisol.....	93
4.2.3.2	Progesterone.....	93
4.2.3.3	Testosterone	93
4.2.4	Statistical Analysis.....	93
4.3	RESULTS	94
4.3.1	Effect of age and birth parameters on salivary testosterone in males:.....	94
4.3.2.	Effect of age and birth parameters on salivary progesterone in females	94
4.3.3	Effect of cortisol on salivary progesterone and testosterone	94
4.4	DISCUSSION.....	96
Chapter 5.....		103

Effect of size at birth, sex and circulating cortisol on glucose homeostasis in the young adult guinea pig.		103
5.1	Introduction	104
5.2	MATERIALS AND METHODS	109
5.2.1	Animals	109
5.2.2	Surgery	109
5.2.3	Intravenous Glucose Tolerance Test (IVGTT)	109
5.2.4	Hormone and metabolite assays	110
5.2.4.1	Plasma cortisol	110
5.2.4.2	Blood Glucose	110
5.2.4.3	Free Fatty Acids	110
5.2.5	Post Mortems	111
5.2.6	Statistical Analysis	111
5.3	RESULTS	112
5.3.1	Size at Birth Tertile in the Guinea Pig	112
5.3.2	Blood Glucose and Glucose Tolerance and Size at Birth	112
5.3.3	Plasma Cortisol	113
5.3.4	Plasma cortisol and blood glucose	114
5.3.5	Plasma cortisol and impact on associations of glucose homeostasis with size at birth.	115
5.3.6	Plasma free fatty acids (FFA)	116
5.3.7	Plasma cortisol and plasma free fatty acids (FFA)	117
5.3.8	Plasma cortisol and impact on associations of plasma free fatty acids (FFA) with size at birth.	118

5.3.9	Relationship between plasma free fatty acids (FFA), fasting blood glucose and glucose tolerance.....	119
5.3.10	Body composition	119
5.3.10.1	Muscles	119
5.3.10.2	Fat	119
5.4	DISCUSSION.....	120
Chapter 6.....		150
Effect of metyrapone on glucose tolerance and body composition in the young adult guinea pig of varying birth weight.....		150
6.1	INTRODUCTION	151
6.2	MATERIALS AND METHODS	153
6.2.1	Animals.....	153
6.2.2	Surgery.....	153
6.2.3	Treatment	153
6.2.4	IVGTT	153
6.2.5	Hormone and metabolite assays	154
6.2.5.1	Plasma cortisol	154
6.2.5.2	Blood Glucose	154
6.2.5.3	Free Fatty Acids	154
6.2.6	Post Mortems	155
6.2.7	Statistical Analysis.....	155
6.3	RESULTS	156
6.3.1	Birth weight class and size at birth in the guinea pig.	156
6.3.2	Effect of birth weight class and gender on blood glucose in the young adult guinea pig.	156

6.3.3	Effect of metyrapone, sex and size at birth on fasting glycaemia and glucose tolerance.	157
6.3.4	Association between circulating cortisol and size at birth	160
6.3.5	Fasting blood glucose, glucose tolerance (GAUC) and cortisol	161
6.3.6	Free fatty acids (FFA).....	162
6.3.7	Body composition	163
6.3.7.1	Muscles:	163
6.3.7.2	Fats	164
6.3.7.3	Organs:.....	165
6.4	DISCUSSION.....	166
Chapter 7	209
General Discussion	209
Chapter 8	xxvi
References	xxvi

LIST OF TABLES AND FIGURES

<i>Table 1.1 Disorders associated with low birth weight in humans.</i>	2
<i>Figure 1.1 Insulin action on glucose fluxes</i>	9
<i>Figure 1.2 HPAA</i>	15
<i>Figure 1.4 Synthesis of cortisol</i>	16
<i>Table 1.2 Physiological processes targeted by glucocorticoids</i>	17
<i>Figure 1.4 Tissue targets of glucocorticoids and the key outcomes</i>	18
<i>Table 1.3 Effect of low birth weight on HPAA</i>	23
<i>Table 1.4 Prenatal and early postnatal perturbation and HPAA outcome</i>	24
<i>Table 2.1 Cohorts of guinea pigs studied</i>	33
<i>Table 2.2 Pup morphometry</i>	34
<i>Figure 2.1 Morphometry of the guinea pig</i>	35
<i>Figure 2.2 Study Design: Effect of fetal growth restriction on salivary cortisol from birth to aged adulthood in the guinea pig (Chapter 3)</i>	36
<i>Table 2.3 Study of effect of fetal growth restriction on salivary cortisol from birth to aged adulthood in the guinea pig (Chapter 3)</i>	37
<i>Figure 2.3 Study design: Relationships of salivary progesterone, testosterone and cortisol to size at birth in the guinea pig (Chapter 4)</i>	40
<i>Table 2.4 Study of relationships of salivary progesterone and cortisol and size at birth in the guinea pigs (Chapter 4)</i>	41
<i>Table 2.5 Study of relationships of salivary testosterone and cortisol and size at birth in the guinea pigs (Chapter 4)</i>	42
<i>Figure 2.4 Study design: Effect of size at birth, sex and circulating cortisol on glucose and lipid homeostasis and body composition in the young adult guinea pig (Chapter 5 and 6)</i>	43

Table 2.6	<i>Study of effect of size at birth, sex and circulating cortisol on glucose and lipid homeostasis and body composition in the young adult (Chapter 5 and 6).....</i>	44
Figure 2.5	<i>Calculation of the glucose area under the curve during intravenous glucose tolerance test (IVGTT) in the guinea pig</i>	46
Figure 2.6	<i>Testosterone validation curve.....</i>	51
Figure 2.7	<i>Progesterone validation curve.....</i>	52
Table 3.1	<i>Size at birth of guinea pigs studied as adults.....</i>	71
Table 3.3	<i>Associations between size at birth and salivary cortisol in the male guinea pig during postnatal life.</i>	74
Table 3.4	<i>Associations between size at birth and salivary cortisol in the female guinea pig during postnatal life.</i>	76
Figure 3.1	<i>Effect of sex on salivary cortisol in male and female guinea pigs.....</i>	78
Figure 3.2	<i>Mean salivary cortisol at each age in male and female guinea pigs throughout postnatal life.....</i>	79
Figure 3.3	<i>Minimum salivary cortisol at each age in male and female guinea pigs throughout postnatal life.....</i>	80
Figure 3.4	<i>Maximum salivary cortisol at each age in male and female guinea pigs throughout postnatal life.....</i>	81
Figure 3.5	<i>Overall Salivary cortisol throughout the day in the guinea pig.....</i>	82
Figure 3.6	<i>Salivary cortisol throughout the day in the male and female guinea pig.....</i>	83
Figure 3.7	<i>Salivary cortisol concentrations throughout the day in male and female guinea pigs between 10 and 60 days of age.</i>	84
Figure 3.8	<i>Salivary cortisol concentrations throughout the day in male and female guinea pigs between 90 and 120 days of age.</i>	85

<i>Figure 3.9 Salivary cortisol concentrations throughout the day in male and female guinea pigs between 180 and 400 days of age.</i>	<i>86</i>
<i>Figure 3.10 Effects of birth weight, sex and age tertile and gender on mean salivary cortisol concentration in the guinea pig.....</i>	<i>87</i>
<i>Figure 4.1 Mean salivary testosterone in the male guinea pigs.</i>	<i>98</i>
<i>Figure 4.2 Associations between mean salivary testosterone and birth weight in the male guinea pigs.....</i>	<i>99</i>
<i>Figure 4.3 Mean salivary progesterone in the female guinea pigs.....</i>	<i>100</i>
<i>Figure 4.4 Associations between mean salivary cortisol and mean salivary testosterone in the male guinea pig.....</i>	<i>101</i>
<i>Figure 4.5 Associations between mean salivary cortisol and mean salivary progesterone in the female aged guinea pig.</i>	<i>102</i>
<i>Figure 5.1 Effect of birth weight tertile on size at birth in the guinea pig.....</i>	<i>126</i>
<i>Figure 5.2 Blood glucose during IVGTT in the male and female adult guinea pigs.....</i>	<i>127</i>
<i>Figure 5.3 Effect of birth weight tertile on blood glucose response to intravenous glucose test in the adult guinea pig.....</i>	<i>128</i>
<i>Figure 5.4 Effect of birth weight tertile on blood glucose response to intravenous glucose tolerance test in male and female adult guinea pigs.....</i>	<i>129</i>
<i>Figure 5.5 Effect of birth weight tertile on glucose homeostasis in the adult guinea pig.....</i>	<i>130</i>
<i>Figure 5.6 Effect of birth weight tertile and sex on glucose homeostasis in male and female adult guinea pig.</i>	<i>131</i>
<i>Figure 5.7 Associations between glucose homeostasis and size at birth in the adult guinea pig.</i>	<i>132</i>
<i>Figure 5.8 Plasma cortisol before and throughout the IVGTT in the male and female guinea pigs.</i>	<i>133</i>

<i>Figure 5.9 Plasma cortisol before and throughout the IVGTT separated by low, medium and high birth weight tertiles</i>	<i>134</i>
<i>Figure 5.10 Effect of birth weight tertile on plasma cortisol before and throughout the IVGTT in male and female guinea pigs.</i>	<i>135</i>
<i>Figure 5.11 Effect of birth weight tertile and sex on plasma cortisol in male and female guinea pigs.</i>	<i>136</i>
<i>Table 5.1 Associations between cortisol concentrations and size at birth in the young adult guinea pig.</i>	<i>137</i>
<i>Figure 5.12 Associations between glucose homeostasis and cortisol in the adult guinea pig. .</i>	<i>138</i>
<i>Table 5.2 Effect of adjusting for circulating cortisol on associations of glycaemia with size at birth in the adult guinea pig.</i>	<i>139</i>
<i>Table 5.3 Effect of adjusting for circulating cortisol on associations of glycaemia with size at birth in the adult male guinea pig.</i>	<i>140</i>
<i>Table 5.4 Effect of adjusting for circulating cortisol on associations of glycaemia with size at birth in the adult female guinea pig.</i>	<i>141</i>
<i>Figure 5.13 Effect of birth weight tertile and plasma cortisol on GAUC in the adult guinea pig.</i>	<i>142</i>
<i>Figure 5.14 Effect of birth weight tertile and plasma cortisol on GAUC in the adult female and male guinea pig.</i>	<i>143</i>
<i>Figure 5.15 Plasma FFA before and throughout the IVGTT</i>	<i>144</i>
<i>Figure 5.16 Plasma FFA during the first 20 minutes of an IVGTT in male and female guinea pigs.</i>	<i>145</i>
<i>Figure 5.17 Effect of birth weight tertile and sex on FFA homeostasis in the young adult guinea pig.</i>	<i>146</i>
<i>Table 5.5 Effect of adjusting for circulating cortisol on associations of FFA with size at birth in the adult guinea pig</i>	<i>147</i>

Table 5.6	<i>Effect of adjusting for circulating cortisol on associations of FFA with size at birth in the adult male guinea pig.....</i>	148
Table 5.7	<i>Effect of adjusting for circulating cortisol on associations of FFA with size at birth in the adult female guinea pig.....</i>	149
Table 6.1	<i>Size at birth in vehicle and metyrapone treated animals of low and high birth weight guinea pigs.....</i>	171
Figure 6.1	<i>Effect of birth weight class on blood glucose throughout 1st IVGTT in male and female guinea pigs.</i>	172
Figure 6.2	<i>Fasting blood glucose during the 1st IVGTT in male and female guinea pigs of high and low birth weight class.</i>	173
Figure 6.3	<i>Maximum blood glucose during the 1st IVGTT in male and female guinea pigs of high and low birth weight class.</i>	174
Figure 6.4	<i>GAUC during the 1st IVGTT in male and female guinea pigs of high and low birth weight class.</i>	175
Figure 6.5	<i>Effect of repeated IVGTT, treatment and birth weight class on fasting blood glucose in the adult guinea pig.....</i>	176
Figure 6.6	<i>Effect of repeated IVGTT, treatment and birth weight class on fasting blood glucose in the young adult male guinea pig.....</i>	177
Figure 6.7	<i>Effect of repeated IVGTT, treatment and birth weight class on fasting blood glucose in young adult female guinea pig.....</i>	178
Figure 6.8	<i>Effect of repeated IVGTT, treatment and birth weight class on maximum blood glucose in the young adult guinea pigs.....</i>	179
Figure 6.9	<i>Effect of treatment on blood glucose before and during IVGTT in the young adult guinea pigs.....</i>	180

<i>Figure 6.10 Effect of treatment on blood glucose before and during IVGTT in the young adult male guinea pig.....</i>	<i>181</i>
<i>Figure 6.11 Effect of treatment on blood glucose before and during IVGTT in the young adult female guinea pig</i>	<i>182</i>
<i>Figure 6.12 Association between indices of glucose homeostasis and size at birth in the young adult guinea pig</i>	<i>183</i>
<i>Figure 6.13 Association between indices of glucose homeostasis and size at birth in the young adult male guinea pig</i>	<i>185</i>
<i>Figure 6.14 Association between indices of glucose homeostasis and size at birth in the young adult female guinea pig.....</i>	<i>187</i>
<i>Figure 6.15 Plasma cortisol during 1st IVGTT.....</i>	<i>189</i>
<i>Figure 6.16 Effect of birth weight class, treatment and test on fasting plasma cortisol in the young adult guinea pig.....</i>	<i>190</i>
<i>Figure 6.17 Effect of birth weight class, treatment and test on mean plasma cortisol in the young adult guinea pig</i>	<i>191</i>
<i>Figure 6.18 Effect of treatment on plasma cortisol during IVGTT in low and high birth weight class adult guinea pig</i>	<i>192</i>
<i>Figure 6.19 Association between cortisol homeostasis and size at birth in the young adult guinea pig.....</i>	<i>193</i>
<i>Figure 6.20 Association between cortisol homeostasis and size at birth in the young adult male guinea pig.....</i>	<i>195</i>
<i>Figures 6.21 Association between cortisol homeostasis and size at birth in the young adult female guinea pig</i>	<i>197</i>
<i>Table 6.2 Effects of treatment and birth weight lass on skeletal muscle mass in the young adult guinea pig</i>	<i>203</i>

Table 6.3 Effect of treatment and birth weight class on adiposity in the young adult guinea pig.
..... 205

Table 6.4 Effect of treatment and birth weight class on organ size in the young adult guinea pig 207

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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	Adrenocorticotrophic Hormone
ANOVA	Analysis of Variance
BW	Birth Weight
11 β HSD	11 β 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

TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

μ l	Microliter
mmol	Millimolar
MR	Mineralocorticoid Receptor
NIDDM	Non-insulin dependent diabetes mellitus
Nmol	Nanomolar
PEPCK	Phosphoenolpyruvate carboxykinase
Pg	Picograms
PM	Post mortem
Pmol	picomolar
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 prenatally 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.