**The long-term effects of being born prematurely and of antenatal steroid administration on subsequent growth, blood pressure, insulin resistance and lipids in later life.**

Thesis submitted for the degree of Doctor of Medicine

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[List of Tables 4](#_Toc161096831)

[List of Figures 6](#_Toc161096832)

[List of Abbreviations 9](#_Toc161096833)

[Summary 10](#_Toc161096834)

[Chapter 1: Introduction 12](#_Toc161096835)

[Chapter 2: The Programming Hypothesis - Birth Weight, Growth and Adult disease 16](#_Toc161096836)

[2.1 Birth weight and coronary heart disease 17](#_Toc161096837)

[2.2 Birthweight and other diseases 17](#_Toc161096838)

[2.3 Birth weight and fetal under nutrition in utero 18](#_Toc161096839)

[2.4 Catch-up growth 19](#_Toc161096840)

[2.5 Insulin Resistance 20](#_Toc161096841)

[2.6 Hypotheses to explain programming 21](#_Toc161096842)

[2.7 Inheritance of low birth weight 23](#_Toc161096843)

[2.8 Mechanisms of programming. 25](#_Toc161096844)

[2.8.1 IGF-1 and programming 25](#_Toc161096845)

[2.8.2 Hypothalamo-pituitary-adrenal axis and programming 27](#_Toc161096846)

[2.8.3 11β hydroxysteroid dehydrogenase activity 29](#_Toc161096847)

[2.8.4 Leptin 32](#_Toc161096848)

[2.9 Antenatal and postnatal steroids 34](#_Toc161096849)

[2.10 The Situation in preterm infants 37](#_Toc161096850)

[2.11 Measuring Growth 42](#_Toc161096851)

[2.12 Rationale for Study 44](#_Toc161096852)

[Chapter 3: Study Population and Methodology 45](#_Toc161096853)

[3.1 Aims 45](#_Toc161096854)

[3.2 Study Population 45](#_Toc161096855)

[3.3 Study Protocol 49](#_Toc161096856)

[3.3.1 Clinical measurement 55](#_Toc161096857)

[3.3.2 Insulin Resistance and Atherogenic Index 56](#_Toc161096858)

[3.4 Statistical Methods 58](#_Toc161096859)

[Chapter 4 : Size at Birth, Size age 5 years and Patterns of Growth since Birth. 60](#_Toc161096860)

[4.1 Demographic data 60](#_Toc161096861)

[4.2 Size at birth 63](#_Toc161096862)

[4.3 Size age 5 years 67](#_Toc161096871)

[4.4 Longitudinal Growth 69](#_Toc161096876)

[Chapter 5: Prematurity, Antenatal Events and Blood Pressure, Glucose Homeostasis and Lipids aged 5 years. 77](#_Toc161096877)

[5.1 Blood Pressure – relationship with current size, prematurity and antenatal intervention 77](#_Toc161096878)

[5.2 Glucose metabolism 85](#_Toc161096883)

[5.3 Lipids 95](#_Toc161096888)

[Chapter 6: IGF-1, Cortisol and Leptin 100](#_Toc161096893)

[Chapter 7: Urinary steroids 108](#_Toc161096898)

[7.1 Urinary Steroids and Blood Pressure 108](#_Toc161096899)

[7.2 Urinary Steroids and Glucose 111](#_Toc161096900)

[7.3 Effect of Antenatal Steroids 112](#_Toc161096901)

[7.4 Infants born less than 34 weeks gestation 114](#_Toc161096902)

[Chapter 8. Catch-up growth 121](#_Toc161096915)

[Chapter 9. Discussion 127](#_Toc161096916)

[9.1 Early growth failure 127](#_Toc161096917)

[9.2 Prematurity, Blood Pressure and Insulin Resistance 128](#_Toc161096918)

[9.3 Catch-up Growth 132](#_Toc161096919)

[9.4 Prematurity and Lipids 135](#_Toc161096920)

[9.5 Multiple Pregnancy 136](#_Toc161096921)

[9.5 Pregnancy Induced Hypertension 136](#_Toc161096922)

[9.6 Mechanisms of programming 137](#_Toc161096923)

[9.6.1 Prematurity and IGF-1 137](#_Toc161096924)

[9.6.2 Prematurity and Cortisol metabolism 139](#_Toc161096925)

[9.6.3 Prematurity, Antenatal steroids and 11βHSD 143](#_Toc161096926)

[9.6.4 Prematurity and Leptin 145](#_Toc161096927)

[9.6.5 Interaction between mechanisms 145](#_Toc161096928)

[9.7 Prematurity and Adrenarche 146](#_Toc161096929)

[9.8 The Fetal Adrenal 147](#_Toc161096930)

[Chapter 10. Conclusions 149](#_Toc161096931)

[10.1 Future study 151](#_Toc161096932)

[Acknowledgements and Thanks. 152](#_Toc161096933)

[Appendix 153](#_Toc161096934)

[References 170](#_Toc161096935)

List of Tables

[Table 1. Baseline data regarding sex, multiple pregnancies, administration of antenatal & postnatal steroids, and family history of diabetes and hypertension for the cohort presented according to gestational age at birth. 62](#_Toc133587015)

[Table 2. Mean gestational age for each group together with size at birth expressed as a standard deviation score (SDS) along with ponderal index, BMI and percentage body fat at birth. 65](#_Toc133587016)

[Table 3. Mean weight, length and head circumference standard deviation scores (SDS) at birth recalculated having excluded all multiple births from the cohort. 66](#_Toc133587017)

[Table 4. Height and weight aged 5 years compared to mid-parental centile together with BMI and measures of fat mass calculated from skin folds. 68](#_Toc133587018)

[Table 5. The relationship between current height, weight, BMI and adiposity and blood pressure & resting heart rate. (Pearson correlation) 81](#_Toc133587019)

[Table 6. Blood pressure and resting heart rate age 5 years and it’s relationship to gestational age at birth 82](#_Toc133587020)

[Table 7. Correlation of blood pressure and heart rate age 5 years with size at birth, antenatal and postnatal steroid administration, maternal and family history of cardiovascular risk factors and CRIB score. (Uncorrected for gestational age at birth) 84](#_Toc133587021)

[Table 8. Size and adiposity aged 5 years and the relationship with measures of glucose metabolism 87](#_Toc133587022)

[Table 9. The effect of Gestation, size at birth, use of perinatal steroids on measures of glucose metabolism aged 5 years. 88](#_Toc133587023)

[Table 10. Gestational age at birth and it’s relationship with measures of glucose metabolism aged 5 years. 89](#_Toc133587024)

[Table 11. Relationship between current height, weight and adiposity and lipids age 5 years 96](#_Toc133587025)

[Table 12. Correlation of lipid profile gestational age, size at birth, antenatal and postnatal steroid administration, maternal eclampsia and CRIB score. 97](#_Toc133587026)

[Table 13. Relationship between gestational age at birth and lipid profile age 5 years 98](#_Toc133587027)

[Table 14. The effect of being born prematurely on levels of Cortisol, IGF-1 and Leptin at 5 years of age 102](#_Toc133587028)

[Table 15. Relationship between Cortisol, IGF-1, Leptin and blood pressure and resting heart rate. 105](#_Toc133587029)

[Table 16. The relationship between glucose metabolism, lipids and IGF-1, Cortisol and Leptin. 107](#_Toc133587030)

[Table 17. The relationship between urinary androgen metabolites, cortisol metabolites, urinary measures of cortisol:cortisone metabolism and blood pressure, insulin resistance and lipids. 110](#_Toc133587031)

[Table 18. The relationship between the administration of antenatal steroids and subsequent urinary androgen metabilites, cortisol metabolites and urinary measures of cortisol:cortisone metabolism. 113](#_Toc133587032)

[Table 19. Characteristics of all children born before 34 weeks gestation at the age of 5 years. 115](#_Toc133587033)

[Table 20. Characteristics singleton children born before 34 weeks gestation at the age of 5 years. 116](#_Toc133587034)

[Table 21. Relationship between between antenatal steroid administration and measures of blood pressure, glucose and lipid metabolism in all children born below 34 weeks gestation at 5 years of age 118](#_Toc133587035)

[Table 22. Effect of antenatal steroid administration on urinary androgen and cortisol:cortisone metabolites in children born < 34 weeks gestation. 120](#_Toc133587036)

[Table 23. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to blood pressure and heart rate. 124](#_Toc133587037)

[Table 24. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to measures of glucose metabolism. 124](#_Toc133587038)

[Table 25. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to lipid metabolism. 125](#_Toc133587039)

List of Figures

[Figure 1. Metabolism of active Cortisol and inactive Cortisone by 11β hydroxysteroid dehydrogenase and the A-ring reductase enzymes 5α reductase and 5β reductase. 30](#_Toc161097142)

[Figure 2: Flow diagram of recruitment to the study 48](#_Toc161097143)

[Figure 3: The CRIB score is composed of the following components: 54](#_Toc161097144)

[Figure 4. Change in mean Weight standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born. 70](#_Toc161097145)

[Figure 5. Change in mean Length and after 2 years mean Height standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born. 71](#_Toc161097146)

[Figure 6. Change in mean Head Circumference standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born. 72](#_Toc161097147)

[Figure 7. Change in mean Weight standard deviation score over the first 8 weeks of life according to gestational age at birth. 73](#_Toc161097148)

[Figure 8. Change in mean Length standard deviation score over the first 8 weeks of life according to gestational age at birth. 74](#_Toc161097149)

[Figure 9. Change in mean Head Circumference standard deviation score over the first 8 weeks of life according to gestational age at birth. 75](#_Toc161097150)

[Figure 10. Systolic blood pressure aged 5 years with correlated with gestational age at birth. (Pearson correlation coefficient). 83](#_Toc161097151)

[Figure 11. Fasting insulin:glucose ratio grouped by gestational age at birth 90](#_Toc161097152)

[Figure 12. Scatter plot showing fasting glucose (mmol/l) grouped by gestational age at birth 91](#_Toc161097153)

[Figure 13. Glucose (mmol/l) at 2 hours following an oral glucose tolerance test ratio grouped by gestational age at birth 92](#_Toc161097154)

[Figure 14. Cholesterol (mmol/l) plotted against gestational age at birth. 99](#_Toc161097155)

[Figure 15. The relationship between fasting Cortisol and Systolic blood pressure 103](#_Toc161097156)

[Figure 16. The relationship between IGF-1 and Systolic blood pressure 104](#_Toc161097157)

[Figure 17. Weight standard deviation score at birth plotted against gestational age at birth in weeks. 154](#_Toc161097158)

[Figure 18. Weight standard deviation score at 1 year of age plotted against gestational age at birth in weeks. 155](#_Toc161097159)

[Figure 19. Weight standard deviation score at 5 years of age plotted against gestational age at birth in weeks. 156](#_Toc161097160)

[Figure 20. Height standard deviation score at birth plotted against gestational age at birth in weeks. 157](#_Toc161097161)

[Figure 21. Height standard deviation score at 1 year plotted against gestational age at birth in weeks. 158](#_Toc161097162)

[Figure 22. Height standard deviation score at 5 years of age plotted against gestational age at birth in weeks. 159](#_Toc161097163)

[Figure 23. Head circumference standard deviation score at birth plotted against gestational age at birth in weeks. 160](#_Toc161097164)

[Figure 24. Head circumference standard deviation score at 5 years plotted against gestational age at birth in weeks. 161](#_Toc161097165)

[Figure 25. Birth Weight standard deviation score grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 162](#_Toc161097166)

[Figure 26. Weight standard deviation score at 1 year grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 163](#_Toc161097167)

[Figure 27. Weight standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 164](#_Toc161097168)

[Figure 28. Length standard deviation score at birth grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 165](#_Toc161097169)

[Figure 29. Length standard deviation score at 1 year of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 166](#_Toc161097170)

[Figure 30. Height standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 167](#_Toc161097171)

[Figure 31. Head circumference standard deviation score at birth grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 168](#_Toc161097172)

[Figure 32. Head circumference standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group. 169](#_Toc161097173)

List of Abbreviations

|  |  |
| --- | --- |
| 11βHSD | 11β hydroxysteroid dehydrogenase |
| 11βHSD1 | 11β hydroxysteroid dehydrogenase type 1 |
| 11βHSD2 | 11β hydroxysteroid dehydrogenase type 2 |
| ACTH | adrenal corticotrophin releasing hormone |
| ALSPAC | Avon longitudinal study of parents and children |
| aTHF | allo-tetrahydrocortisol |
| BMI | body mass index |
| CRIB | clinical risk index for babies |
| DHEA | dihydroepiandrosterone |
| DHEAS | dihydroepiandrosterone sulphate |
| GH | growth hormone |
| GH-IGF-1 axis | growth hormone - insulin-like growth factor 1 axis |
| HbA1C | glycosylated haemoglobin |
| HDL | high density lipoprotein |
| HOMA | Homeostasis model assessment |
| HPA axis | hypothalamo-pituitary-adrenal axis |
| IGF-1 | insulin-like growth factor 1 |
| IUGR | intrauterine growth retardation |
| LDL | low density lipoprotein |
| MODY | maturity onset diabetes of the young |
| mRNA | messenger ribonucleic acid |
| OGTT | oral glucose tolernace test |
| PIH | pregnancy induced hypertension |
| SDS | standard deviation score |
| SGA | small for gestational age |
| THE | tetrahydrocortisone |
| THF | tetrahydrocortisol |
| THF+aTHF:THE ratio | an indirect measure of overall 11β hydroxysteroid dehydrogenase activity |

Summary

Many studies have demonstrated the link between small size at birth and the risk of hypertension, insulin resistance and hyperlipidaemia in later life. The process whereby events in fetal or early life “program” for the “metabolic syndrome” or “syndrome X” is widely accepted. However few studies have looked at the long-term effects of prematurity. Preterm infants represent the extremes of low birth weight. They are frequently exposed to steroids (which in animal models cause hypertension and diabetes) as they are given routinely in threatened preterm labour to mature the fetal lungs. The aim of this study was to investigate whether there were any effects on blood pressure and glucose metabolism as a consequence of being born prematurely and whether antenatal steroids have any adverse long-term effects.

We studied a group of 158 children born prematurely who, together with a group of matched term controls, were recruited at birth into a longitudinal growth study. The cohort where evaluated aged 5-6 years. In addition to blood pressure and measures of insulin resistance we also studied IGF-1 and the enzyme 11β hydroxysteroid dehydrogenase (11βHSD) which have been proposed as candidate mechanisms whereby programming occurs.

Children born prematurely had higher blood pressure, evidence of insulin resistance and severe early growth failure followed by prolonged catch-up growth. Those born most prematurely had the highest blood pressure, greatest insulin resistance and most marked growth failure. Antenatal steroids had an adverse effect on blood pressure and insulin resistance. Both IGF-1 and 11βHSD appear to be potential mechanisms for programming. We found clear evidence of altered 11βHSD activity in particular (in urinary steroid profiles) as a consequence of antenatal steroids.

Prematurity predisposes to hypertension and insulin resistance. Antenatal steroids exacerbate the effect. Early growth failure, IGF-1 and 11βHSD appear to be potential mechanisms by which the effects are mediated.

Chapter 1: Introduction

Over the last decade an increasingly large number of studies have reported on the link between small size at birth and an increased risk of disease in adult life. Low birth weight has been associated with a wide range of subsequent illness including heart disease (Barker *et al.* 1989), stroke (Martyn *et al.* 1995), diabetes (Hales *et al.* 1991), hypertension (Barker *et al.* 1990) and polycystic ovaries (Ibanez *et al.* 1998). The concept that events in fetal and early life can permanently alter the structure or function of an individual's homeostasis and predispose, or program, susceptibility to disease in adult life is now widely accepted. Programming is said to occur when an insult or stimulus applied at a critical or sensitive period in development, causes a long-lasting or lifelong effect on the structure or function of an organism (Lucas 1994). The theory underpinning the fetal origins hypothesis is that stresses, such as nutritional deprivation, that occur during critical periods of development in fetal and early life force the baby to resort to adaptive survival strategies. These entail the resetting or programming of various metabolic, physiological and anatomical parameters. These adaptations to nutritional deprivation in early life can become maladaptive if the child encounters contrasting nutritional circumstances in later life (Lucas 1994).

The vast majority of the work investigating the link between size at birth and subsequent adult disease has focused on individuals born at term. This study will focus on infants born prematurely and the possible programming effect of prematurity. In the USA approximately 12% of the population are born prematurely, in Denmark 6-7%, and the proportion of preterm births is rising (Martin *et al.* 2005;Langhoff-Roos *et al.* 2006). It is important to establish whether prematurity predisposes to hypertension, insulin resistance and hyperlipidaemia in later life. Children born prematurely represent the extremes of low birth weight. As this thesis will illustrate, they frequently show significant early growth failure and subsequent catch-up growth. The development of insulin resistance appears to be a key mechanism in programming (Reaven 1988) and it is an important predictor of subsequent diabetes, hypertension and heart disease (Facchini *et al.* 2001). Low birth weight, nutritional deprivation, growth failure and catch-up growth have all been implicated in the development of insulin resistance (Eriksson *et al.* 1999;Huxley, Shiell, and Law 2000). Several mechanisms have been proposed as possible contributors to the evolution of insulin resistance. These include programming the hypothalamo-pituitary-adrenal axis (HPA axis) (Benediktsson *et al.* 1993), programming the growth hormone - IGF-1 axis (GH - IGF-1 axis) (Kajantie *et al.* 2003) and altered activity of 11β hydroxysteroid dehydrogenase (Seckl, Cleasby, and Nyirenda 2000). Studying preterm infants may provide important insights into the mechanisms underlying programming as this group exhibit a number of features which in term populations have been implicated in programming for subsequent insulin resistance. In addition to representing the extremes of low birth weight, exhibiting postnatal growth failure and catch-up growth preterm infants are frequently exposed to steroids in the antenatal and postnatal period. Antenatal steroids have been implicated in programming of the hypothalamo-pituitary-adrenal axis in animal models (Benediktsson *et al.* 1993).

The purpose of this study is to look at the effects of prematurity, neonatal and postnatal growth and perinatal steroid administration of subsequent blood pressure, glucose metabolism and lipids in childhood. We sought to examine two hypotheses:

* that being born prematurely can in itself program for raised blood pressure and insulin resistance in childhood and that it is a consequence of prematurity rather than being small for gestational age.
* that glucocorticoid steroids in the antenatal and early neonatal period may have a programming effect on blood pressure and glucose homeostasis in preterm infants.

This study examines a cohort of children born prematurely in 1994 and matched at birth to healthy term controls. They have been part of a longitudinal study of growth in prematurity and data on their patterns of growth is available as they have been measured regularly throughout childhood. Prior to 1994 maternal antenatal steroid administration in threatened preterm labour to promote fetal lung maturation was not routine practice. Following the National Institutes of Health consensus statement supporting the administration of steroids in threatened preterm labour prior to 34 weeks gestation, which was published in 1995, it became routine practice to do so (National Institutes of Health Consensus Developement Conference statement 1995). Prior to 1994 antenatal steroid use in our unit was approximately 30%, during 1994 70% and in 1995 92%. We therefore fortuitously had a group in whom detailed growth data was available and who were born during the period when antenatal steroid use was rising but was not yet universal.

We have revisited this group aged five-six years to examine effects of prematurity, subsequent growth and exposure in particular to antenatal steroids on:

* blood pressure in childhood
* insulin resistance in childhood
* lipids in childhood

In order to try and elucidate the mechanisms by which any programming effect demonstrated may be mediated we have also examined markers of the growth hormone IGF-1 axis and hypothalamo-pituitary-adrenal axis.

Chapter 2: The Programming Hypothesis - Birth Weight, Growth and Adult disease

It is worthwhile initially reviewing how the fetal origins hypothesis evolved before moving on to focus on key areas of the current debate surrounding programming in populations born at term and review what is known about preterm populations. The chapter will discuss the importance of patterns of growth and of adiposity, the various hypotheses that have been proposed to explain the phenomena before moving on to focus on insulin resistance and possible mechanisms - in particular modulation of the hypothalamo-pituitary-adrenal axis and of the growth hormone – IGF-1 axis that have been proposed as possible mediators for programming.

The “fetal origins hypothesis” arose from the paradox noted by David Barker and co-workers in the 1980’s that the incidence of ischaemic heart disease which had been attributed to increased prosperity was now most marked in poorer areas and in those individuals in low income groups. They noted that areas with the highest *i*maternal and infant mortality last century had the highest current incidence of death from ischaemic heart disease and stroke (Barker and Osmond 1986;Barker and Osmond 1987). Based on the premise that maternal and infant mortality correlated closely with poor maternal nutrition they proposed that adverse conditions in pregnancy & in early life, in particular poor nutrition, predisposed to ischaemic heart disease. The original fetal origins hypothesis stated that “under nutrition in middle to late gestation leads to disproportionate fetal growth and programs later coronary heart disease” (Barker 1995). This elegantly explained the changing aspects of the epidemiology of heart disease. Fifty years ago it was more common in social classes 1 and 2 but by the 1960’s the trend had reversed and death was more common in social classes 4 and 5 (Marmot 1980). If the population becomes predisposed to heart disease by early nutritional deprivation maladaptive responses in the presence of excess nutrition and obesity in later life it would manifest initially in higher socio-economic groups but later across the whole population as affluence spread (Barker and Osmond 1986).

2.1 Birth weight and coronary heart disease

Since Barker’s initial studies were published several large epidemiological studies have examined birth records and demographic data confirming the link between low birthweight and subsequent coronary heart disease (Barker *et al.* 1989). These studies have been replicated in the different populations in a number of different countries with differing levels of affluence (Stein *et al.* 1996;Leon *et al.* 1998). An important aspect of the studies is that they demonstrated a continuum of increasing risk across the birthweight spectrum. It is not only individuals with the lowest birth weights at increased risk. Individuals with a below average birthweight have increased mortality rate from coronary heart disease compared to those with above average birthweight (Barker *et al.* 1989).

2.2 Birthweight and other diseases

Links between low birthweight and later adult have been described for a wide range of disorders in addition to coronary heart disease. These include hypertension (where over 20 studies have confirmed the link) (Barker *et al.* 1990), stroke (Martyn *et al.* 1995), diabetes (Hales *et al.* 1991) and polycystic ovary syndrome (Ibanez *et al.* 1998). Links between low birth weight and risk factors for adult disease such as raised serum lipids and altered clotting factors are also reported (Fall *et al.* 1992). In many of these conditions a gradation of risk across the birthweight spectrum has been described. Once again these associations have been seen in ethnically diverse populations (Yajnik *et al.* 1995;Law *et al.* 2001). Studies have described similar inverse relationships between birth weight and blood pressure in childhood (Woelk *et al.* 1998). Risk factors are also evident in children (Law *et al.* 1991;Forrester *et al.* 1996). The association of hypertension with birthweight becomes stronger with increasing age (Law *et al.* 1993). This is consistent with Folkow’s hypothesis that there is an initiating and then amplification mechanism involved in the genesis of high blood pressure (Folkow 1978).

2.3 Birth weight and fetal under nutrition *in utero*

As the fetal origins hypothesis evolved attention initially focused on poor fetal nutrition *in utero*. Small placental size and thinness at birth are associated with poor fetal nutrition (Phipps *et al.* 1993). A low ponderal index at birth, a high placenta to birth weight ratio and thinness at birth rather than simply small size at birth were linked to cardiovascular disease and diabetes in later life (Phillips *et al.* 1994). Studies in both the developed and developing world established that poor intrauterine nutrition and thinness at birth were important markers for subsequent disease (Yajnik *et al.* 1995).

2.4 Catch-up growth

It has become clear that in addition to intrauterine growth restriction, low birth weight and thinness at birth, subsequent growth and in particular subsequent weight gain are at least as important (Dietz 1994). Low birth weight has been linked to an increased risk of obesity in later life (Gale *et al.* 2001). Individuals subjected to intrauterine growth restraint tend to overshoot their genetic centile and to have a higher fat mass in childhood (Ong *et al.* 2000). Babies born thin tend to store fat centrally rather than peripherally in later life (Hales *et al.* 1991). Children born small for gestational age have reduced lean muscle mass and a higher percentage of body fat in later life (Crowther *et al.* 1998). This has been demonstrated in childhood populations and in large epidemiological studies looking at birth weight and waist hip ratios (Crowther *et al.* 1998;Valdez *et al.* 1994). Individuals born small who later end up large are at highest risk of adult disease. The prevalence of type 2 diabetes is highest in those who are small at birth and become overweight as adults (Hales *et al.* 1991). This has been demonstrated most elegantly in studies from India (Bavdekar *et al.* 1999). There are a number of adverse outcomes to rapid postnatal catch up growth. Rapid weight gain in infancy is a risk factor for future obesity and is associated with earlier pubertal maturation in both boys and girls (Mills *et al.* 1986;Dietz 1994). Different risk factors for heart disease are related to different patterns of the early growth. Blood pressure is related to birthweight but not weight at one year of age suggesting that the critical period for programming blood pressure occurs in fetal life rather than in infancy (Barker 1992). Fibrinogen is related to weight at one year but not birthweight (Barker 1992). Cholesterol is related to patterns of infant feeding (Fall *et al.* 1992) and reflects animal experiments that suggest programming of lipid metabolism occurs in infancy and may be influenced by feeding practices (Mott, Lewis, and McGill, Jr. 1991). Patterns of subsequent weight gain and catch up growth would now seem to be at least as important as low birth weight. This fits well with the concept that the programming effects on fetal growth and it’s metabolic and physiological adaptations can become maladaptive in later life if subsequently challenged by obesity. The relationship between patterns of growth and fetal programming may, as we shall discuss, be particularly pertinent in preterm birth where poor nutrition, growth failure and later catch-up growth are common.

2.5 Insulin Resistance

Attention has moved towards identifying the underlying mechanisms and processes contributing to programming. Insulin resistance appears to be a central factor in developing subsequent adult disease and it has been suggested that this may be a key factor in the process of programming (Reaven 1988). The association of hypertension, impaired glucose tolerance and hyperlipidaemia has been called termed “syndrome X” or “the metabolic syndrome” with insulin resistance as the proposed a link between the these abnormalities (Reaven 1988). Insulin resistance and hypertension are key risk factors in the subsequent development of diabetes, stroke, and heart disease (Facchini *et al.* 2001) and these associations are apparent even from relatively early in childhood.

2.6 Hypotheses to explain programming

A number of hypotheses have been proposed to explain the link between low birth weight, growth and insulin resistance. These include:

* The “thrifty phenotype” hypothesis – The original thrifty phenotype hypothesis as previously mentioned proposed that maternal and fetal under nutrition resulted in a long term adaptive changes or programming of metabolic or hormonal activity in the offspring (Hales and Barker 1992). It suggests that these events in fetal life or infancy could alter the homeostasis of hormones such as insulin, insulin-like growth factors or the adrenal axis predisposing to insulin resistance. These adaptive changes whilst advantageous *in utero* and in early life may become deleterious if the individual whilst adapted to lean conditions then encounters nutritional excess later.
* The “thrifty genotype hypothesis” – The thrifty genotype or surviving small baby hypothesis suggested that the relationship between low birthweight and adult diseases could be explained could by the selective survival (given the higher mortality of low birthweight infants) of those genetically predisposed to increase risk disease in later life (Neel 1999).
* The “fetal insulin hypothesis” - Individuals with a rare glucokinase gene defect responsible for maturity onset diabetes of the young (MODY) were noted to have reduced birthweight infants. It was therefore proposed that specific genetic defects affecting insulin secretion or responsiveness to insulin could link small size of birth and the risk of diseases in adult life (Hattersley and Tooke 1999).

There is a variety of evidence to support all of the hypotheses. The intrauterine environment is a major factor influencing fetal growth. Maternal size (Ong *et al.* 2000), maternal nutrition (Godfrey and Barker 1995;Kramer 2003) and maternal parity (Ong *et al.* 2002) all have an effect on size of birth. Embryo transfer and cross breeding experiments between Shire horses and Shetland ponies elegantly illustrate the constraining effect of maternal size on fetal growth (Snow 1989). Animal models of maternal nutritional deprivation have demonstrated reduced birthweight in offspring and subsequent hypertension and insulin resistance (Ozanne *et al.* 1996;Lewis *et al.* 2001;Langley, Browne, and Jackson 1994;Langley and Jackson 1994;Crowe *et al.* 1995). The role of nutrition in programming in man is more complex. Except in extreme circumstances nutritional supplementation of women during pregnancy has relatively little effect on birth weight. Examination of the studies of survivors from the Dutch famine of 1944-1945 further illustrates the complexity of the problem. Those individuals exposed to the famine during the last trimester of pregnancy and in the initial months of postnatal life had reduced birthweight and the highest post load glucose levels aged twenty-five (Ravelli *et al.* 1998) whereas those individuals exposed to famine in the first and second trimesters of pregnancy and whose mothers subsequently had normal nutrition had higher birth weights and a lesser degree of impaired glucose tolerance despite higher rates of obesity as young adults (Ravelli, Stein, and Susser 1976). Paradoxically though individuals born during the siege of Leningrad don’t show evidence of the metabolic syndrome (Stanner *et al.* 1997). The timing of a nutritional insult and of any catch-up growth may be crucial. This may be particularly important in preterm infants as the insult occurs in the 3rd trimester.

Fetal growth is characterised by rapid cell division and tissues exhibit so-called critical periods of even more rapid cell division (Widdowson and McCance 1975). Depending on the timing of impaired nutrition disproportionate growth can occur because different tissues have critical periods of growth. Even a brief period of impaired nutrition *in utero* can have persistent effects on blood pressure, cholesterol, glucose metabolism, and the immune system (Widdowson 1974;Barker 1998). Permanent physiological changes have been demonstrated as a result of altered nutrition during critical developmental periods in a range of tissues including the pancreas, liver, and blood vessels (Persson and Jansson 1992;Mott *et al.* 1991).

2.7 Inheritance of low birth weight

Low birthweight may also have had a hereditary component. Although mothers with type 2 diabetes tend to have larger babies because of the metabolic effects of their diabetes (hyperinsulinism in the fetus as a consequence of maternal hyperglycaemia promotes fetal growth), type 2 diabetes in the father is associated with low birthweight (Hypponen, Smith, and Power 2003). This lends support to concept of a genetic component. Twins studies are always important when assessing the genetic or environmental component in a condition. Studies of monozygotic and dizygotic twins and their offspring suggest that fetal genes may contribute up to 50-80% of the variance in birthweight (Magnus 1984). In contrast, in monozygotic twin pairs, the twin with the lower birthweight demonstrates higher blood pressure and evidence of impaired glucose handling suggesting that the environment at the placental level maybe significant given that they share common genes and a identical maternal nutritional environment (Levine, Hennekens, and Jesse 1994;Poulsen *et al.* 1997). In man rare mutations that affect, for example, the insulin receptor (Wertheimer *et al.* 1993) and glucokinase (Hattersley *et al.* 1998) result in small size of birth and intrauterine growth retardation. Within the normal population size at birth has been associated with a genetic polymorphism that controls the insulin gene, the VNTR microsatellite (Dunger *et al.* 1998). This functional polymorphism that controls transcription of the insulin gene has 2 discrete allele classes (dependant on the number of tandem repeats) which are associated with differences in birth weight. Many genes that control size at birth are imprinted and birthweight correlates much more closely with maternal birthweight than with paternal birthweight (Little 1987). Genomic imprinting may well have an evolutionary role in controlling birth weight (Moore and Haig 1991). Maternal contributions to low birth weight may also be transmitted via mitochondrial DNA, such as the 16189 variant which has been associated with thinness at birth and insulin resistance (Poulton *et al.* 1998;Casteels *et al.* 1999).

The aetiology of type 2 diabetes and cardiovascular disease is multifactorial and there is evidence to support both a genetic and environmental contribution to the associations between low birth weight and hypertension, impaired glucose tolerance and hyperlipidaemia.

2.8 Mechanisms of programming.

Various mechanisms have been suggested to explain the likely interaction between genes and the environment and early growth. These include increased concentrations of plasma insulin like growth factor in childhood (Fall *et al.* 1995), maternal iron deficiency anaemia (Godfrey and Barker 1995) and mpaired vascular structure and loss of elasticity in vessel walls in man (Martyn *et al.* 1995). Animal experiments in rat models suggest maternal protein deprivation leads to higher concentrations angiotensin converting enzyme (Langley and Jackson 1994) and that numbers of nephrons are affected by birth weight (Mackenzie and Brenner 1995). Increased exposure to maternal glucocorticoid hormones in rats resulted in raised blood pressure (Edwards *et al.* 1993). Leptin has also been proposed as a potential mechanism for programming.

Two mechanisms have been explored in detail in the literature and would seem most plausible in the context of prematurity. Programming of the GH-IGF-1 axis and programming of the hypothalamo-pituitary-adrenal axis and 11β hydroxysteroid dehydrogenase.

2.8.1 IGF-1 and programming

Alterations in the GH-IGF-1 axis may contribute to programming (Barker *et al.* 1993b). The insulin-like growth factors are thought to have a central role in fetal growth and may be affected by growth failure. There is evidence that children who fail to put on weight early in life develop resistance to GH as adults (Barker *et al.* 1993a). They exhibit an exaggerated response to growth hormone releasing factor and have lower plasma IGF-1 levels suggesting a degree of growth hormone resistance (Job *et al.* 1990;Lassarre *et al.* 1991). Growth hormone is essential to the growth of pancreatic beta cells and the link between reduced beta cell mass as a consequence for intrauterine growth retardation GH-IGF-1 in animal models (van Assche and Aerts 1979). Maternal starvation reduces IGF-1 levels in fetal sheep (Oliver *et al.* 1993).

In children plasma IGF-1 levels are related to systolic blood pressure (Fall *et al.* 1995). Those children with the lowest birthweights had the highest levels of IGF-1 in childhood and the highest systolic blood pressure. This was evident both in populations from the UK and in populations in India (Fall *et al.* 1995). Reports are however conflicting. In children with intrauterine growth retardation there was no evidence from euglycaemic clamp studies that the insulin resistance observed was related to the IGF-1 system (Cianfarani *et al.* 2001). Conversely in other studies children born small for gestational age had evidence of increased growth hormone secretion which correlated with fasting insulin resistance (Woods *et al.* 2002). Similarly other studies have shown an association of IGF binding protein 1 with impaired glucose tolerance (Heald *et al.* 2001) and an association between intrauterine growth retardation and elevated growth hormone concentrations (Haymond, Karl, and Pagliara 1974).

2.8.2 Hypothalamo-pituitary-adrenal axis and programming

Programming of the HPA axis is an attractive hypothesis to link events *in utero* and later disease as excess glucocorticoids can cause raised blood pressure and glucose intolerance. The metabolic syndrome shares many features of Cushing’s Syndrome. Dexamethasone has been shown to programme the HPA axis in rats (Levitt *et al.* 1996). There is some data to link reduced fetal and infant size with altered activity of the HPA axis. Fetuses with weights below the 10th centile have raised cord cortisol levels and levels of adrenal corticotrophin releasing hormone (ACTH) when compared to heavier fetuses matched for gestational age (Economides *et al.* 1988). Intrauterine growth retardation is also associated with higher cortisol levels (Haymond *et al.* 1974). Light-for-dates infants also have increased 24 hour urinary adrenal steroid excretion measured by gas chromatography age 9 years (Clark *et al.* 1996). Plasma cortisol is usually normal or low in essential hypertension but fasting 9am cortisols have been shown to be highest in those hypertensive men who were lightest at birth (Phillips *et al.* 2000).

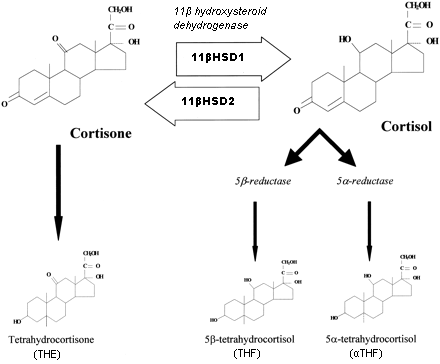
The most impressive evidence for the role of steroids in programming comes from animal experiments. In animals there is evidence that exposure to maternal glucocorticoids results in reduced birth weight and hypertension in later life. The fetus is normally protected from the effects of active maternal glucocorticoids by the placental enzyme 11β hydroxysteroid dehydrogenase type 2 (11βHSD2) which rapidly converts active cortisol to inactive cortisone (Lindsay *et al.* 1996). Cortisone is also converted back into active cortisol by 11β hydroxysteroid dehydrogenase type 1 (11βHSD1). Overall levels of active cortisol in tissues are determined by the balance between 11βHSD1 and 11βHSD2 activity. Eleven beta hydroxysteroid dehydrogenase type 2 could play an important role in maintaining the autonomy and development of the fetal HPA axis or the enzyme system itself (together with 11β hydroxysteroid dehydrogenase type 1) may be a target for programming. Dexamethasone (which is not metabolised by 11βHSD2 and therefore reaches the fetus), when administered to rats resulted in reduced birth weight and subsequent hypertension (Benediktsson *et al.* 1993). Pregnant rats treated with carbenoxalone, which inhibits the activity of 11β hydroxysteroid dehydrogenase (11βHSD), also gave birth to low birth weight pups who subsequently had higher blood pressure (Lindsay *et al.* 1996).

Placental size and birthweight are predictors of blood pressure. Those infants with the largest placenta and lowest birth weight had the highest blood pressure as adults (Barker *et al.* 1990). In rat placenta the activity of 11βHSD2 varies and was inversely related to the birth weight of the offspring (Benediktsson *et al.* 1993). This suggests that in those rats with lower placental enzyme activity more glucocorticoid reaches the fetus resulting in lower birth weight. Although there is no data on human placental 11βHSD activity and blood pressure, it has been demonstrated that enzyme activity in fresh human placentas was inversely related to the birth weight of the infant (Benediktsson *et al.* 1997). It has been proposed that a relative deficiency in the placental barrier may alter the balance of the pituitary adrenal axis. A deficiency in the placental barrier by impaired nutrition resulting in hypertension can be demonstrated in rats (Langley and Jackson 1994).

2.8.3 11β hydroxysteroid dehydrogenase activity

It has also been suggested that programming of 11β hydroxysteroid dehydrogenase (11βHSD) activity at a cellular level may be responsible for the programming of hypertension and insulin resistance. Levels of active cortisol within the tissues are determined by the equilibrium between the two isoenzymes of 11βHSD (Quinkler, Oelkers, and Diederich 2001). Eleven beta hydroxysteroid dehydrogenase type 2 (11βHSD2) is a high affinity enzyme that rapidly converts cortisol into inactive cortisone. Cortisone is converted back into active cortisol by 11 beta hydroxysteroid dehydrogenase type 1 (11βHSD1) (Quinkler *et al.* 2001). The two isoforms are also tissue specific. 11βHSD2 is mainly located in the kidney where it protects mineralocorticoid receptors from exposure to cortisol (which would elevate blood pressure) (Quinkler *et al.* 2001). 11βHSD1 is located principally in the liver and in fatty tissue (Stewart, Toogood, and Tomlinson 2001). In addition to the inactivation and reactivation of cortisol by 11βHSD tissue levels are also dependant on the A-ring reductase enzymes 5α reductase and 5β reductase. These irreversibly inactivate cortisol and cortisone. Cortisol is converted to allo-tetrahydrocortisol (αTHF) and tetrahydrocortisol (THF) by 5α reductase and 5β reductase respectively. Cortisone is converted to tetrahydrocortisone (THE) (Palermo *et al.* 1996). See Figure 1.

Figure 1. Metabolism of active Cortisol and inactive Cortisone by 11β hydroxysteroid dehydrogenase and the A-ring reductase enzymes 5α reductase and 5β reductase.



The overall activity of 11 beta hydroxysteroid dehydrogenase type 1 and type 2 can be measured from the ratio of cortisol to cortisone metabolites in the urine (THF + αTHF: THE) (Quinkler and Stewart 2003). A higher ratio suggests either proportionately higher activity of 11βHSD1 or lower activity of 11βHSD2 resulting in a relative increase of active cortisol metabolites compared to the metabolites of inactive cortisone. Conversely a low ratio suggests reduced activity of 11βHSD1 (reduced reactivation of cortisol from cortisone) or increased activity of 11βHSD2 (increased deactivation of cortisol to cortisone). 11βHSD1 effectively amplifies local cortisol levels (Seckl and Walker 2001). The ratio of tetrahydrocortisol (THF) to allo-tetrahydrocortisol (αTHF) gives an estimate of the relative activity of 5β reductase and 5α reductase.

Several previous studies suggest that the metabolic syndrome may be linked to altered 11βHSD activity. In obesity there is evidence of reduced reactivation of cortisone by 11βHSD1 in the liver and there is evidence of enhanced 5α reductase activity (Stewart *et al.* 1999;Andrew, Phillips, and Walker 1998). There is some evidence that insulin may upregulate 5α reductase (Kerstens *et al.* 2000). In women with PCOS there is elevated 5α reductase activity and altered 11βHSD activity (Stewart *et al.* 1990;Rodin *et al.* 1994). Antenatal steroids may exert a programming effect as the fetus is normally protected from maternal cortisol by placental 11βHSD2 (Benediktsson *et al.* 1997). Fetal expression of 11βHSD1 is usually very low prior to delivery (Hingre *et al.* 1994).

2.8.4 Leptin

Leptin has been proposed as a possible component or contributing factor to the metabolic syndrome (de Court *et al.* 1997). Leptin has been demonstrated to be related to insulin resistance and other markers of the metabolic syndrome independently of body mass in lean nondiabetic men (de Court *et al.* 1997;Haffner *et al.* 1999;Haffner *et al.* 1997). Relationships between size at birth and leptin levels in adult life have been described. Levels of leptin were highest in men with low birth weights when compared to individuals with similar levels of obesity in adult life (Phillips *et al.* 1999). Cord blood leptin it is associated with size at birth weight and predicts infancy weight gain (Ong *et al.* 1999). Leptin levels are reduced in children with intrauterine growth retardation (Cetin *et al.* 2000). In premature infants leptin is associated with poor weight gain in the postnatal period. An inverse exponential relationship with weight gain was demonstrated (Shekhawat *et al.* 2000).

Perhaps most impressive evidence for a potential role of leptin comes from interventional animal experiments. In rat models maternal nutritional deprivation leads to hyperinsulinism, hypertension and elevated levels of leptin in offspring (Vickers *et al.* 2005). Administration of leptin to their offspring in the neonatal period prevented subsequent hypertension and insulin resistance and appears to reverse the undesirable effects of undernutrition *in utero* (Vickers *et al.* 2005). Offspring whose mothers were given leptin during pregnancy whilst also subjected to nutritional deprivation similarly did not show evidence of the hyperinsulinism and hypertension seen in control animals (Stocker, Arch, and Cawthorne 2005).

Leptin levels are closely linked to adiposity in both children and adults. It is involved in the complex processes that control satiety, energy balance and adiposity in later life. A role for leptin in the perinatal programming of appetite has been postulated (Dootsch, Rascher, and Meissner 2004). In animal models overfeeding in the perinatal period has long-lasting effects on levels of plasma leptin in adult life (Lopez *et al.* 2006). Animal experiments also suggests that prenatal nutrition may influence the quantity and distribution of adipose tissue into adult life (McMillen *et al.* 2006). In animals levels of leptin mRNA in fetal fat are related to fetal body weight (Bernard *et al.* 1999).

In a study of nutrition in human preterm infants examining the effects of breast milk compared to a nutrient enriched preterm formula the ratio of leptin to fat mass was significantly higher in adolescence in those that received the calorie enriched preterm formula (Singhal *et al.* 2002). This suggested that early nutrition may influence leptin and later fat mass.

Leptin levels have also been linked to prematurity and administration of antenatal steroids. Levels of leptin were lowest in preterm infants and positively correlated with increasing gestational age (Hytinantti *et al.* 2000;Cetin *et al.* 2000). Levels were up to three times higher following administration of antenatal steroids (Shekhawat *et al.* 1998). Other studies have demonstrated a similar association between leptin and antenatal steroids (Hytinantti *et al.* 2000).

2.9 Antenatal and postnatal steroids

Many children born prematurely are exposed to steroids *in utero*. Since 1994, following the National Institutes of Health Consensus statement and a detailed meta-analysis on the use of antenatal steroids, it has been routine practise to give glucocorticoids to those mothers threatening to deliver before 34 weeks gestation as they promote lung maturation and significantly reduce neonatal deaths (National Institutes of Health Consensus Developement Conference statement 1995;Crowley 1995). Approximately 90% of women in preterm labour now receive antenatal steroid treatment to promote lung maturation.

Liggins first noted the benefits of antenatal steroids in improving survival in preterm infants by promoting lung maturation in the 1970’s. Antenatal steroid therapy is associated with improved survival and with improved neurologically intact survival (Salokorpi *et al.* 1997;Crowley 1995). Their use was initially controversial as there were concerns about an effect on linear growth, a reduction in birth weight and on potential neurological development. In monkeys there was evidence of neuronal damage (Epstein *et al.* 1977) and evidence that corticosteroid therapy adversely affected brain growth (Barrada, Blomquist, and Kotts 1980). In man, important developmental disturbances have not been reported (MacArthur *et al.* 1981;MacArthur *et al.* 1982;Doyle *et al.* 2000b) although one study reported an adverse effect on behaviour (French 1998). This group also reported a significant reduction in birth weight particularly with repeated doses of steroids (French *et al.* 1999) in a cohort of 652 infants born between 20-32 weeks gestation. Although birth weight was reduced, by 3 years of age there was no difference in height between those who received antenatal steroids and those who did not. However other studies have not shown that antenatal steroids reduce birthweight and showed no evidence of a detrimental effect on growth aged 4 years (Hasbargen *et al.* 2001). No evidence of an effect on growth age 10-12 years was seen in those entered in a randomised trial, except perhaps later puberty in boys (Smolders-de Haas *et al.* 1990).

Antenatal steroids have a number of other effects on the hormonal milieu of the fetus whose mother is given antenatal steroids. In addition to the effects on the adrenal various other endocrine changes have been noted (Padbury, Ervin, and Polk 1996;Ballard *et al.* 1980). The thyroid economy is altered. Levels of TSH and T4 are unaffected but levels of T3 and reverse T3 are elevated (Osathanondh, Chopra, and Tulchinsky 1978). Antenatal glucocorticoids modulate the amplitude of pulsatile cortisol secretion in the premature neonate (Arnold *et al.* 1998). There is a short term suppressive effect on the adrenal but no evidence of long term effects on ACTH testing in the neonatal period (Ng *et al.* 1997;Ng *et al.* 1999).

Given the potential effect on growth, birthweight and the programming effect demonstrated in rat models there has been concern that antenatal glucocorticoids may have an adverse programming effect in man.

Although in animal models antenatal steroid exposure has been shown to cause hypertension in offspring in man the situation is not so clear cut. A study from Western Australia of 210 preterm survivors born <1500g reported significantly higher blood pressure at 14 years of age in children born to mothers who were randomised to receive antenatal corticosteroids as part of a trial compared to those whose mothers did not receive corticosteroids (Doyle *et al.* 2000a). The mean difference in systolic blood pressure was 4.1 mmHg and the mean difference in diastolic blood pressure 2.8 mmHg. In a study from New Zealand of 223, six year old children whose mothers had taken part in a prospective randomised trial of prenatal betamethasone for the prevention of respiratory distress syndrome the authors found no significant difference between those children whose mothers had received antenatal betamethasone and those that did not (Dalziel *et al.* 2004).

Similar concerns about growth and possible adverse neurological effects have been raised in neonates given dexamethasone in the postnatal period to treat chronic lung disease of prematurity In a study looking at the outcome at two years of infants who took part in a double blind controlled trial of early dexamethasone to prevent chronic lung disease there was a significant reduction in the somatic growth of boys and a significantly higher incidence of neuromotor dysfunction than the controls (Yeh *et al.* 1998). The dexamethasone treated group also had transient hyperglycaemia, hypertension, cardiac hypertrophy, hyperparathyroidism, and a transient delayed in weight gain although by day twenty-eight there was no significant difference between the groups in any of these variables (Yeh *et al.* 1998). Animal experiments with pharmacological doses of dexamethasone have revealed adverse effects on brain cell division, differentiation, and myelination (Weichsel 1977). It has been suggested that dexamethasone therapy for chronic lung disease produces a significant increase in blood pressure within 48 hours which does not return to baseline levels after treatment (Marinelli, Burke, and Herson 1997).

2.10 The Situation in preterm infants

Whilst the evidence for a link between hypertension, glucose intolerance and low birth weight is fairly conclusive in individuals born at term the situation in children who were born prematurely has not been clearly elucidated. Most children born prematurely have a low birth weight compared to term infants. But their low birth weight is a consequence of premature birth rather than of intrauterine growth restriction. The majority of infants born prematurely are an appropriate size for their gestation.

Initial epidemiological studies indicated that there was no relationship between gestational age and subsequent glucose intolerance and hypertension (Barker *et al.* 1993a;Whincup and Cook 2000;Robinson *et al.* 1992;Phipps *et al.* 1993)*.* 1993). While suggesting that gestation was not associated with subsequent hypertension and glucose intolerance these studies looked at historical cohorts in which the mortality associated with prematurity 50 to 60 years ago precluded the inclusion of significant numbers of preterm births within the cohorts. In essence they were examining the effect of gestation in infants born between 35 to 36 weeks and 42 weeks gestation. Few of these studies had the power to separate the effects of low birthweight and prematurity (Law 2002). The only large epidemiological study with sufficient statistical power comprised 165,000 Swedish adults and looked at individuals born between 35 and 44 weeks gestation (Leon, Johansson, and Rasmussen 2000). It showed an effect of gestational age and also of being small for gestation on subsequent adult blood pressure. Prematurity was in itself associated with higher blood pressure in later life (Leon *et al.* 2000).

The results from studies examining contemporary cohorts including children born before 34 weeks gestation are conflicting. A large study from Cambridge of 616 children born before 34 weeks gestation found no relationship between gestation and subsequent blood pressure (Morley *et al.* 1994). However this study was originally established to look at the effect of different feeding regimes in very low birthweight babies (less than 1850 g). As a consequence the majority of infants within the study had significant intrauterine growth retardation as well as prematurity. There was no term control group and comparisons were within the cohort. The authors demonstrated lower blood pressure in the lightest infants. The authors concluded that the data did not support the view that fetal growth retardation before 34 weeks gestation programmed for raised blood pressure in later life and refuted the link between low birthweight and subsequent hypertension. Other studies reached different conclusions. A study comparing low birth weight rates in infants with normal birthweight controls at 10 years of age found a difference in systolic blood pressure of 5 mmHg (Cater and Gill 1984). A similar study in Scotland comparing very low birthweight children with normal birthweight controls at aged nine years of age found in elevation in systolic blood pressure of 5 mmHg in girls and 2.7 mmHg in boys (Mutch and Mcleod 1995). A large study comprising nearly 700 children in New Zealand demonstrated an inverse relationship between birth weight and subsequent systolic blood pressure (Simpson *et al.* 1981). A further study of 172 children born weighing less than 1500 g demonstrated that they were shorter and lighter than term controls with higher systolic blood pressure in childhood (Pharoah, Stevenson, and West 1998). These studies did not though, address the question as to whether it was prematurity or being small for gestation that was the key factor. All these cohorts included a substantial number of babies with intrauterine growth retardation as birth weight was the principal criterion used to recruit them.

A few small studies have specifically addressed the relationship of prematurity as well as being small for gestational age with subsequent blood pressure and glucose metabolism. A group of young adults were identified from a cohort originally recruited between 1973 and 1975 (Irving *et al.* 2000). A total of 15 individuals born with low birth weight and intrauterine growth retardation (mean gestation 35 weeks), and 19 individuals of low birth weight but appropriate to their gestation (mean gestation 31.9 weeks) were compared to 27 term controls at 23 years of age. Blood pressure was significantly higher in both groups compared to term controls. Systolic blood pressure was 123mmHg in those born simply premature, 120mmHg in those born IUGR compared to 115mmHg in healthy term controls. Diastolic blood pressure was similarly elevated at 80mmHg in the preterm group, 77mmHg in the IUGR group and 77mmHg in term controls. Fasting blood glucose was higher in the preterm group (5.6 mmol), but not the group born IUGR (4.3 mmol) compared to the term controls (4.9mmol). Although not significant there were trends for other adverse metabolic profiles such as are higher insulin, triglyceride, and cholesterol. The study concluded that premature delivery alone was a risk factor for hypertension and hyperglycaemia as an adult (Irving *et al.* 2000).

A further study supported these findings in that higher levels of both systolic and diastolic blood pressure were noted in a group of 38 individuals born prematurely but appropriate for gestational age compared to 30 healthy term controls (Szathmari *et al.* 2000). A further group of 32 individuals born both small for gestational age and prematurely had higher blood pressure than controls of a similar magnitude to those simply born prematurely. Interestingly, in view of findings to be presented in this thesis, in this study plasma cortisol was associated with a raised systolic blood pressure and raised heart rate in men but not women whereas higher levels of the adrenal steroid dihydroepiandrosterone (DHEA) was associated with raised systolic blood pressure and heart rate in women but not in men (Szathmari *et al.* 2000).

A follow-up study from Sweden examined the blood pressure of a small group (44 individuals) of 49 yr old men who were part of a larger cohort in whom the link between birth weight and adult blood pressure was being investigated and found a strong inverse correlation between gestational age and adult blood pressure. Those born most prematurely had the highest blood pressure (Siewert-Delle and Ljungman 1998).

Using measurements from the intravenous glucose tolerance test researchers in New Zealand demonstrated that prematurity was linked to later insulin resistance. Children born prematurely were insulin resistant when compared to term controls. They found no effect between the degree of prematurity and insulin resistance within their cohort (infants born below 32 weeks gestation) (Hofman *et al.* 2004). On the other hand in a study of Indian children at 4 years of age there was no evidence of altered glucose homeostasis in children who had been admitted to special care (mean gestation 34 .8 weeks and mean birth weight 1.5kg) compared to routine deliveries (mean wt 2.7 kg) though no gestation was recorded. The authors focused principally on the relationship between low birth weight and glucose metabolism rather than prematurity. Many of the term infants had significant IUGR and only 61% of the group admitted to SCBU survived which may have distorted the data (Yajnik *et al.* 1995).

Flow mediated endothelial dependant vasodilatation is a marker for cardiovascular risk. A group in Cambridge found no difference between a group born prematurely who were appropriate for their gestation compared to preterm infants with intrauterine growth retardation and term controls. Similarly there was no difference in the systolic and diastolic blood pressure, lipid profiles or levels of fasting glucose and insulin (Singhal *et al.* 2001).

2.11 Measuring Growth

Despite the importance of growth as a barometer of wellbeing and it’s link to ill health in adult life it is an area frequently neglected particularly in the increasingly complex neonatal unit. Measurement of newborn babies and in particular preterm infants has often been considered too inaccurate to make it worth while (Gibson *et al.* 2003). Growth is often equated simply with weight gain with no assessment made of linear growth. Accurate measurements can be readily obtained with training, appropriate equipment and techniques (Betts, Voss, and Bailey 1992).

In addition to the general measurement of neonates more detailed and specialised techniques are available to look at patterns of growth. In particular knemometry which uses a micrometer to accurately measure the lower leg (Michaelsen *et al.* 1991). It is possible to make a very accurate sequential measurements even in the most preterm infants. It is ideal for looking at short term growth. This is particularly useful where an evaluation of the response to therapeutic interventions is required eg. use of dexamethasone in the postnatal period (Gibson, Pearse, and Wales 1993).

Interpretation of measurements can be more difficult. The widely used 1990 UK standards do not give a reference range for length or BMI below 33 weeks gestation (Freeman *et al.* 1995;Cole, Freeman, and Preece 1995). The standards suggested by Keen and Pearse (Keen and Pearse 1988) for length and weight in preterm infants from 23-42 weeks gestation are not concordant with the 1990 UK standards. The discrepancy between the two standards is most apparent approaching term. At 33 weeks the discrepancy is minimal (1990 UK mean 44.9 cm vs 44.7cm for Keene & Pearse in males) but towards term the standards diverge. In term males the mean length was 51.0 cm by the 1990 standards compared to 53.7 cm in the Keane & Pearse standards. This creates problems converting measurements into standard deviation scores (SDS).

Similarly much of the early work on programming has looked at ponderal index (weight/height3) and body mass index (weight/height2) as a measure of thinness at birth. These are significantly lower in preterm infants compared to term infants because of the disproportionate effect of height2 or height3. There are no published standards for body mass index in the preterm infant below 33 weeks. It is therefore not possible to establish BMI standard deviation scores for this group.

Assessment of body fat and body composition is also challenging. There are published standards for subscapular and triceps skinfold thicknes but once again there is discrepancy between them (Oakley, Parsons, and Whitelaw 1977;Vaucher *et al.* 1984). Prior to 32 weeks gestation neonates have very little subcutaneous fat. Techniques to measure fat mass such as DEXA or MRI pose problems in extremely premature neonates and are not suitable for sequential measurements. The percentage of body fat can be calculated from skin fold thicknesses (Brook 1971) but these equations have not been validated against more precise methods in neonates.

Care therefore needs to be taken in interpreting neonatal measurements.

2.12 Rationale for Study

Based on the preceding information we examined a cohort of children then aged 5-6 years, born at gestational ages ranging from 24 weeks to term, who had been under regular follow up to monitor their long-term growth to investigate whether there was any evidence of programming of the HPA axis following administration of glucocorticoids. If events in the antenatal or neonatal period had influenced the HPA axis we would expect to see raised blood pressure or evidence of glucose intolerance. It is conceivable that there may be critical periods during fetal development when steroid administration may have particularly marked effects in later life.

During 1994 antenatal dexamethasone/betamethasone administration became routine obstetric practice in threatened preterm delivery. Because of the variation in the pace of change in the clinical practice of individual obstetricians, of the infants born prematurely and subsequently admitted to our neonatal unit their mother had received steroids antenatally in 30-40% cases. These children thus represent a unique cohort in whom to study any long-term effects of steroid administration as their treatment was in all other respects identical.We obtained data on the growth, blood pressure and glucose tolerance of this cohort. Urinary steroid profiles and fasting cortisol provide some descriptive information on the hypothalamic-pituitary-adrenal axis. The information the study provided allowed us to test the hypothesis that steroids in the antenatal and neonatal period can programme for hypertension and impaired glucose tolerance in childhood. Measurements of insulin, leptin and IGF-1 allowed assessment of their importance in this process.

Chapter 3: Study Population and Methodology

3.1 Aims

The study aimed to test two hypotheses:

* Whether being born prematurely can in itself programme for raised blood pressure and insulin resistance in childhood and that any observed effects are a consequence of prematurity rather than of being small for gestational age.
* Whether administration of glucocorticoid steroids in the antenatal and early neonatal period have a programming effect on blood pressure and glucose homeostasis in preterm infants at 5 years of age.

3.2 Study Population

In 1994 all preterm infants admitted to the neonatal unit at the Jessop Hospital in Sheffield were invited to take part in a long-term longitudinal study to monitor growth in children born prematurely. Detailed measuremenst were undertaken by the same auxologist within 48 hours of birth, weekly until discharge from the neonatal unit, and then at 8 weeks of age (uncorrected for gestation), at 6 months of age (uncorrected for gestation) and thereafter annually within 2 weeks of their birth date (not original due date). A random sample of 50 healthy term singleton infants were approached and recruited from the post-natal wards contemporaneously to act as controls. These children were closely matched for sex, social class and maternal smoking. A total of 254 children (including controls) gave consent and were originally enrolled into the study and measured in the neonatal period (PedoBaby ruler TM - J.M.B. Ets, Brussels, Belgium). A number of children born prematurely unfortunately died in the neonatal period and tertiary referrals from distant units (> 20 miles from Sheffield) were excluded from the long term follow-up component of the study for practical reasons. Children in whom complex neurological disabilities were anticipated were included. A cohort of 197 children was therefore followed up long-term. These children were measured annually at home around their birth date by the same research nurse. Measurements were made of:

* Height using a portable stadiometer (“Leicester meter”™ - Child Growth Foundation, London, UK)
* Sitting height and leg length using a Leicester meter™. In the initial neonatal period only lower leg length was measured using knemometry (Neonatal knemometer, Force Institute, Copenhagen) (Gibson, Pearse, and Wales 1993b)
* Head circumference using a Lasso-o tape TM (Child Growth Foundation, London, UK)
* Skin fold thickness - Triceps and subscapular in the neonatal period and of triceps, biceps, subscapular and suprailiac skinfolds (Holtain calipers™ - Holtain Ltd, Crosswell, Pembrokeshire, UK).
* Midarm circumference using a tape measure (Lasso-o tape TM).

At approximately 5 years of age all the families enrolled in the existing longitudinal growth study were approached initially by telephone, followed up by a home visit and invited to participate in the current metabolic study. The study protocol was approved by the South Sheffield Research Ethics Committee. One hundred and thirty children from the cohort were recruited to the study (representing 81% of the cohort still under follow up at that time). In addition to those participating in the longitudinal growth study children born prematurely at the other smaller neonatal unit in Sheffield situated at the Northern General Hospital (identified from the Unit’s birth register), of the same age as the original cohort, were also invited to take part. Data on size at birth and early growth up to 2 years of age was available for these children. Recruitment rates were similar to those from families in the growth study with 75% of those contacted taking part (28 children). (See figure 2 for recruitment)

The following additional exclusion criteria were applied:

* Children who had received systemic steroids within the last month or who were taking inhaled steroids.
* Children with major co-existing medical problems in which growth impairment is a recognised feature that would make interpretation of the data difficult. For example complex congenital heart disease, chromosomal disorders, severe cerebral palsy.
* Children with whom current parental responsibility rested with social services

In total 158 children and their families gave written informed consent and took part in the study.

Figure 2: Flow diagram of recruitment to the study

254 Children recruited to the study at birth. (204 preterm infants & 50 term controls). All measured in neonatal period

197 children followed up long term (147 preterm infants & 50 controls)

57 children lost to follow-up in the neonatal period.

- deaths

- children transferred back to distant neonatal units

- didn’t want to continue after discharge

160 still under follow-up age 5 years (116 preterm & 44 term controls). All approached to take part in the study

37 lost to follow-up over the years

130 children agreed to take part. (91 preterm and 39 controls)

30 declined to take part or didn’t meet inclusion criteria

37 preterm infants from NGH neonatal unit approached

28 families agreed to take part

158 families participated in the study. 118 children born prematurely (91 Jessop & 28 NGH) and 39 term controls

3.3 Study Protocol

Each child and their parents were invited to attend the Day Care Unit at Sheffield Children's Hospital on one morning for a glucose tolerance test. All were fasted from midnight and all had applied a topical local anaesthetic cream (EMLA™) at home at least an hour previously. Each child was seen in the same room, at the same time of day (8.00-9.00am) and at the same ambient temperature (approximately 20 C). On arrival after a rest period of 15 minutes the blood pressure was checked with the child seated on a parents knee using an automated device and the same appropriately sized cuff (Critikon 8100 Dinamap™ and 12cm Dinamap™ cuff that covered 2/3 of the upper arm). After an initial reading to accustom the child to the device 3 blood pressure readings were taken at 2 minute intervals and the systolic, diastolic, mean blood pressure and heart rate recorded (by the Dinamap). An automated device was chosen to avoid any potential observer bias, or number preference, which are problems associated with mercury syphygmanometers (Barker, Shiell, and Law 2000). There is also debate as to whether the muffling (korotkoff 4) or complete disappearance of the heart sounds (korotkoff 5) should be used in children as it is difficult to differentiate the two (Sinaiko, Gomez-Marin, and Prineas 1990). The Dinamap 8100 oscillometric device has been found to be an appropriate device for use in both clinical practice and research in children (Barker *et al.* 2000;Jin *et al.* 2001). It is the reference automated device used to produce the latest UK standards (Jackson, Thalange, and Cole 2006). Resting heart rate was obtained from the automated readout of the Dinamap to avoid observer bias whilst the child was sat quietly for measurement of blood pressure. Again the mean of 3 measurements was taken. A single venepuncture was then carried out to insert a cannula and fasting blood samples collected for:

* Insulin. - Measured by Micropartical immunoenzymometric assay - Abbott AxSYM, Abbott Diagnostic, USA. An automated assay system based on a mouse monoclonal antibody. There is no cross reactivity with pro-insulin (0.016%). Range 1.0-300 mU/l. Sensitivity 1.0 mU/ml. Intra-assay coefficient of variation 1.9%, inter-assay coefficient of variation 2.9%.
* Glucose – Measured on whole blood by a glucose oxidase methodology using a YSI 2300 STAT analyser (YSI UK Ltd ). Blood glucose measurement range 0.2-50.0 mmol/l. Precision ±2% or 0.2 mmol.
* Glycosylated Haemoglobin (HbA1c) – Measured by high performance borate affinity chromatography – Primus, USA. Coefficient variation (at the midpoint of the precision curve where HbA1c = 7.5%) was 1.8%.
* Leptin - (Human Leptin RIA kit, Linco Research, St Charles, USA). Measures human leptin using a rabbit monoclonal antibody. Range 0.5-100ng/ml. Sensitivity 0.5ng/ml. Intra-assay coefficient of variation 3.9%, inter-assay coefficient of variation 4.7% at mid range values.
* Cortisol - Measured by a commercially available solid phase competitive chemiluminescent enzyme immunoassay (DPC Immulite, Diagnostics Product Corporation, USA). The Immulite system utilizes assay-specific, antibody or antigen-coated plastic beads as the solid phase, alkaline phosphate-labelled reagent, and a chemiluminescent enzyme substrate. The analytical sensitivity was 5.5 nmol/l. Measurement range 28-1300nmol/l. Coeffiecients of variation vary from 6.4 to 10.8 over the analytical range.
* IGF-1 - Measured by 2 site radioimmunoassay using murine antibodies following acid-ethanol IGF-1 extraction from binding protein – (Medgenix, Brussels, Belgium). The range over which the with a coefficient of variation remains <10% is 17-1073 ng/ml. Sensitivity 0.25ng/ml.
* Fasting lipids – Assayed by high performance liquid chromatography (HPLC) with lipoproteins separatedby gel permeation column (TSKgel LipopropakXL; Tosoh).

Samples were assayed at the laboratories at Sheffield Children’s Hospital (Glucose, Lipids) under the supervision of Dr Jim Bonham and at the Royal Hallamshire Hospital (Insulin, HbA1c, Cortisol, IGF-1) under the supervision of Dr Kevin Page. Leptin was measured by Sue Justice at the Northern General Hospital. The child was then asked to drink a glucose load (1.75g/kg) in the form of Lucozade. A single further blood sample for blood glucose was taken via the cannula at 2 hours to complete the glucose tolerance test.

During the morning the second voided urine of the day was collected for a spot urinary steroid profile. Urinary steroid metabolites were measured as a ratio to creatinine on a spot morning sample. This method was chosen as it was felt that obtaining 24-hour urine collections would significantly reduce compliance and the quantity of data available. Using spot urine profiles has limitations in that it doesn’t represent the total steroid metabolite output over the day. In particular it fails to take account of the diurnal variation in cortisol secretion and androgen production. However important qualitative and semiquantitative information can be obtained by expressing metabolite excretion as a ratio against urinary creatinine (Honour 2001). In clinical practice the value and efficacy of spot urine profiles approaches that of 24 hour collections particularly in younger children. The control population within the study provided robust normative data for comparative purposes.

Urinary creatinine was measured by spectrophotometer using a method traceable to an isotope dilution mass spectrometry (IDMS) reference method. Urinary steroid profile assays were performed at the Royal Hallamshire Hospital by Ailsa Rogers. Samples were subjected to enzymatic hydrolysis followed by reverse phase solid chromatography to separate contaminants in the aqueous phase. An internal standard was added and a bicarbonate wash performed to remove the phenolic steroids. Samples were then subjected to gas chromatography with separation and quantification of compounds in relation to internal standards. The urinary metabolites of the adrenal androgen Dihydroepiandrosterone sulphate (DHEAS) principally androsterone and aetiocholanolone were summed and called the total androgen metabolites. Various cortisol and cortisone metabolites were measured including tetrahydrocortisone (THE), tetrahydrocortisol (THF), allo-tetrahydrocortisol (αTHF), alpha and beta-cortolone and alpha and beta -cortol. These were summed to give a measure of the levels of cortisol metabolites present. From the ratio of cortisol metabolites to cortisone metabolites the activity of 11 beta hydroxysteroid dehydrogenase can be inferred. The ratio of the cortisol metabolites (THF and αTHF) to the cortisone metabolite (THE), the THF+αTHF:THE ratio, gives an indirect measure of overall 11βHSD activity. We also used an additional measure of 11βHSD activity derived from all the cortisol and cortisone metabolites:

THF + αTHF + α cortol + β cortol

THE + α cortolone + β cortolone

The THF: αTHF ratio gives a measure of the A ring reductase activity.

Many of the steroid metabolites were not normally distributed. They were subjected to a logarithmic transformation to a normal distribution before statistical analysis.

Details of maternal hypertension, diabetes, pre-eclampsia and of antenatal and postnatal steroid administration were obtained from a series of databases. The CRIB score (clinical risk index for babies – see figure 3) was used as a measure of illness severity in the preterm infants (The International Neonatal Network 1993). This data had been collected prospectively at birth and was obtained from:

* Neonatal Unit database (data relating to mode of delivery, fetal distress, measures of illness severity in the neonatal period and steroid use)
* Hospital PROTOS database (maternal history, family history, social class, smoking)
* Trent Regional Neonatal survey (maternal history, steroid use, delivery and neonatal course)
* Discharge summaries.

Figure 3: The CRIB score is composed of the following components:

|  |  |
| --- | --- |
| **Factor** | **Score** |
| **Birth weight (g)** |  |
| >1350 | 0 |
| 851-1350 | 1 |
| 701-850 | 4 |
| ≤700 | 7 |
| **Gestation (week)** |  |
| >24 | 0 |
| ≤24 | 1 |
| **Congenital malformations** |  |
| None | 0 |
| Not acutely life threatening | 1 |
| Acutely life threatening | 3 |
| **Maximum base excess in first 12 hours** |  |
| >-7.0 | 0 |
| -7.0 to -9.0 | 1 |
| -10.0 to -14.9 | 2 |
| ≤ - 15.0 | 3 |
| **Minimum FiO2 in first 12 hours** |  |
| ≤0.40 | 0 |
| 0.41-0.80 | 2 |
| 0.81-0.90 | 3 |
| 0.91-1.00 | 4 |
| **Maximum FiO2 in first 12 hours** |  |
| ≤0.40 | 0 |
| 0.41-0.80 | 1 |
| 0.81-0.90 | 3 |
| 0.91-1.00 | 5 |

Gestational age was taken from the dating scan where performed and if not from the last menstrual period. The steroid regimen in use at the time in threatened preterm labour was two doses of betamethasone 12mg given intramuscularly 12 hours apart. In some cases mothers received repeated courses given at weekly intervals where the risk of premature labour remained high.

3.3.1 Clinical measurement

All measurements of height, weight and body mass index (BMI - weight/height2) were converted to standard deviation scores (SDS) or Z-scores. The 1990 UK standards were used to calculate SDS for all measurements of weight and for measurements of length taken beyond 42 weeks gestation (Freeman *et al.* 1995;Cole *et al.* 1995). The standards suggested by Keen and Pearse were for all children, for length and weight, (including term infants) up to 42 weeks gestation (Keen and Pearse 1988).

Standard deviation score = (patient measurement – population mean)

Population standard deviation

Although the children participated in the metabolic study around 5 years of age, the longitudinal study of their growth has continued. Data is available on the children’s growth trajectories up 7 years of age and is presented.

There are no published standards for body mass index in the preterm infants below 33 weeks. It was not possible to establish BMI standard deviation scores for this group so the data was examined in its raw form. All infants had their triceps and subscapular skinfold measurement taken at birth. The percentage of body fat was calculated from biceps and subscapular skin fold thicknesses using the equation proposed by Brook (Brook 1971). This equation was used at all time points. The equation has not been validated in the preterm population and may not be entirely appropriate. However there are no validated methods to measure fat in this group. Magnetic resonance imaging has been used latterly but this was not available at the time.

Maternal and paternal heights were measured in the neonatal period. The midparental or target adult height was calculated for each child as below (Freeman *et al.* 1995):

Males = (maternal height + paternal height) + 7

2

Females = (maternal height + paternal height) - 7

2

The target height centile SDS was calculated and a comparision between the childs actual height SDS and their expected or target height SDS was made (Actual height SDS – Target height SDS).

3.3.2 Insulin Resistance and Atherogenic Index

Various methods of assessing insulin resistance have been proposed (Ferrannini and Mari 1998). There is considerable debate about the most appropriate measures to use (Cutfield and Hofman 2005). We selected the fasting insulin:glucose ratio (Vuguin, Saenger, and Dimartino-Nardi 2001;Legro, Finegood, and Dunaif 1998), and the Homeostasis model assessment (HOMA) index of insulin resistance (Matthews *et al.* 1985). Both of these methods are simple and correlate well with more sophisticated measures such as the euglycaemic hyperinsulinaemic clamp (Matthews *et al.* 1985;Vuguin *et al.* 2001;Cutfield and Hofman 2005). We also had difficulty obtaining ethics committee approval for the study which restricted us to only zero and 120 minute samples on the oral glucose tolerance test.

HOMA score = Glucose (mmol/l) x Insulin (mU/l)

22.5

Cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) were measured. The Atherogenic index was used to combine aspects of the lipid profile as it is a reliable marker of cardiovascular risk (Despres *et al.* 2000):

Atherogenic index = (Cholesterol –HDL)

HDL

3.4 Statistical Methods

Advice as to statistical methods was taken from a statistician (Alan Rigby – Lecturer in Medical Statistics – Sheffield University). All the statistical tests were carried out by myself using SPSS version 9.0.

For clarity of presentation much of the data is presented grouped, although gestational age is a continuous variable, according to the degree of prematurity (< 30 weeks, 30-34 weeks, 34 –37 weeks and term) as it’s importance was a key hypothesis we wished to test. These categories were adopted because they delineate subgroups within the cohort. Less than 30 weeks was the gestation at which neonates were usually electively ventilated initially on our unit at that time. Onset of labour at or below 34 weeks is the criteria for considering antenatal steroid administration to the mother to promote fetal lung maturation. Term delivery was considered to be beyond 37 weeks completed gestation. The longitudinal growth data was further subdivided into those born between 23-26 weeks and 27-30 weeks.

A combination of T-tests and ANOVA were used to assess the significance of differences between the groups where appropriate. Pearson correlation coefficients and scatter plots were also used to examine relationships as continuous variables (without grouping). Where relationships were identified a detailed examination of data was performed using multiple regression with binary categorical variables, eg. gender, entered as dummy variables. A normal distribution was confirmed using the Kolmogorov-Smirnov test. Data that was not normally distributed (principally urinary steroid metabolites and CRIB scores) was subjected to logarithmic transformation to obtain a normal distribution prior to statistical analysis.

Prior to the study power calculations suggested that to detect a difference in mean systolic blood pressure of 5 mmHg (80% power with 95% confidence) a sample of approximately 130 children was required and to detect a difference in HbA1c of 0.1% (80% power with 95% confidence) approximately 190 children and a difference of 0.2% (80% power with 95% confidence) approximately 50 children were required.

Chapter 4 : Size at Birth, Size age 5 years and Patterns of Growth since Birth.

4.1 Demographic data

The demographic background data of the cohort is presented in Table 1. As previously stated the cohort was divided into four groups according to gestational age at birth in order facilitate analysis and provide clarity in presentation of the results.

The groups were similar in age when they took part in the study and well matched for gender. Gestational age at birth ranged from 23 weeks - 42 weeks. The control group comprised only singleton infants. The preterm group included infants born following a multiple pregnancy (35/119) - these were mainly twins but include two sets of triplets. The incidence of multiple birth was highest in the group of born 34-37 weeks gestation – 38% (which is understandable as twin pregnancies frequently do not progress to 40 weeks). As anticipated the majority of infants who received antenatal steroids were born before 34 weeks gestation. Approximately 25 per cent of those born between 34-37 weeks received antenatal steroids. These were pregnancies where there was concern regarding possible premature delivery before 34 weeks but where the pregnancy continued and the infants were subsequently delivered after thirty-four weeks completed gestation. In those individuals born most prematurely (<30 weeks gestation) a proportion (23%) received postnatal steroids (dexamethasone) for chronic lung disease of prematurity. A history of maternal pregnancy induced hypertension was significantly higher in all the preterm groups – particularly those born between 34-37 weeks gestation. This was anticipated given that it is a significant factor in obstetric decisions to deliver prematurely. At between 34-37 weeks gestation the risks to the baby of preterm delivery are considered small and delivery may be precipitated by concern for maternal well-being.

There were no significant differences between the groups in the incidence of a family history of diabetes, heart disease or hypertension.

Table 1. Baseline data regarding sex, multiple pregnancies, administration of antenatal & postnatal steroids, and family history of diabetes and hypertension for the cohort presented according to gestational age at birth.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | <30 weeks (n=31) | 30-34 weeks (n=51) | 34-37 weeks (n=37) | > 37 weeks (n=39) |
| Age when glucose tolerance test performed | | 5.0 years (SD±0.5) | 5.1 years (SD±0.6) | 5.1 years (SD±0.6) | 5.4 years (SD±0.7) |
| Sex | Male | 48% (15) | 47% (24) | 49% (18) | 53% (21) |
|  | Female | 52% (16) | 53% (17) | 51% (19) | 47% (18) |
| Percentage of individuals born following a multiple pregnancy | | 19% (6) | 29% (15) | 38% (14) | 0% |
| Percentage who received antenatal steroids | | 65% (20) | 71% (36) | 24% (9) | 0% |
| Percentage who received multiple courses of antenatal steroids | | 22% (7) | 29% (15) | 5% (2) | 0% |
| Percentage who received postnatal steroids | | 23% (7) | 2% (1) | 3% (1) | 0% |
| Percentage with maternal pregnancy induced hypertension | | 13% (4) | 33% (17) | 38% (14) | 3% (1) |
| Family history diabetes | | 20% (6) | 31% (16) | 29% (11) | 16% (6) |
| Family history hypertension | | 23% (7) | 18% (9) | 27% (10) | 23% (9) |
| Family history heart disease | | 19% (6) | 25% (13) | 32% (12) | 26% (10) |

Figures in brackets represent actual number of patients

n = number of subjects in each group

4.2 Size at birth

Details of the infants size at birth and proportion of body fat at birth are given in Table 2. Preterm infants as anticipated had a lower birthweight than term controls. In order to differentiate whether the infants were small because of prematurity or whether there was an additional component of intrauterine growth retardation standard deviation scores were compared. The group born between 34-37 weeks gestation were significantly smaller and lighter but with sparing of the head (lower birth weight SDS and lower birth length SDS) compared to term controls, those infants born before 30 weeks gestation and those born between 30-34 weeks gestation. We postulated that this may be due to an increased incidence of twins in this group. The data were then analysed having excluded multiple births and although the differences were now less marked and no longer statistically significant (perhaps in part due to the reduced number of subjects) this trend persisted (see Table 3). However when adjusted for parental height (by calculating the mid parental centile and its standard deviation score) it became apparent that their parents were minus 0.5 standard deviations smaller than the population mean (see Table 4) and smaller than parental heights in other groups. It seems most likely that these children were smaller because their parents were smaller although we are unable to account for why this should be the case. Although they were smaller there was no evidence that they were growth retarded or thinner than other children in the study group.

The ponderal index and body mass index at birth are presented. These are significantly lower in preterm infants and were positively correlated with gestational age at birth. There are no reliable standards from which to calculate standard deviation scores for these values so the data is therefore presented in its raw form. Similarly all infants had their triceps and subscapular skinfold measurement taken at birth. Data is presented in raw form and as a percentage of body fat (calculated using Brook’s equation). Preterm infants as anticipated had a significantly lower sum of their triceps and subscapular skinfolds. All measures of adiposity indicated that the more preterm an infant the lower the percentage body fat.

Table 2. Mean gestational age for each group together with size at birth expressed as a standard deviation score (SDS) along with ponderal index, body mass index and percentage body fat at birth.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | | ANOVA |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD | P- value |
| Gestational age | **28.1** | 1.4 | **32.2** | 1.2 | **35.5** | 0.7 | **40.8** | 0.8 |  |
| Birth weight (g) | **1124** | 263 | **1767** | 392 | **2234** | 432 | **3518** | 503 |  |
| Birth weight SDS | **-0.2** | 0.8 | **-0.2** | 1.0 | **-0.8\*** | 1.2 | **-0.3** | 1.0 | **0.04** |
| Birth length SDS | **-0.5** | 0.6 | **-0.5** | 0.8 | **-1.1\*** | 1.2 | **-0.4** | 1.0 | **0.02** |
| Head circumference SDS at birth | **-0.2** | 0.6 | **0.1** | 1.0 | **0.0** | 1.0 | **-0.2** | 0.8 |  |
| Ponderal index at birth | **22.8** | 1.7 | **23.8** | 2.2 | **24.1** | 2.0 | **27.0** | 2.1 | **<0.001** |
| Body mass index at birth | **8.3** | 1.0 | **10.0** | 1.3 | **10.8** | 1.1 | **13.7** | 1.2 | **<0.001** |
| Sum of triceps & subscapular skin folds (mm) | **5.2** | 0.7 | **6.1** | 1.4 | **6.7** | 1.5 | **8.8** | 1.4 | **<0.001** |
| Percentage fat at birth | **4.2%** | 0.9 | **5.2%** | 1.6 | **5.9%** | 1.7 | **8.3%** | 1.5 | **<0.001** |

Figures in table are Mean (bold) and standard deviation (SD)

Significance values related to ANOVA

\* = p< 0.05

n = number of subjects in each group

Table 3. Mean weight, length and head circumference standard deviation scores (SDS) at birth recalculated having excluded all multiple births from the cohort.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=25) | | 30-34 weeks (n=36) | | 34-37 weeks (n=23) | | > 37 weeks (n=39) | | ANOVA |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD | P- value |
| Birth weight SDS | **-0.1** | 0.9 | **-0.2** | 1.1 | **-0.4** | 1.2 | **-0.3** | 1.0 | **0.70** |
| Birth length SDS | **-0.5** | 0.6 | **-0.6** | 0.8 | **-0.8** | 1.1 | **-0.2** | 1.0 | **0.12** |
| Head circumference SDS | **-0.3** | 0.7 | **0.1** | 1.0 | **0.1** | 1.1 | **-0.2** | 0.8 | **0.39** |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

There were no statistically significant differences

4.3 Size age 5 years

At the age of 5 years the children born most prematurely, were significantly smaller (Height SDS -0.9) and lighter (Weight SDS -0.9) than those born either at term or beyond 30 weeks gestation (see Table 4). The most preterm children were significantly smaller than would be expected from their target mid-parental height (-0.8 SD). There was no difference in head circumference. There was no significant difference in terms of BMI but they had a reduced percentage of their total body mass as fat. This trend for the preterm infants to be leaner was evident across the range of prematurity (Pearson correlation coefficient 0.14, p= 0.08) and not just in the most premature children.

Table 4. Height and weight aged 5 years compared to mid-parental centile together with BMI and measures of fat mass calculated from skin folds.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Age (years) | **5.0** | 0.5 | **5.1** | 0.6 | **5.1** | 0.6 | **5.4** | 0.7 |
| Height (cm) | **105.1\*\*** | 6.8 | **109.5** | 5.3 | **108.2** | 6.8 | **110.9** | 6.8 |
| Height SDS | **-0.9\*\*** | 1.4 | **-0.0** | 0.9 | **-0.4** | 1.1 | **-0.1** | 1.1 |
| Weight (kg) | **17.0\*** | 3.2 | **18.3** | 2.5 | **18.0** | 3.3 | **19.0** | 3.3 |
| Weight SDS | **-0.9\*** | 1.6 | **-0.2** | 0.9 | **-0.5** | 1.4 | **-0.2** | 1.2 |
| BMI (kg/m2) | **15.3** | 1.6 | **15.2** | 1.7 | **15.3** | 2.0 | **15.4** | 1.3 |
| BMI SDS | **-0.3** | 1.1 | **-0.4** | 1.0 | **-0.4** | 1.7 | **-0.2** | 1.0 |
| Head circumference SDS | **-0.5** | 1.3 | **-0.1** | 1.1 | **-0.4** | 1.1 | **-0.1** | 1.2 |
| Sum of Triceps & Subscapular skin folds (mm) | **13.3** | 3.3 | **13.9** | 4.0 | **14.6** | 3.6 | **14.5** | 3.5 |
| Fat mass as a percentage of total body mass | **12.8\*** | 3.1 | **13.3** | 3.4 | **14.0** | 3.3 | **13.9** | 3.1 |
| Mid-Parental height SDS | **-0.2** | 1.0 | **0.1** | 0.9 | **-0.5\*** | 1.2 | **0.0** | 0.8 |
| Difference between individuals height SDS and their mid-parental SDS | **-0.8\*\*** | 1.4 | **-0.1** | 0.8 | **0.1** | 1.0 | **0.0** | 0.9 |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

ANOVA \* = p < 0.05, \*\* = p<0.001

4.4 Longitudinal Growth

Figure 4 illustrates the change in weight SDS during childhood according to the gestational age at birth. Similarly Figure 5 illustrates the change in length SDS according to the gestational age at birth. The data illustrates the dramatic early failure of both linear growth and weight gain associated with being born prematurely. The more extreme the degree of prematurity the more marked the growth failure. From being an appropriate weight and length for their gestation at birth those infants at the extremes of prematurity (23-27 weeks gestation) demonstrated very poor growth and weight gain during the first two years of life falling almost 2.5 standard deviations in both length and weight. Poor early growth continues well beyond the period of time when the infants are nursed on the neonatal unit. Continued poor growth is evident after discharge from hospital. Following early growth failure preterm infants show steady catch up growth continuing over many years. At 5 years when this metabolic study took place those infants born most prematurely were still showing catch up growth – the data shows that it continued until at least 7 years of age. Figure 6 illustrates patterns of head growth (change in head circumference SDS). Those born before 30 weeks gestation demonstrate poor early growth.

Much of the early growth failure occurs within the first few weeks of life. Figures 7, 8 and 9 illustrate this rapid early fall across centiles with poor weight gain, poor linear growth and reduced early head growth.

Figure 4. Change in mean Weight standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born.

Figure 5. Change in mean Length and after 2 years mean Height standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born.

Figure 6. Change in mean Head Circumference standard deviation score with age (months - nonlinear scale) according to the gestational age at which the child was born.

Figure 7. Change in mean Weight standard deviation score over the first 8 weeks of life according to gestational age at birth.

Figure 8. Change in mean Length standard deviation score over the first 8 weeks of life according to gestational age at birth.

Figure 9. Change in mean Head Circumference standard deviation score over the first 8 weeks of life according to gestational age at birth.

Additional more detailed data on the cohort’s size at birth, size at 1 year and size age 5 years is presented in the Appendix. Figures 17, 18 and 19 show scatterplots of weight standard deviation score at birth, 1 year and 5 years plotted against gestational age at birth. Figures 20, 21 and 22 show scatterplots of length and height standard deviation score at birth, 1 year and 5 years plotted against gestational age at birth. Figures 23 and 24 show scatterplots of head circumference standard deviation score at birth and 5 years plotted against gestational age at birth.

Data is also shown grouped according to gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and term). Weight standard deviation score at birth, 1 year and 5 years is shown in figures 25, 26 and 27. Length and height standard deviation score at birth, 1 year and 5 years is shown in figures 28, 29 and 30. Head circumference standard deviation score at birth and 5 years is shown in figures 31 and 32.

Chapter 5: Prematurity, Antenatal Events and Blood Pressure, Glucose Homeostasis and Lipids aged 5 years.

One of the key objectives of the study was to examine the effects of prematurity on blood pressure, glucose metabolism and lipids at five years of age

5.1 Blood Pressure – relationship with current size, prematurity and antenatal intervention

As would be expected systolic, diastolic and mean blood pressure were all positively correlated with current height, weight and BMI. Higher blood pressure was also positively associated with increasing adiposity. Resting heart rate was inversely related to height. There was no correlation between heart rate and weight, BMI and percentage body fat (see Table 5). There was no relationship between gender and blood pressure or resting heart rate.

The most important relationships were those between blood pressure, resting heart rate, gestational age at birth and the administration of antenatal glucocorticoid steroids (see Table 6). Systolic, diastolic, mean blood pressure and a higher resting heart rate were all significantly inversely correlated to gestational age at birth. The more preterm the infant the higher the blood pressure and the higher the resting heart rate (see Figure 10). As preterm infants were also smaller and lighter this inverse relationship was strengthened if the data were controlled for current size (height and weight). Prior to correction for current height and weight the correlation between systolic blood pressure and gestation was r = -0.29 (p <0.0001) and after correction r = -0.37 (p < 0.0001). The administration of antenatal steroids was positively correlated with systolic blood pressure, mean blood pressure and resting heart rate (see Table 7). Those infants who received antenatal steroids had higher blood pressure age 5 years. The number of courses doses of antenatal steroids given prior to delivery also correlated with a systolic blood pressure and resting heart rate. The use of postnatal steroids was not significantly related to blood pressure but was related to resting heart rate with those who received post natal steroids having a higher resting heart rate. However antenatal and postnatal steroid use are all related to the degree of prematurity.The positive relationship between systolic blood pressure and the use of antenatal steroids remained statistically significant having controlled for current size and gestational age at birth. The relationship with the number doses received and postnatal steroids was no longer apparent.

The strongest relationship was between blood pressure, heart rate and gestational age. But systolic blood pressure, diastolic blood pressure and resting heart rate were also initially appeared to be associated with a number of other variables. These included, a history of maternal pregnancy induced hypertension, birthweight, birth weight SDS, ponderal index and body mass index at birth and CRIB score (a measure of illness severity in the neonatal period) (The International Neonatal Network 1993). However gestation is an important confounder for all these variables as they are often dependant on each other. When gestational age was controlled for the relationships disappeared except for the relationship between maternal eclampsia and resting heart rate.

There was no correlation between a family history of hypertension or diabetes and later blood pressure or resting heart rate. There was no relationship between multiple pregnancy (i.e. twins or triplets) and blood pressure but there was a positive correlation with resting heart rate.

Given the complexity of the data and the dependence of many variables upon other variables, stepwise multiple regression models were constructed to examine the most important predictors of blood pressure and heart rate. Variables included in the models were gestation, current height and weight SDS, birth length height and weight SDS, gender, multiple pregnancy, antenatal steroids, postnatal steroids, maternal eclampsia and CRIB score. Systolic blood pressure was predicted by gestational age at birth, administration of antenatal steroids, low birth weight SDS and weight SDS age 5 years ( r2 = 0.27). Diastolic blood pressure was predicted by weight SDS age 5 years and a history of maternal pregnancy induced hypertension. Gestational age and weight SDS age 5 years ( r2 = 0.08) predicted mean blood pressure. Gender, being born following a multiple pregnancy and the CRIB score were not significant factors in any of the models.

Regression models therefore suggested that prematurity, having received antenatal steroids, low birthweight for gestation, high current weight and maternal preeclampsia were most important in determining subsequent blood pressure and heart rate. Of these variables gestation was the most important predictor in the models. Systolic blood pressure was almost 7mmHg higher in those born before 30 weeks compared to term controls. Based on the slope of the regression line describing the relationship between systolic blood pressure and gestation each week of prematurity increased systolic blood pressure by 0.5mmHg

Table 5. The relationship between current height, weight, BMI and adiposity and blood pressure & resting heart rate. (Pearson correlation coefficient)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Systolic Blood Pressure | | Diastolic Blood Pressure | | Mean Blood Pressure | | Resting Heart Rate | |
|  | r | P value | r | P value | r | P value | r | P value |
| Height | **0.157** | **0.05** | **0.179** | **0.026** | 0.110 | 0.174 | **-0.209** | **0.009** |
| Weight | **0.269** | **<0.001** | **0.247** | **0.002** | **0.210** | **0.009** | -0.132 | 0.100 |
| BMI | **0.258** | **<0.001** | **0.198** | **0.014** | **0.221** | **0.006** | 0.015 | 0.853 |
| Height SDS | **0.255** | **<0.001** | **0.212** | **0.008** | **0.184** | **0.022** | -0.148 | 0.065 |
| Weight SDS | **0.321** | **<0.001** | **0.278** | **<0.001** | **0.260** | **0.001** | -0.098 | 0.222 |
| BMI SDS | **0.238** | **0.003** | **0.219** | **0.006** | **0.206** | **0.011** | 0.002 | 0.975 |
| % of body mass as fat | 0.166 | 0.06 | 0.151 | 0.087 | 0.164 | 0.063 | 0.037 | 0.679 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

Table 6. Blood pressure and resting heart rate age 5 years and its relationship to gestational age at birth

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | | ANOVA |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD | P- value |
| Systolic Blood Pressure (mmHg) | **101.9** | 8.7 | **100.2** | 8.0 | **95.9** | 7.3 | **95.1** | 7.1 | **< 0.001** |
| Diastolic Blood Pressure (mmHg) | **53.8** | 6.6 | **54.7** | 5.6 | **54.7** | 5.0 | **51.9** | 5.4 | **0.084** |
| Mean Blood Pressure (mmHg) | **73.2** | 6.2 | **73.4** | 6.3 | **71.4** | 7.3 | **69.2** | 5.6 | **0.011** |
| Resting Heart Rate (beats/min) | **101.7** | 11.4 | **97.7** | 11.8 | **98.1** | 10.0 | **95.5** | 11.7 | **0.028** |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

Figure 10. Systolic blood pressure aged 5 years with correlated with gestational age at birth. (Pearson correlation coefficient).

r = -0.29, P<0.001

Table 7. Correlation of blood pressure and heart rate age 5 years with size at birth, antenatal and postnatal steroid administration, maternal and family history of cardiovascular risk factors and CRIB score, (Uncorrected for gestational age at birth)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Systolic Blood Pressure | | Diastolic Blood Pressure | | Mean Blood Pressure | | Resting Heart Rate | |
|  | r | P value | r | P value | r | P value | r | P value |
| Gestational Age at birth | **-0.286** | **<0.001** | -0.134 | 0.095 | **-0.243** | **0.002** | **-0.176** | **0.028** |
| Multiple birth | 0.061 | 0.452 | 0.013 | 0.873 | 0.086 | 0.289 | **0.168** | **0.036** |
| Birth Weight | **-0.205** | **0.010** | -0.113 | 0.160 | -**0.186** | **0.021** | **-0.204** | **0.011** |
| Birth Weight SDS | **0.173** | **0.031** | 0.094 | 0.241 | 0.116 | 0.150 | -0.069 | 0.392 |
| Birth Length SDS | **0.218** | **0.017** | -0.029 | 0.749 | 0.102 | 0.268 | -0.117 | 0.201 |
| Antenatal steroid administration | **0.201** | **0.012** | 0.075 | 0.352 | **0.177** | **0.028** | **0.207** | **0.010** |
| Number doses of antenatal steroid | **0.177** | **0.027** | 0.043 | 0.592 | 0.141 | 0.080 | **0.196** | **0.014** |
| Postnatal steroids | 0.011 | 0.895 | 0.080 | 0.323 | 0.072 | 0.374 | **0.230** | **0.004** |
| Maternal pre-eclampsia | 0.053 | 0.512 | **0.253** | **0.001** | **0.209** | **0.009** | **0.189** | **0.018** |
| Family history of Diabetes | 0.009 | 0.909 | -0.014 | 0.863 | 0.070 | 0.391 | -0.091 | 0.259 |
| Family history of Hypertension | 0.019 | 0.813 | -0.018 | 0.825 | -0.032 | 0.691 | -0.064 | 0.430 |
| CRIB score | **0.181** | **0.024** | 0.057 | 0.478 | 0.141 | 0.081 | 0.095 | 0.238 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

5.2 Glucose metabolism

All the children underwent a standard oral glucose tolerance test with samples taken at zero and 2 hours. Glycosylated haemoglobin was also measured although this is a poor measure of glucose tolerance. The fasting insulin:glucose ratio and the HOMA index were used as a measure of insulin resistance.

The blood glucose and insulin levels of all children were within the normal range. Plasma blood glucose at 120 minutes, the fasting insulin:glucose ratio and the HOMA index of insulin resistance were all positively and significantly correlated with measures of current size, weight and adiposity ( height, weight and BMI standard deviation scores and percentage body fat). The heaviest children with the highest proportion of body fat were most insulin resistant. Interestingly glycosylated haemoglobin was inversely related to current height and weight standard deviation scores. Female gender was also associated with insulin resistance (see Table 8).

The data were examined for relationships between the measures of glucose homeostasis and the various factors associated with prematurity. Gestational age at birth was inversely related to the fasting insulin:glucose ratio (see Table 9). Prematurity was strongly associated with subsequent insulin resistance and the more premature the infant the greater the degree of insulin resistance age 5 years (see Table 10 and Figure 11).

There was no evidence of any relationship between antenatal and postnatal steroid use, multiple birth or a family history of diabetes and subsequent insulin resistance. A history of maternal pregnancy induced hypertension was associated with later insulin resistance. It initially appeared that there may be a relationship between severity of illness (CRIB score) and a raised fasting insulin:glucose ratio, though it just failed to reach statistical significance. However when corrected for gestation there was no relationship between severity of illness and glucose metabolism.

There was no relationship between fasting glucose and gestation (see figure 12). Two hour plasma blood glucose was positively related to gestation (see figure 13). The blood glucose two hours after a glucose load was highest in term infants and lowest in those born prematurely. There were also statistically significant relationships between being born from a multiple pregnancy and having received antenatal steroids and plasma glucose at 2 hours. Two hour glucose was lower in those born following multiple pregnancies and in those who had received antenatal steroids.

Table 8. Size and adiposity aged 5 years and the relationship with measures of glucose metabolism

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Fasting glucose | | Glucose  2 hours | | HbA1c | | Fasting Insulin:glucose ratio | | HOMA Index Insulin resistance | |
|  | r | P | r | P | r | P | r | P | r | P |
| Female Sex | 0.069 | 0.438 | 0.077 | 0.440 | -0.078 | 0.390 | **0.288** | **0.001** | **0.249** | **0.005** |
| Height SDS | 0.115 | 0.201 | **0.260** | **0.009** | -0.171 | 0.057 | **0.183** | **0.044** | **0.188** | **0.035** |
| Weight SDS | 0.150 | 0.091 | **0.299** | **0.002** | **-0.195** | **0.029** | **0.262** | **0.003** | **0.263** | **0.003** |
| BMI SDS | 0.105 | 0.242 | **0.197** | **0.048** | -0.133 | 0.140 | **0.220** | **0.015** | **0.212** | **0.018** |
| % body fat | 0.025 | 0.784 | **0.307** | **0.002** | -0.029 | 0.753 | 0.173 | 0.065 | 0.170 | 0.066 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

Table 9. The effect of Gestation, size at birth, use of perinatal steroids on measures of glucose metabolism aged 5 years.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Fasting glucose | | Glucose  2 hours | | HbA1c | | Fasting Insulin:glucose ratio | |
|  | r | P | r | P | r | P | r | P |
| Gestational Age at birth | 0.115 | 0.199 | **0.247** | **0.012** | -0.094 | 0.297 | **-0.184** | **0.042** |
| Multiple birth | -0.030 | 0.735 | **-0.256** | **0.009** | 0.004 | 0.962 | -0.010 | 0.916 |
| Birth Weight SDS | -0.016 | 0.861 | 0.016 | 0.877 | 0.017 | 0.852 | -0.058 | 0.526 |
| Birth Length SDS | 0.029 | 0.782 | -0.025 | 0.820 | -0.050 | 0.632 | -0.065 | 0.536 |
| Percentage body fat at birth | -0.003 | 0.976 | 0.157 | 0.163 | 0.001 | 0.994 | -0.115 | 0.290 |
| Antenatal steroids | -0.073 | 0.413 | **-0.195** | **0.049** | 0.069 | 0.445 | -0.018 | 0.842 |
| Number doses of antenatal steroid | -0.045 | 0.616 | -0.187 | 0.060 | -0.011 | 0.901 | -0.023 | 0.798 |
| History maternal pre-eclampsia | 0.097 | 0.279 | 0.050 | 0.618 | -0.021 | 0.815 | **0.180** | **0.046** |
| Family history of Diabetes | 0.050 | 0.575 | 0.010 | 0.922 | 0.142 | 0.116 | 0.034 | 0.708 |
| CRIB score | -0.012 | 0.896 | -0.065 | 0.514 | 0.105 | 0.243 | 0.172 | 0.057 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

Table 10. Gestational age at birth and its relationship with measures of glucose metabolism aged 5 years.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Fasting Glucose (mmol/l) | **4.2** | 0.5 | 4.1 | 0.4 | **4.3** | 0.6 | **4.3** | 0.5 |
| Glucose at 2 hours following OGTT (mmol/l) | **4.4\*** | 1.0 | **4.6\*** | 1.0 | **4.9\*** | 0.9 | **5.1\*** | 0.9 |
| Glycosylated Haemoglobin (%) | **4.8** | 0.3 | 4.8 | 0.3 | **4.8** | 0.3 | **4.7** | 0.3 |
| Fasting Insulin (mU/l) | **3.4\*** | 2.3 | **3.4\*** | 2.4 | **2.7\*** | 2.1 | **2.6\*** | 1.6 |
| Fasting Insulin:Glucose ratio | **1.31\*** | 0.76 | **1.26\*** | 0.78 | **1.05\*** | 0.70 | **1.01\*** | 0.52 |
| HOMA Index – Insulin resistance | **0.68\*** | 0.49 | **0.63\*** | 0.52 | **0.54\*** | 0.47 | **0.52\*** | 0.34 |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

\* = p<0.05

Figure 11. Fasting insulin:glucose ratio grouped by gestational age at birth



<30 weeks 30-34 weeks 34-37 weeks >37 weeks

Figure 12. Scatter plot showing fasting glucose (mmol/l) grouped by gestational age at birth



<30 weeks 30-34 weeks 34-37 weeks >37 weeks

Figure 13. Glucose (mmol/l) at 2 hours following an oral glucose tolerance test ratio grouped by gestational age at birth



<30 weeks 30-34 weeks 34-37 weeks >37 weeks If the data are adjusted for the effect of current weight and height then the inverse relationship between gestational age at birth and subsequent insulin resistance persists. The relationship between 2 hour glucose and being born following a multiple pregnancy is weakened and just fails to reach statistical significance ( r = -0.19, p= 0.08). Having corrected for the effect of current height and weight the severity of illness in the neonatal period then becomes significantly associated with later insulin resistance. The CRIB score becomes correlated with the fasting insulin:glucose ratio ( r = 0.22, p = 0.04) and the HOMA index ( r = 0.23, p = 0.03). Controlling for gender in addition did not affect the relationships.

If this data is corrected for the effect of gestation then antenatal and postnatal steroid usage becomes related to later insulin resistance (antenatal steroids r = 0.23, p = 0.03 and r = 0.21, p = 0.04, postnatal steroids r = 0.23, p = 0.03 and r = 0.26, p = 0.01 for Insulin:glucose ratio and HOMA respectively). This suggests that steroids may promote a weak tendency to develop insulin resistance later which may have initially been masked by the adverse effect of prematurity. Adjusting for gender did not alter the relationships.

Stepwise multiple regression models were constructed with the following variables gestation, current height and weight SDS, birth length height and weight SDS, gender, multiple pregnancy, antenatal steroids, postnatal steroids, maternal eclampsia and CRIB score. Insulin resistance was predicted by current weight SDS, female sex and gestational age at birth ( r2 = 0.19). Only current weight SDS predicted the fasting blood glucose and 2 hour glucose ( r2 = 0.07 and r2 = 0.13 respectively) – multiple delivery and administration of antenatal steroids were not significant in the model.

5.3 Lipids

An adverse lipid profile with elevated cholesterol, triglyceride and low density lipoprotein (LDL) with reduced levels of high density lipoprotein (HDL) was evident as one might have anticipated in those with the highest BMI and highest levels of body fat (see Table 11).

Administration of postnatal steroids was positively associated with a raised HDL and as a result a reduced atherogenic index (see Table 12). Female gender correlated with higher levels of cholesterol and LDL. A higher CRIB score in the neonatal period was also positively correlated to a higher HDL value although this did not quite reach statistical significance. Birth weight standard deviation score was correlated with a higher atherogenic index. Being small for gestational age was associated with an adverse lipid profile.

We found no significant associations between lipids and prematurity (see Table 13 and Figure 14)

Multiple regression models were used to examine the predictor variables for the various lipid components. Cholesterol was predicted only by BMI SDS and female sex ( r2 = 0.12). Triglyceride, LDL and atherogenic index were all predicted by current percentage body fat (r2 = 0.08, r2 = 0.09 and r2 = 0.07 respectively). HDL was positively predicted by postnatal steroids (r2 = 0.09). Current size and adiposity appear to be the key determinants of the lipid profile with no effect of prematurity or antenatal interventions.

Table 11. Relationship between current height, weight and adiposity and lipids age 5 years

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cholesterol | | High density lipoprotein | | Triglycerides | | Low density lipoprotein | | Atherogenic index | |
|  | r | P | r | P | r | P | r | P | r | P |
| Height SDS age 5 years | -0.08 | 0.389 | -0.18 | 0.064 | 0.10 | 0.289 | 0.00 | 0.977 | 0.12 | 0.224 |
| Weight SDS age 5 years | 0.07 | 0.469 | **-0.25** | **0.009** | **0.19** | **0.035** | 0.16 | 0.106 | **0.28** | **0.004** |
| BMI SDS age 5 years | **0.21** | **0.021** | **-0.19** | **0.054** | **0.18** | **0.047** | **0.28** | **0.004** | **0.31** | **0.001** |
| Percentage body fat age 5 years | **0.23** | **0.015** | -0.18 | 0.071 | **0.20** | **0.029** | **0.25** | **0.012** | **0.31** | **0.002** |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

Table 12. Correlation of lipid profile gestational age, size at birth, antenatal and postnatal steroid administration, maternal eclampsia and CRIB score.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cholesterol | | High density lipoprotein | | Triglycerides | | Low density lipoprotein | | Atherogenic index | |
|  | r | P | r | P | r | P | r | P | r | P |
| Gestational age | -0.03 | 0.758 | -0.06 | 0.543 | 0.00 | 0.983 | -0.01 | 0.896 | 0.03 | 0.768 |
| Sex | **0.24** | **0.007** | 0.12 | 0.216 | 0.08 | 0.405 | **0.30** | **0.001** | 0.13 | 0.198 |
| Multiple pregnancy | 0.05 | 0.553 | -0.07 | 0.504 | 0.10 | 0.264 | 0.02 | 0.876 | 0.07 | 0.470 |
| Birth weight SDS | 0.06 | 0.524 | -0.17 | 0.086 | 0.06 | 0.497 | 0.13 | 0.185 | **0.20** | **0.038** |
| Birth length SDS | 0.05 | 0.652 | -0.10 | 0.368 | -0.09 | 0.419 | 0.13 | 0.224 | 0.12 | 0.282 |
| Percentage boy fat at birth | -0.01 | 0.958 | -0.01 | 0.936 | -0.16 | 0.146 | 0.05 | 0.652 | 0.03 | 0.804 |
| Antenatal steroids | 0.04 | 0.639 | -0.06 | 0.527 | 0.04 | 0.691 | -0.01 | 0.946 | 0.04 | 0.650 |
| Postnatal steroids | -0.08 | 0.405 | **0.26** | **0.007** | -0.05 | 0.564 | -0.16 | 0.110 | **-0.25** | **0.009** |
| Maternal eclampsia | 0.06 | 0.519 | 0.02 | 0.860 | 0.09 | 0.348 | 0.05 | 0.616 | 0.06 | 0.536 |
| Neonatal CRIB score | 0.02 | 0.788 | 0.18 | 0.059 | -0.08 | 0.364 | 0.02 | 0.823 | -0.13 | 0.170 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant

Table 13. Relationship between gestational age at birth and lipid profile age 5 years

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Cholesterol (mmol/l) | **4.3** | 0.8 | **4.2** | 0.7 | **4.0** | 0.6 | **4.2** | 0.6 |
| HDL (mmol/l) | **1.5** | 0.2 | **1.2** | 0.3 | **1.2** | 0.3 | **1.3** | 0.3 |
| Triglyceride (mmol/l) | **0.8** | 0.3 | **0.8** | 0.3 | **0.9** | 0.4 | **0.8** | 0.3 |
| LDL (mmol/l) | **2.5** | 0.7 | **2.7** | 0.6 | **2.4** | 0.6 | **2.5** | 0.6 |
| Atherogenic index | **2.0** | 0.6 | **2.7** | 1.0 | **2.4** | 1.0 | **2.3** | 1.7 |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

ANOVA showed no significant relationships.

Figure 14. Cholesterol (mmol/l) plotted against gestational age at birth.



<30 weeks 30-34 weeks 34-37 weeks >37 weeks

Chapter 6: IGF-1, Cortisol and Leptin

Various mechanisms have been proposed to explain the link between low birth weight and subsequent programming for hypertension, diabetes and hyperlipidaemia. Amongst the potential mechanisms are programming of the GH-IGF- 1 axis, programming of the pituitary adrenal axis and alteration of the biological activity of 11β hydroxysteroid dehydrogenase (11βHSD) and programming of leptin. We examined the effect of IGF-1, fasting cortisol and leptin on blood pressure, measures of insulin resistance and lipids. We examined the relationship of these variables with prematurity. The role of 11βHSD will be considered in more detail in the next chapter.

There was no evidence of any relationship between the degree of prematurity and plasma cortisol and leptin. However there was an inverse relationship between levels of IGF-1 and prematurity. Levels of IGF-1 were highest in those children born most prematurely (see Table 14). There was no evidence of a relationship between fasting cortisol levels and childhood blood pressure blood pressure (see Figure 15). There was however a very clear relationship between IGF-1 and blood pressure (see Figure 16). Higher levels of IGF-1 were assosciated with higher blood pressure. Leptin was also independantly positively correlated with blood pressure (see Table 15).

Independent correlations found associations between gender and both IGF-1 (r = 0.31, p <0.001) and leptin (r = 0.34, p < 0.001). Leptin was, as anticipated, highly correlated with fat mass ( r = 0.68, p <0.001). IGF-1 was also correlated with fat mass (r = 0.23, p= 0.015). Current weight SDS and height SDS were also correlated with levels of IGF-1, with taller heavier children having higher levels. (r= 0.36, p<0.001 and r = 0.28, p = 0.002). Cortisol was also inversely related to fat mass ( r = -0.28, p = 0.002). Adjusting for current height and weight did not affect the relationship between blood pressure, resting heart rate and both IGF-1 and leptin. Children born prematurely are smaller and lighter at 5 years than those born at term. Adjusting for current height and weight strengthened the relationship between prematurity and IGF-1.

Multiple regression models were constructed incorporating the following variables (gestation, current height, weight and BMI SDS, percentage body fat, birth length height and weight SDS, sex, multiple pregnancy, antenatal steroids, postnatal steroids, maternal eclampsia and CRIB score). The model suggested that current weight SDS and sex were the best predictors of IGF-1 (r2 = 0.18) and that current percentage body fat best predicted leptin (r2 = 0.52).

Table 14. The effect of being born prematurely on levels of Cortisol, IGF-1 and Leptin at 5 years of age

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | <30 weeks (n=31) | | 30-34 weeks (n=51) | | 34-37 weeks (n=37) | | > 37 weeks (n=39) | |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Fasting morning Cortisol (nmol/l) | **262** | 77 | **292** | 88 | **256** | 76 | **289** | 88 |
| IGF-1 (mcg/l)\* | **122** | 45 | **125** | 47 | **108** | 54 | **98** | 54 |
| Leptin (ng/ml) | **2.8** | 1.3 | **3.2** | 2.3 | **3.2** | 2.3 | **2.5** | 1.3 |

Figures in table are Mean (bold) and standard deviation (SD)

n = number of subjects in each group

\* IGF-1 inversely correlated with gestation.

Pearson correlation coefficient = -0.18, p = 0.04

Figure 15. The relationship between fasting Cortisol and Systolic blood pressure

Correlation coefficient r = 0.04, p =0.7

Figure 16. The relationship between IGF-1 and Systolic blood pressure

Correlation coefficient r = 0.26, p<0.001

Table 15. Relationship between Cortisol, IGF-1, Leptin and blood pressure and resting heart rate.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Systolic Blood Pressure | | Diastolic Blood Pressure | | Mean Blood Pressure | | Resting Heart Rate | |
|  | r | P value | r | P value | r | P value | r | P value |
| Fasting Cortisol | 0.030 | 0.739 | 0.053 | 0.558 | 0.056 | 0.537 | 0.155 | 0.084 |
| IGF-1 | **0.300** | **0.001\*** | 0.131 | 0.144 | **0.221** | **0.013\*** | 0.141 | 0.117 |
| Leptin | **0.184** | **0.044\*** | 0.157 | 0.085 | **0.188** | **0.038\*** | 0.103 | 0.262 |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant.

\* = p<0.05

Both IGF-1 and leptin were associated with insulin resistance correlating strongly with a raised insulin:glucose ratio and raised HOMA index. They were also both associated with an adverse lipid profile. Higher levels of IGF-1 and leptin were correlated with higher cholesterol, triglycerides, LDL and a poorer athrrogenic index. (See Table 16). Adjusting the data for current height and weight did not affect the relationship between IGF-1, leptin and insulin resistance. Adjusting for current size and adiposity negated the relationship between both IGF-1, leptin and lipids but the association with insulin resistance remained just as strong.

Table 16. The relationship between glucose metabolism, lipids and IGF-1, Cortisol and Leptin.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Cortisol | | IGF- 1 | | Leptin | |
|  | r | P value | r | P value | r | P value |
| Fasting Insulin : glucose ratio | -0.02 | 0.803 | **0.40** | **< 0.0001** | **0.33** | **< 0.0001** |
| HOMA Index | -0.06 | 0.517 | **0.34** | **< 0.0001** | **0.36** | **< 0.0001** |
| Cholesterol | 0.03 | 0.719 | **0.19** | **0.033** | **0.21** | **0.019** |
| HDL | 0.03 | 0.759 | -0.08 | 0.387 | -0.05 | 0.640 |
| Triglyceride | 0.01 | 0.877 | **0.19** | **0.033** | 0.15 | 0.101 |
| LDL | 0.05 | 0.603 | **0.22** | **0.020** | **0.23** | **0.017** |
| Atherogenic index | 0.02 | 0.852 | **0.25** | **0.009** | **0.21** | **0.032** |

r = Pearson correlation coefficient

Figures in **bold** are those where the relationship was statistically significant

Chapter 7: Urinary steroids

7.1 Urinary Steroids and Blood Pressure

Although there was no relationship between fasting cortisol, blood pressure and resting heart rate within the cohort there was evidence of a positive correlation between resting heart rate and total cortisol metabolites (Table 17). Higher overall levels of cortisol metabolites were associated with a higher resting heart rate. There was also evidence of a relationship between diastolic blood pressure and markers of 11 beta hydroxysteroid dehydrogenase activity. A higher ratio of cortisol:cortisone metabolites (raised THF+αTHF:THE ratio) was associated with higher diastolic blood pressure. This was expected given that 11 beta hydroxysteroid dehydrogenase type 2 is present in the kidney and protects the nephron from cortisol by converting active cortisol to inactive cortisone (Walker *et al.* 1993;Edwards *et al.* 1993). Previous studies have shown a similar pattern with a reduction in the relative activity of 11 beta hydroxysteroid dehydrogenase type 2 linked to raised blood pressure (Soro *et al.* 1995).

There was no evidence of any direct association between markers of cortisol and cortisone metabolism and prematurity.

In order to address potential confounders multiple regression models were constructed to control for these. Current weight SDS and the ratio of cortisol:cortisone metabolites predicted diastolic blood pressure (r2 = 0.28). If the THF+αTHF:THE ratio and the ratio including the cortols and cortolones were excluded from the model then antenatal steroid use (as discussed earlier) became an important predictor of diastolic blood pressure in addition to weight SDS. An increasing weight SDS predicted higher diastolic blood pressure as did the cortisol:cortisone ratio and administration of antenatal steroids. Multiple regression models also suggested that weight SDS, antenatal steroids, gestational age and the ratio of cortisol:cortisone metabolites were predictors of systolic blood pressure. Higher systolic blood pressure was associated with increased weight SDS, administration of antenatal steroids, a raised cortisol:cortisone ratio in urinary steroid metabolites and with prematurity. Those individuals with the lowest relative levels of 11 beta hydroxysteroid dehydrogenase type 2 had the highest levels of systolic and diastolic blood pressure.

Data was examined both with and without correction for lean muscle mass in case this influenced the urinary steroid:creatinine ratios via differences in the urinary creatinine (as creatinine levels are dependant on lean muscle mass). All data presented is uncorrected as correction made no difference to any of the results.

Table 17. The relationship between urinary androgen metabolites, cortisol metabolites, urinary measures of cortisol:cortisone metabolism and blood pressure, insulin resistance and lipids.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total Androgen metabolites | | Total cortisol metabolites | | THF+αTHF/THE ratio | | THF:αTHF ratio | | Cortisol:cortisone metabolite ratio | |
|  | r | p | r | p | r | p | r | p | r | p |
| Systolic blood pressure | -0.04 | 0.605 | 0.10 | 0.238 | 0.04 | 0.626 | -0.09 | 0.254 | 0.03 | 0.699 |
| Diastolic blood pressure | 0.02 | 0.776 | 0.04 | 0.591 | **0.18** | **0.030** | 0.00 | 0.983 | **0.17** | **0.037** |
| Mean blood pressure | 0.03 | 0.683 | 0.11 | 0.196 | 0.05 | 0.514 | -0.07 | 0.377 | 0.05 | 0.542 |
| Mean resting heart rate | 0.12 | 0.162 | **0.24** | **0.004** | 0.00 | 0.968 | 0.00 | 0.967 | -0.04 | 0.611 |
| Fasting insulin:glucose ratio | 0.03 | 0.750 | 0.03 | 0.710 | **-0.30** | **0.001** | 0.18 | 0.052 | **-0.38** | **0.000** |
| HOMA Index | 0.02 | 0.789 | 0.02 | 0.862 | **-0.30** | **0.001** | 0.06 | 0.549 | **-0.38** | **0.000** |
| Cholesterol | -0.04 | 0.640 | 0.07 | 0.455 | -0.03 | 0.780 | -0.02 | 0.852 | -0.06 | 0.528 |
| HDL | -0.21 | 0.039 | -0.09 | 0.368 | -0.13 | 0.189 | -**0.21** | **0.037** | -0.15 | 0.128 |
| Triglyceride | 0.14 | 0.140 | 0.15 | 0.116 | -0.03 | 0.711 | 0.12 | 0.204 | -0.10 | 0.282 |
| LDL | -0.04 | 0.713 | 0.02 | 0.809 | 0.00 | 0.994 | 0.04 | 0.699 | -0.02 | 0.875 |
| Atherogenic index | 0.15 | 0.136 | 0.13 | 0.187 | 0.05 | 0.610 | **0.25** | **0.010** | 0.05 | 0.610 |

r = Pearson correlation coefficient

Figures in **bold** are those where the relationship was statistically significant.

Androgen and cortisol metabolites measured in µg:mmol creatinine

7.2 Urinary Steroids and Glucose

With respect to glucose metabolism the relationship with urinary steroid metabolites was different (Table 17). Whereas higher blood pressure was associated with a raised cortisol: cortisone metabolite ratio the converse was true for insulin resistance. The fasting insulin:glucose ratio and HOMA index were negatively correlated with the cortisol:cortisone metabolite ratios (THF+αTHF:THE ratio). Increasing insulin resistance was associated with a reduced ratio of cortisol:cortisone metabolites. This had been anticipated in that previous studies had demonstrated that as a consequence of the tissue specific distribution of the two isoenzymes of 11βHSD a reduced THF+αTHF:THE ratio was linked to insulin resistance (Andrews *et al.* 2002;Valsamakis *et al.* 2004). The associated alteration in liver 11βHSD1 activity affects tissue levels of cortisol and promotes insulin resistance.

Multiple regression was again used to control for confounders and the predictors of insulin resistance were an increased weight SDS, prematurity and a reduced ratio of cortisol:cortisone metabolites (r2 = 0.26).

7.3 Effect of Antenatal Steroids

We looked directly at the effect of the administration of antenatal steroids on the various urinary steroid metabolites across the cohort as a whole (including term infants). We also examined whether the number of doses of antenatal steroids was a factor. Although there appears to be a trend to an excess of androgen metabolites following antenatal steroid administration this does not quite reach statistical significance (Table 18).

Similarly there appeared to be an effect on A ring reductase (THF:αTHF ratio) which did not quite reach statistical significance and did not correlate with the number of courses of steroids received. There was evidence that administration of antenatal steroids affected 11 beta hydroxysteroid dehydrogenase metabolism with a reduced THF+αTHF:THE ratio.

Gestational age showed no significant correlations with any markers of cortisol:cortisone metabolism and 11 beta hydroxysteroid dehydrogenase activity. Controlling for gestation (given the relationship between gestation and steroid use) the association between the THF+αTHF:THE ratio and antenatal steroids was weakened but that with number of treatment courses persisted.

Table 18. The relationship between the administration of antenatal steroids and subsequent urinary androgen metabolites, cortisol metabolites and urinary measures of cortisol:cortisone metabolism.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Administration of Antenatal steroids | | Number of courses of antenatal steroids | | |
|  | | r | P value | r | P value |
| Total Androgen metabolites | | 0.15 | 0.07 | 0.02 | 0.83 |
| Total cortisol metabolites | | 0.09 | 0.28 | 0.08 | 0.32 |
| THF+ αTHF:THE ratio | | -0.13 | 0.11 | -0.18 | 0.03 |
| THF:αTHF ratio | | 0.15 | 0.06 | 0.05 | 0.58 |
| Cortisol : cortisone metabolite ratio | | -0.14 | 0.09 | -0.21 | 0.01 |

r = Pearson correlation coefficient

Androgen and cortisol metabolites measured in µg:mmol creatinine

7.4 Infants born less than 34 weeks gestation

In order to investigate the potential effect of antenatal steroids alone, whilst excluding as many confounding variables associated with prematurity as possible, we looked at a subgroup of children within the cohort. All were born at or below 34 weeks completed gestation.

We further subdivided the group into 3 cohorts – those who received no antenatal steroids, those who received a single dose and those who received multiple courses. The characteristics of the groups at birth and at 5 years are given in Table 19. There were more twins and triplets in the group that received more than one course of antenatal betamethasone. When only singletons were included in analysis the results remained essentially unchanged (see Table 20).

Those who received multiple courses were of significantly lower birth weight and remained lighter aged 5 years. An identical pattern was seen in those born following a singleton pregnancy compared to those born following a multiple pregnancy.

Table 19. Characteristics of all children born before 34 weeks gestation at the age of 5 years.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | No antenatal steroids (n=26) | | Single course antenatal steroids (n=34) | | Multiple courses of antenatal steroids (n=22) | |
|  | Mean | SD | Mean | SD | Mean | SD |
| Male | 38% (10)  62% (16)  7% (2) | | 52% (18)  48% (16)  20% (7) | | 50% (11)  50% (11)  54% (12) | |
| Female |
| Multiple pregnancy |
| Gestational age | **30.7** | 2.5 | **30.6** | 2.4 | **30.8** | 2.5 |
| Birth weight SDS | **0.2\*** | 0.8 | **-0.4\*** | 1.1 | **-0.4\*** | 0.7 |
| Birth length SDS | **-0.6** | 0.7 | **-0.5** | 0.8 | **-0.4** | 0.6 |
| Percentage fat at birth | **5.7** | 1.6 | **4.8** | 1.6 | **4.8** | 1.2 |
| Neonatal CRIB score | **2.1** | 2.3 | **2.5** | 3.0 | **2.0** | 1.9 |
| Height SDS age 5 years | **-0.4** | 0.9 | **-0.3** | 1.1 | **-0.3** | 1.6 |
| Weight SDS age 5 years | **-0.2** | 1.1 | **-0.5** | 1.1 | **-0.7** | 1.6 |
| BMI SDS age 5 years | **0.0\*** | 1.1 | **-0.4\*** | 0.9 | **-0.7\*** | 1.1 |
| Mid parental SDS | **0.1** | 1.0 | **0.0** | 1.1 | **0.2** | 0.9 |
| Percentage body fat age 5 years | **15.6** | 5.9 | **15.1** | 3.7 | **14.1** | 2.9 |

Figures in table are Mean (shown in **bold**) and standard deviation (SD)

n = number of subjects in each group

\* p= <0.05 by ANOVA

Table 20. Characteristics singleton children born before 34 weeks gestation at the age of 5 years.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | No antenatal steroids (n=24) | | Single course antenatal steroids (n=27) | | Multiple courses of antenatal steroids (n=10) | |
|  | Mean | SD | Mean | SD | Mean | SD |
| Gestational age | **31.0** | 2.3 | **30.6** | 2.4 | **30.2** | 2.5 |
| Birth weight SDS | **0.2\*** | 0.8 | **-0.3\*** | 1.2 | **-0.5\*** | 0.6 |
| Birth length SDS | **-0.6** | 0.7 | **-0.6** | 0.8 | **-0.6** | 0.6 |
| Percentage fat at birth | **5.7** | 1.6 | **4.8** | 1.7 | **4.0** | 0.9 |
| Neonatal CRIB score | **1.8** | 2.2 | **2.2** | 2.9 | **1.9** | 2.2 |
| Height SDS age 5 years | **-0.4** | 0.9 | **-0.3** | 1.2 | **-0.7** | 1.8 |
| Weight SDS age 5 years | **-0.1** | 1.1 | **-0.4** | 1.1 | **-1.0** | 2.0 |
| BMI SDS age 5 years | **0.1\*** | 1.1 | **-0.3\*** | 0.9 | **-0.7\*** | 1.4 |
| Mid parental SDS | **0.1** | 1.0 | **-0.2** | 1.1 | **-0.23** | 0.9 |
| Percentage body fat age 5 years | **16.5** | 5.2 | **15.3** | 3.5 | **13.9** | 2.9 |

Figures in table are Mean (shown in **bold**) and standard deviation (SD)

n = number of subjects in each group

\* p= <0.05 by ANOVA

The relationships between blood pressure variables, measures of insulin resistance and lipids are shown in Table 21. There were no statistically significant differences in blood pressure and glucose metabolism between those children born before 34 weeks who received steroids and those who did not. The apparent trend for lower blood pressure and a reduced insulin:glucose ratio (Table 21) following administration of steroids (which could have suggested potential benefit) disappears completely when the fact that children receiving steroids remained smaller and lighter aged 5 years was controlled for. LDL cholesterol was significantly lower in those receiving steroids or multiple doses of steroids. This effect persisted after correction for current size. Leptin was significantly lower in those receiving steroids or multiple doses of steroids but this effect disappeared after correction for current size.

Table 21. Relationship between antenatal steroid administration and measures of blood pressure, glucose and lipid metabolism in all children born below 34 weeks gestation at 5 years of age

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | No antenatal steroids (n=26) | | Single course antenatal steroids (n=34) | | Multiple courses of antenatal steroids (n=22) | |
|  | Mean | SD | Mean | SD | Mean | SD |
| Systolic blood pressure | **102.6** | 9.1 | **100.3** | 7.1 | **99.5** | 9.0 |
| Diastolic blood pressure | **54.5** | 6.4 | **55.5** | 5.8 | **52.4** | 5.2 |
| Mean blood pressure | **73.8** | 7.3 | **74.0** | 5.4 | **71.9** | 6.1 |
| Mean resting heart rate | **96.9** | 10.7 | **102.0** | 11.8 | **97.8** | 12.6 |
| Fasting insulin:glucose ratio | **1.45** | 0.68 | **1.29** | 0.88 | **1.12** | 0.62 |
| HOMA Index | **0.69** | 0.58 | **0.69** | 0.57 | **0.56** | 0.34 |
| Cholesterol | **4.4** | 0.9 | **4.4** | 0.7 | **4.1** | 0.7 |
| HDL | **1.3** | 0.3 | **1.3** | 0.3 | **1.3** | 0.3 |
| Triglyceride | **0.8** | 0.3 | **0.8** | 0.4 | **0.8** | 0.2 |
| LDL | **3.1\*** | 0.7 | **2.6\*** | 0.5 | **2.4\*** | 0.6 |
| Atherogenic index | **2.8** | 1.1 | **2.4** | 0.9 | **2.2** | 0.9 |
| Fasting Cortisol | **257.9** | 76.5 | **307.3** | 88.0 | **266.5** | 81.2 |
| IGF-1 | **132.6** | 58.3 | **124.8** | 37.7 | **113.9** | 43.6 |
| Leptin | **3.8\*** | 3.1 | **2.9\*** | 1.2 | **2.5\*** | 1.0 |

Figures in table are Mean (shown in **bold**) and standard deviation (SD)

n = number of subjects in each group

\* p= <0.05 by ANOVA

Examining the various urinary steroid metabolites there was a trend for increasing total cortisol and androgen metabolites with increased exposure to antenatal steroids though it did not reach statistical significance (see Table 22). The THF + αTHF: THE ratio and ratio of cortisol:cortisone metabolites which give an indirect measure of 11β hydroxysteroid dehydrogenase activity were significantly inversely related to glucocorticoid exposure. Controlling for other differences within the cohort using stepwise multiple regression (variables entered in the model: gestation, sex, antenatal steroid administration, postnatal steroid administration, birth weight and length SDS, adiposity at birth, current adiposity, and current height, weight and BMI SDS) found that antenatal steroid use was the only predictor of the THF+αTHF:THE ratio accounting for 22% of the variance. Antenatal steroids predicted a reduced ratio.

The influence of repeated doses of antenatal steroids can be seen.

The overall ratio was associated with an elevation of systolic, diastolic and mean blood pressure. Conversely though both the THF+αTHF:THE ratio and overall metabolite ratio was associated with a reduction in the insulin:glucose ratio and HOMA index. There was no association with lipids.

Table 22. Effect of antenatal steroid administration on urinary androgen and cortisol:cortisone metabolites in children born < 34 weeks gestation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | No antenatal steroids (n=26) | | Single course antenatal steroids (n=34) | | Multiple courses of antenatal steroids (n=22) | | ANOVA |
|  | Mean | SD | Mean | SD | Mean | SD | P- value |
| Total Androgen metabolites | **10.9** | 13.6 | **21.4** | 22.8 | **23.8** | 27.1 | **0.45** |
| Total cortisol metabolites | **925.0** | 436.5 | **1001.8** | 444.4 | **1038.6** | 357.7 | **0.64** |
| THF: αTHF ratio | **0.86** | 0.39 | **1.99** | 6.10 | **1.23** | 0.72 | **0.55** |
| THF + αTHF / THE ratio | **0.99** | 0.37 | **0.86** | 0.32 | **0.74** | 0.18 | **0.03** |
| Cortisol : cortisone metabolite ratio | **0.84** | 0.30 | **0.75** | 0.40 | **0.70** | 0.27 | **0.07** |

Figures in table are Mean (shown in **bold**) and standard deviation (SD)

n = number of subjects in each group

Androgen and cortisol metabolites measured in µg:mmol creatinine

Chapter 8. Catch-up growth

It has been suggested that growth failure and catch up growth are instrumental in the subsequent development of the metabolic syndrome. We have examined the relationships between patterns of growth and subsequent blood pressure, insulin resistance and lipids. Prematurity is an important confounder – as we have demonstrated individuals born prematurely show significant growth failure and then catch-up growth.

We initially examined simple correlations between the changes in height SDS, weight SDS, BMI SDS and percentage body fat over specific periods of time (birth- 8 weeks, birth-12 months, birth-5 years and 1-5 years). Associations with current height, weight and adiposity have already been presented.

Systolic and diastolic blood pressure were highest in those who demonstrated the greatest increase in weight standard deviation score since birth (Table 23). The individuals with the highest blood pressure were those light for dates at birth who became heavier subsequently. Diastolic blood pressure but not systolic blood pressure was associated with catch-up growth in length. Both systolic and diastolic blood pressure were increased in those whose BMI SDS increased from the end of the first year of life onwards.

For glucose metabolism an increase in both weight and height SDS from birth was associated with a reduction in insulin sensitivity (Table 24). Those individuals who were short and light and who showed the greatest catch-up in height and weight had the most significant insulin resistance. An increase in adiposity from the end of the first year of life onwards was also strongly associated with insulin resistance.

Within the cohort an adverse lipid profile (reduced HDL, high triglyceride and elevated atherogenic index) was associated with poor early linear growth and with later increase in adiposity (Table 25).

Multiple regression was used to identify the key phases of growth that predicted blood pressure, glucose homeostasis and lipids. Gestation is an important predictor of subsequent growth but was not controlled for in initial analyses (Tables 23, 24 & 25). Using stepwise multiple regression the predictors of systolic blood pressure were change in length standard deviation score between birth and 12 months of age and change in body mass index from age 2 years to age 5 years ( r2 = 0.27). For diastolic blood pressure and mean blood pressure only change in length standard deviation score between birth and 12 months of age was significant in the model ( r2 = 0.16 and r2 = 0.22 respectively). The change in length standard deviation score between birth and 12 months also predicted the insulin:glucose ratio and HOMA (r2 = 0.12 and r2 = 0.29 respectively). For lipids, only for the atherogenic index and triglyceride levels were statistically significant models constructed. Predictors of atherogenic index were current percentage body fat and change in length SDS between 8 weeks of age and 12 months. (r2 = 0.34). Triglyceride levels were predicted only by change in percentage body fat between 12 months of age and 5 years (r2 = 0.39). Current height and weight were not predictors in the models when early growth was included. Including gestational age and use of antenatal steroids in the models altered the predictors only for systolic blood pressure. In addition to change in length standard deviation score between birth and 12 months of age and change in body mass index from age 2 years to age 5 years, current height standard deviation score and change in weight from birth to 8 weeks now became significant ( r2 = 0.53).

Given the enormous amount of growth data regression models were highly complex. Subtle alterations in the order in which variables were entered and controlled for affected the outcomes slightly. The variables were also related in many aspects - early growth failure was associated with catch-up growth and with gestational age etc. The key findings were that failure of linear growth in the first year of life followed by an increase in body mass index and adiposity from the 2nd year of life onwards were the most important predictors in the models of an adverse effect on blood pressure, glucose metabolism and lipid profile. Failure of linear growth in the first year of life was the most important predictor in the models.

Table 23. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to blood pressure and heart rate.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Systolic Blood Pressure | | Diastolic Blood Pressure | | Mean Blood Pressure | | Resting Heart Rate | |
|  | r | P value | r | P value | r | P value | r | P value |
|  |  |  |  |  |  |  |  |  |
| Difference between birth weight SDS and weight SDS at 8 weeks | -0.08 | 0.394 | 0.07 | 0.434 | 0.00 | 0.959 | -0.10 | 0.241 |
| Difference between birth weight SDS and weight SDS at 12 months | 0.07 | 0.429 | 0.14 | 0.114 | 0.05 | 0.585 | -0.09 | 0.288 |
| Difference between birth weight SDS and weight SDS at 5 years | **0.20** | **0.023** | **0.26** | **0.003** | **0.23** | **0.009** | -0.02 | 0.796 |
| Difference between weight SDS at 12 months and weight SDS at 5 years | **0.18** | **0.038** | 0.17 | 0.063 | 0.24 | 0.006 | 0.10 | 0.271 |
|  |  |  |  |  |  |  |  |  |
| Difference between birth length SDS and length SDS at 8 weeks | -0.04 | 0.706 | 0.18 | 0.057 | 0.03 | 0.747 | -0.18 | 0.054 |
| Difference between birth length SDS and length SDS at 12 months | 0.12 | 0.179 | **0.25** | **0.006** | 0.16 | 0.080 | -0.02 | 0.849 |
| Difference between birth length SDS and height SDS at 5 years | 0.13 | 0.173 | **0.24** | **0.008** | 0.17 | 0.059 | -0.03 | 0.768 |
| Difference between length SDS at 12 months and height SDS at 5 years | 0.01 | 0.922 | 0.01 | 0.896 | 0.06 | 0.489 | -0.03 | 0.745 |
|  |  |  |  |  |  |  |  |  |
| Difference between BMI SDS at 8 weeks and BMI SDS at 5 years | 0.17 | 0.063 | **0.18** | **0.050** | 0.17 | 0.059 | 0.02 | 0.825 |
| Difference between BMI SDS at 12 months and BMI SDS at 5 years | **0.24** | **0.007** | **0.23** | **0.008** | 0.29 | 0.001 | **0.18** | **0.045** |
|  |  |  |  |  |  |  |  |  |
| Difference in percentage body fat at birth and at 8 weeks | 0.08 | 0.420 | 0.18 | 0.058 | 0.15 | 0.118 | 0.03 | 0.727 |
| Difference in percentage body fat at birth and at 12 months | 0.11 | 0.256 | 0.15 | 0.114 | 0.18 | 0.058 | **0.21** | **0.027** |
| Difference in percentage body fat at 12 months and at 5 years | 0.10 | 0.283 | 0.17 | 0.084 | 0.18 | 0.058 | 0.14 | 0.151 |

r = Pearson correlation coefficient

Figures in **bold** are those where the relationship was statistically significant.

Table 24. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to measures of glucose metabolism.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Fasting Insulin:glucose ratio | | HOMA score | |
|  | r | P value | r | P value |
|  |  |  |  |  |
| Difference between birth weight SDS and weight SDS at 8 weeks | -0.077 | 0.444 | -0.077 | 0.444 |
| Difference between birth weight SDS and weight SDS at 12 months | 0.014 | 0.889 | 0.052 | 0.597 |
| Difference between birth weight SDS and weight SDS at 5 years | **0.325** | **0.001** | **0.366** | **<0.0001** |
| Difference between weight SDS at 12 months and weight SDS at 5 years | **0.409** | **<0.0001** | **0.417** | **<0.0001** |
|  |  |  |  |  |
| Difference between birth length SDS and length SDS at 8 weeks | -0.093 | 0.376 | -0.108 | 0.306 |
| Difference between birth length SDS and length SDS at 12 month | 0.111 | 0.285 | 0.159 | 0.127 |
| Difference between birth length SDS and height SDS at 5 years | **0.283** | **0.006** | **0.318** | **0.002** |
| Difference between length SDS at 12 month and height SDS at 5 years | **0.286** | **0.003** | **0.284** | **0.004** |
|  |  |  |  |  |
| Difference between BMI SDS at 8 weeks and BMI SDS at 5 years | **0.250** | **0.013** | **0.286** | **0.004** |
| Difference between BMI SDS at 12 months and BMI SDS at 5 years | **0.349** | **<0.0001** | **0.361** | **<0.0001** |
|  |  |  |  |  |
| Difference in percentage body fat at birth and at 8 weeks | 0.036 | 0.744 | 0.070 | 0.526 |
| Difference in percentage body fat at birth and at 12 months | -0.066 | 0.549 | -0.075 | 0.499 |
| Difference in percentage body fat at 12 months and at 5 years | **0.340** | **0.001** | **0.373** | **<0.0001** |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant.

Table 25. Pearson Correlation coefficients relating changes in Weight, Length, BMI and body fat over the first 5 years of life to lipid metabolism.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cholesterol | | HDL | | Triglyceride | | LDL | | Atherogenic index | |
|  | r | p | r | p | r | p | r | p | r | p |
|  |  |  |  |  |  |  |  |  |  |  |
| Difference between birth weight SDS and weight SDS at 8 weeks | -0.05 | 0.593 | -0.14 | 0.179 | 0.10 | 0.308 | -0.04 | 0.699 | 0.10 | 0.334 |
| Difference between birth weight SDS and weight SDS at 12 months | -0.01 | 0.928 | **-0.20** | **0.050** | 0.07 | 0.507 | 0.02 | 0.819 | 0.13 | 0.217 |
| Difference between birth weight SDS and weight SDS at 5 years | 0.02 | 0.815 | -0.11 | 0.279 | **0.24** | **0.016** | 0.01 | 0.942 | 0.12 | 0.256 |
| Difference between weight SDS at 12 months and weight SDS at 5 yrs | 0.03 | 0.737 | 0.08 | 0.431 | **0.23** | **0.022** | -0.02 | 0.846 | 0.01 | 0.958 |
|  |  |  |  |  |  |  |  |  |  |  |
| Difference between birth length SDS and length SDS at 8 weeks | -0.04 | 0.693 | **-0.29** | **0.006** | **0.23** | **0.026** | 0.02 | 0.841 | 0.23 | 0.031 |
| Difference between birth length SDS and length SDS at 12 month | -0.07 | 0.507 | -0.16 | 0.138 | **0.19** | **0.070** | -0.07 | 0.505 | 0.04 | 0.684 |
| Difference between birth length SDS and height SDS at 5 years | -0.07 | 0.535 | -0.02 | 0.840 | 0.15 | 0.149 | -0.11 | 0.314 | -0.04 | 0.715 |
| Difference between length SDS at 12 month and height SDS at 5 yrs | -0.01 | 0.934 | 0.14 | 0.177 | 0.03 | 0.791 | -0.05 | 0.647 | -0.07 | 0.495 |
|  |  |  |  |  |  |  |  |  |  |  |
| Difference between BMI SDS at 8 weeks and BMI SDS at 5 years | 0.10 | 0.320 | -0.19 | 0.066 | **0.20** | **0.047** | 0.15 | 0.161 | **0.24** | **0.024** |
| Difference between BMI SDS at 12 months and BMI SDS at 5 years | 0.06 | 0.547 | 0.05 | 0.657 | **0.28** | **0.004** | 0.00 | 0.976 | 0.04 | 0.736 |
|  |  |  |  |  |  |  |  |  |  |  |
| Difference in percentage body fat at birth and at 8 weeks | 0.15 | 0.164 | -0.03 | 0.790 | 0.18 | 0.109 | 0.11 | 0.346 | 0.12 | 0.307 |
| Difference in percentage body fat at birth and at 12 months | 0.09 | 0.416 | -0.01 | 0.912 | 0.11 | 0.302 | 0.01 | 0.936 | 0.03 | 0.810 |
| Difference in percentage body fat at 12 months and at 5 years | 0.25 | 0.022 | -0.04 | 0.723 | **0.43** | **0.000** | **0.22** | **0.050** | **0.23** | **0.037** |

r = Pearson correlation coefficient

Figures in bold are those where the relationship was statistically significant.

Chapter 9. Discussion

9.1 Early growth failure

The longitudinal growth component of this study demonstrates that individuals born prematurely show dramatic early growth failure. Both weight gain and linear growth were similarly affected. This is followed by a period of sustained catch-up growth which lasts many years. Those born most prematurely show the most marked growth failure. Many individuals born before 28 weeks gestation were still showing evidence of continued catch-up growth at seven years of age. Previous studies have suggested that there is early growth failure but that the normal growth trajectory is maintained from approximately 14 days onwards (Berry, Conrod, and Usher 1997). This is not the pattern we observed. In those infants born at the extremes of prematurity, poor linear growth and poor weight gain was evident up to one to two years of age. At seven years of age those individuals born before 30 weeks gestation remain significantly smaller and lighter, they are also leaner with a lower BMI and lower percentage body fat. Most previous follow-up cohort studies of preterm infants have been based on birthweight, for example following up all babies born weighing less than 1500 g (Ford *et al.* 2000;Ericson and Kallen 1998). As a consequence these cohorts include a higher proportion of individuals who had intrauterine growth retardation. Whilst a proportion of individuals within our cohort were small for gestation ( 6.9% below 3rd centile [-1.88 SD] and 17.7% below 10th centile [-1.28 SD] )the majority were appropriate for gestation. Nevertheless there are parallels with other previous growth studies. Longitudinal studies following very low birthweight infants until age 20 years have shown evidence of catch up growth although some individuals particularly boys remained at shorter and lighter (Ericson and Kallen 1998;Hack *et al.* 2003). Evidence of catch-up growth up until 14 years has been demonstrated in some studies although a proportion of individuals remained smaller and lighter (Ford *et al.* 2000). Other studies suggest that infants born weighing less than 1000 g and remain small and light aged 12 to 16 years with continuing catch-up growth during adolescence (Saigal *et al.* 2001).

9.2 Prematurity, Blood Pressure and Insulin Resistance

Some of the most significant findings from the study are the association between prematurity and higher blood pressure and insulin resistance during childhood. There was a clear link between the degree of prematurity and the magnitude of subsequent blood pressure and insulin resistance. These associations were strengthened further if current height, current weight and current fat mass were controlled. In the general childhood population blood pressure is highest in the tallest and heaviest individuals. Despite individuals born prematurely, particularly those born at the extremes of prematurity being smaller, lighter and leaner their blood pressure and insulin resistance were higher. We also found evidence of programming of the autonomic nervous system with a relationship between resting heart rate and prematurity. Individuals born prematurely had a higher resting heart rate. A raised resting heart rate has been identified as a marker for the subsequent development of the metabolic syndrome (Flanagan *et al.* 1999;Bergholm *et al.* 2001). Several epidemiological studies have shown that the various components of the metabolic syndrome are associated with a raised resting heart rate (Stern *et al.* 1992;Moan *et al.* 1995). Insulin resistance has been linked to an increase in resting heart rate together with evidence of activation of the sympathetic nervous system (Flanagan *et al.* 1999). It has been suggested that insulin itself may increase sympathetic nervous system activity (Hausberg *et al.* 1997).

The large epidemiological studies which have extensively examined the links between low birthweight and later hypertension and type 2 diabetes refuted a link with prematurity (Whincup and Cook 2000;Barker *et al.* 1993a;Phipps *et al.* 1993). They concluded that it was low birthweight that was the key predictor for subsequently developing the metabolic syndrome. However, as previously discussed, these studies involved historical cohorts and few of these studies included significant proportions of preterm infants. Studies of contemporary cohorts only include small numbers of individuals born prematurely. A study of 750 children from Zimbabwe aged six years confirmed an inverse relationship between the systolic blood pressure and birthweight but concluded that gestational age was not a significant factor (Woelk *et al.* 1998). However, only 27 of the individuals in the study were born before 37 weeks gestation.

More recent epidemiological studies have supported a link between prematurity and risk factors for the metabolic syndrome. In a study of Swedish male army conscripts prematurity appears to be a significant factor in determining adult blood pressure (Gennser, Rymark, and Isberg 1988). In another Swedish study of men approaching 50 born in the 1920s prematurity appeared to have a marked effect on adult blood pressure (Siewert-Delle and Ljungman 1998). The study did not investigate infants at the extremes of prematurity (gestation ranged from 30-38 weeks and only 4 individuals <34 weeks) we examined but noted that a one-week difference in gestation increased systolic blood pressure by seven mmHg. In our cohort each week of prematurity increased systolic blood pressure by 0.5 mmHg based on the slope of the regression line describing the relationship between systolic blood pressure and gestation. The blood pressure values we obtained within the term control group (mean systolic blood pressure 95.1 ±7.1 mmHg) are comparable with published standards relating to the general population (mean systolic blood pressure age 5 years 96.2 ± 9.4mmHg) (de Swiet, Fayers, and Shinebourne 1992).

In studies specifically examining or commenting upon individuals born prematurely a study from Cambridge which examine 616 children born before 34 weeks gestation and weighing less than 1850 g showed no link between low birthweight or prematurity and subsequent blood pressure (Morley *et al.* 1994). Indeed they found the reverse of previous studies in that lower birthweight was associated with lower subsequent blood pressure. Further studies from the same group concluded that low birthweight whether due to prematurity or intrauterine growth retardation were associated with later insulin resistance but there was no link with gestation (Fewtrell *et al.* 2000). They suggested that relative under nutrition and poor postnatal growth may be protective in preterm infants (Singhal *et al.* 2003). Other studies have suggested, as discussed earlier, that prematurity does in itself lead to raised blood pressure and evidence of insulin resistance in later life (Irving *et al.* 2000;Szathmari *et al.* 2000;Hofman *et al.* 2004). None of the studies though demonstrated a clear relationship with the degree of prematurity. Indeed the most prominent study suggested that there was no link between insulin resitance and the degree of prematurity (Hofman *et al.* 2004). This is most likely a result of small numbers of subjects, a high proportion of small for gestational age children, a wide age range and a very narrow range of variation in gestational age. We believe we have demonstrated quite clearly that prematurity does predispose to hypertension and insulin resistance and that the more preterm infants the more significant this problem will be.

The systolic blood pressure in those born before 28 weeks gestation was 7 mmHg higher than in term controls. The association of hypertension with birthweight appears to become stronger with age (Law *et al.* 1993). The tracking (maintenance of rank order throughout life) of blood pressure has been repeatedly observed in longitudinal studies in both children and adults (Arnlov *et al.* 2005). It is consistent with Folkows hypothesis that there is an initiating and then amplification mechanism involved in the development of high blood pressure (Folkow 1978). Other risk markers such as insulin resistance have also been observed tracking from childhood into adult life and predict the onset of cardiovascular disease (Arnlov *et al.* 2005;Berenson *et al.* 1998). Blood pressure within the upper limit of the normal range is associated with an increased risk of cardiovascular disease (Vasan *et al.* 2001). We would postulate that as they move into adult life the differences noted in childhood in preterm infants will increase and their risk of developing hypertension, insulin resistance and subsequent cardiovascular and cerebrovascular disease will be significantly increased.

9.3 Catch-up Growth

The relative contributions of intra-uterine growth retardation, size at birth and postnatal growth to the evolution of the metabolic syndrome has been widely debated. Some observers suggest that current size is a more important predictor of blood pressure than size at birth (Fewtrell *et al.* 2000;Taylor *et al.* 1997). Many studies correct for current size when attempting to assess the effect of size at birth. It has been suggested that this correction removes the effects of subsequent growth (Lucas, Fewtrell, and Cole 1999). Current size is certainly an important predictor of the metabolic syndrome (Whincup *et al.* 1997). Catch-up growth, in particular weight gain, is also important (Crowther *et al.* 1998). Numerous studies investigating the programming hypothesis and the differing contributions of intrauterine growth failure, postnatal catch-up growth and postnatal weight gain have demonstrated that subsequent catch-up growth may have detrimental effects on blood pressure and glucose metabolism particularly in those who subsequently become overweight (Osmond *et al.* 1993;Leon *et al.* 1996). In the Avon longitudinal study of parents and children (ALSPAC), a large prospective follow-up study of 14,000 expectant mothers and their children recruited in 1985, postnatal catch-up growth was associated with insulin resistance aged eight (Ong *et al.* 2004a). The critical period for programming appeared to be the first two to three years of life with weight gain being the major determinant of later insulin resistance although insulin secretion was related early height gain (Ong *et al.* 2004a). The catch-up growth hypothesis suggests that over-activation of various metabolic pathways in an attempt to induce catch-up growth may induce a long-term metabolic maladaptation (Cianfarani, Germani, and Branca 1999).

Our cohort is unique in that we have detailed longitudinal growth data of individuals born prematurely. Patterns of growth were determined largely by gestational age at birth. Within our cohort the degree of prematurity was the single most important factor in predicting metabolic abnormalities. Being born small for gestational age was, in line with many other exponents of the Barker hypothesis, a factor, but was much less important. Similarly whilst size age 5 years was important in determining the blood pressure and insulin resistance gestational age was more important. We attempted to establish from the comprehensive growth data we had which periods of growth seemed to be most critical in terms of influence on later blood pressure and glucose metabolism. Poor linear growth in the first year of life and excessive weight gain from age 2 years onwards were the most important factors and strongest predictors of raised blood pressure and insulin resistance, of which poor linear growth in the first year of life was the strongest and most important predictor. It should be noted that preterm infants differ from term cohorts and children born IUGR in that the majority show post-natal growth failure. It seems likely that early growth failure is related to the propensity to raised blood pressure and insulin resistance in later life that we demonstrated within the cohort. Those children born most prematurely show the most marked growth failure and subsequently had the highest blood pressure and the greatest evidence of insulin resistance.

It has been suggested that significant under nutrition is almost inevitable in prematurity (Embleton, Pang, and Cooke 2001). Preterm infants are frequently undernourished and rarely achieve current recommendations for daily intake. However there are considerable differences in growth velocity in extremely preterm infants between neonatal units (Olsen *et al.* 2002). Adjusted weight gain ranged from 10.4 to 14.3 g/kg/d among the 6 sites studied. Many of these differences were explained by differences in nutritional intake. The models constructed suggested that adding 1 g/kg/d protein to the infants mean intake would increase growth by 4.1 g/kg/d. Others have suggested that improvements in total caloric intake and total protein intake may be associated with improved rates of growth in preterm infants born weighing less than 1000 g at birth (Berry, Abrahamowicz, and Usher 1997). The authors suggest that rates of growth not dissimilar to the intrauterine environment can be maintained (Berry *et al.* 1997). The link between patterns of early growth and markers of risk for later cardiovascular and cerebrovascular disease make it imperative that further investigations of the links between growth failure and subsequent blood pressure and insulin resistance in preterm infants are more thoroughly investigated. Within our cohort it was the most preterm infants that grew most poorly. These were not always the sickest infants. Those born at the extremes of gestation were sickest with the highest CRIB scores but the link between gestation and growth failure was stronger than that between CRIB score and growth failure. Indeed when gestation was controlled for, there was no evidence of a significant relationship between CRIB score and growth failure. It has been suggested that reduced early nutrition in preterm infants may have a protective effect for subsequent blood pressure and insulin resistance (Singhal *et al.* 2003). We found no evidence to support this. Indeed if one assumes that growth failure is related to nutritional insufficiency there is evidence to refute the suggestion.

9.4 Prematurity and Lipids

No significant relationship between prematurity and lipids was found. It has been suggested that in children born prematurely who subsequently show rapid catch-up growth that there is an adverse effect on lipid profiles (Mortaz *et al.* 2001). However it was markers of cholesterol biosynthesis (lathosterol) and absorption efficiency (campesterol) that were altered and there was no effect on cholesterol or lipoprotein fractions. We did not examine lathosterol or campesterol metabolism. The authors considered that size at birth and future weight gain was most important and not prematurity. Individuals born with intrauterine growth retardation at term have evidence of raised lipid in childhood (Tenhola *et al.* 2000). Other studies have shown that the key factor in developing hyperlipidaemia is the rate of subsequent weight gain and subsequent obesity (Ong *et al.* 2000). It may well be that we showed no evidence of an effect on lipids as the preterm group remain leaner than term controls. It is conceivable that if they become overweight in adult life they may be at increased risk of developing hyperlipidaemia. An effect of prematurity on lipid metabolism may at this stage be masked by the fact that the children are relatively lean.

9.5 Multiple Pregnancy

The effects of multiple pregnancy is now considered. In the preterm groups a significant proportion of children were born following multiple pregnancy. Twins tend to be smaller than singletons and are routinely delivered at an earlier gestation. In a study of twin pregnancies, delivered at a mean gestation of 35 weeks, the systolic blood pressure was on average 2 mmHg higher in twins than term singletons (Dwyer *et al.* 1999). The authors documented an inverse relationship between birth weight and blood pressure. They concluded that gestational age was not a factor independent of birthweight however birthweight is directly related to the duration of pregnancy. The lighter twin had a higher mean blood pressure though this did not reach statistical significance in this study.

Excluding twin pregnancies from the analysis did not significantly affect the results. Including twins strengthened some of the relationships but purely as a consequence of increased sample size increasing statistical power. We found no evidence that individuals born following twin/triplet pregnancies had higher subsequent blood pressure once their earlier gestation was taken into account.

9.5 Pregnancy Induced Hypertension

Pregnancy induced hypertension, independently of gestation, also appears to be associated with raised blood pressure in the children, particularly with diastolic blood pressure and an elevated resting heart rate in childhood. Maternal blood pressure in pregnancy has been associated with higher blood pressure in offspring (Tenhola *et al.* 2003). Elevated maternal blood pressure in pregnancy is also associated with a reduced birthweight. Maternal hypertension outside pregnancy is associated with an increased risk of hypertension in the offspring (Stamler *et al.* 1979). It has been speculated that there may be both an inherited effect and an environmental effect of maternal blood pressure on the fetus (Walker *et al.* 1998a). Intriguingly there is evidence that activity of 11 beta hydroxysteroid dehydrogenase type 2, which would predispose to hypertension, may be reduced in pregnancy induced hypertension (Heilmann *et al.* 2001).

9.6 Mechanisms of programming

A number of mechanisms have been postulated by which programming of blood pressure, glucose and lipid metabolism by low birth weight may occur. We focussed on the growth hormone-IGF-I axis, leptin, the pituitary-adrenal axis and in particular modulation of 11β hydroxysteroid dehydrogenase

9.6.1 Prematurity and IGF-1

The growth hormone-IGF-I axis has been proposed as a potential link between fetal and early growth and later cardiovascular disease (Langford *et al.* 1994). In our study levels of IGF-I were highest in those born most prematurely and there was a clear relationship between gestational age and IGF-I. Higher levels of IGF-I correlated strongly with higher blood pressure and insulin resistance. The strength of these relationships was enhanced by adjusting the data for current height and weight. In the normal childhood population the highest levels of IGF-I are usually seen in the tallest individuals. Within our cohort despite being small and lighter compared to term controls children born prematurely had significantly higher levels of IGF-I. In children and in adults higher levels of IGF-I have been associated with raised blood pressure (Fall *et al.* 1995) and with an increased risk of type 2 diabetes (Kajantie *et al.* 2003). Studies in adults have contradicted some of these findings in that it has been suggested that raised levels of IGF-I are associated with a reduced risk of impaired glucose tolerance and cardiovascular disease (Sandhu *et al.* 2002;Juul *et al.* 2002). In a group of 400 children from Pune in India and Salisbury in the UK IGF-I levels were related to birthweight in a term cohort. Children of low birthweight had higher levels of IGF-I subsequently (Fall *et al.* 1995). Systolic blood pressure was highest in those children with the highest IGF-I levels (Fall *et al.* 1995). IGF-1 remains an attractive hypothetical link between low birthweight and catch-up growth. It has been suggested that IGF-I concentrations may be linked to catch-up growth (Gluckman *et al.* 1996). Rapid postnatal growth is associated with higher levels of IGF-I (Ong *et al.* 2004a).

9.6.2 Prematurity and Cortisol metabolism

Cortisol, cortisol metabolism and the pituitary adrenal axis have also been proposed as potential mechanisms by which programming can occur. Animal studies supporting its role are amongst the most elegant. In animal studies antenatal steroids lead to subsequent hypertension and insulin resistance (Benediktsson *et al.* 1993). In rat models maternal protein restriction during pregnancy results in elevated blood pressure which can be prevented by the inhibition of maternal corticosteroid biosynthesis during pregnancy (Langley-Evans *et al.* 1996).

Several epidemiological studies have suggested that low birthweight is associated with raised levels of fasting cortisol (Phillips *et al.* 1998). This has been demonstrated in several populations (Phillips *et al.* 2000) and in both children and adults (Phillips *et al.* 1998;Clark *et al.* 1996;Levitt *et al.* 2000). In Asian populations (a population at high risk of the metabolic syndrome) the relationship between fasting cortisol and low birthweight and the correlation between raised fasting cortisol and elevated blood pressure and insulin resistance was much stronger than that seen in the Caucasian population (Ward *et al.* 2003). Not all studies though are concordant. A large study from Finland looking at term infants found no evidence of correlation between size at birth and cortisol secretion in adult life as measured by fasting cortisol (Kajantie *et al.* 2002). In a Swedish study there was no evidence of a significant difference in levels of cortisol, which were measured seven times daily, in children born small for gestational age and children appropriate for gestation or age (Dahlgren *et al.* 1998). A further study suggested an association between low birthweight and raised fasting cortisol in three different populations but noted that the effect on systolic blood pressure appeared dependant on an interaction with obesity. It seems that those individuals who were born small who later became obese were most likely to develop overt hypertension (Phillips *et al.* 2000). We found no link between fasting cortisol or either gestation or birthweight SDS – even after adjustment for the fact that children born prematurely within the cohort were leaner.

More complex studies have looked at the response of plasma cortisol to ACTH. Evidence of increased activity of the hypothalamo-pituitary-adrenal axis has been demonstrated with enhanced responses to ACTH and increased total urinary cortisol metabolite excretion (Reynolds *et al.* 2001;Levitt *et al.* 2000). Modulation of the pituitary-adrenal axis and cortisol metabolism may influence programming in different ways. Animal experimentation has demonstrated that prenatal dexamethasone alters receptor numbers in the rat hippocampus (Levitt *et al.* 1996). This may well underpin the reduced feedback/reduced sensitivity to glucocorticoid steroids that has been seen in individuals prone to the metabolic syndrome.

When we looked at patterns of urinary steroid excretion we found significant correlations with blood pressure and glucose metabolism. Whilst urinary steroid profiles give more detailed information about cortisol metabolism, interpretation of the various metabolite ratios is complex as the various isoenzymes are tissue specific. In knockout mouse models absent 11βHSD1 results in increased insulin sensitivity and reduced insulin resistance (Morton *et al.* 2001). Conversely mice over expressing 11βHSD1 develop central obesity, hyperlipidaemia and diabetes (Masuzaki *et al.* 2001) .

In this study we demonstrated what may initially appear to be paradoxical results. Across the whole cohort systolic blood pressure was associated with a raised cortisol:cortisone metabolite ratio (raised THF+αTHF:THE), suggesting either increased activity of 11βHSD1or reduced activity of 11βHSD2. Insulin resistance appeared to be related to a reduced cortisol:cortisone metabolite ratio (reduced THF+ αTHF:THE). Previous studies have demonstrated that individuals with essential hypertension have reduced inactivation of cortisol by 11βHSD2 (Walker *et al.* 1993;Soro *et al.* 1995). We confirmed that those children with the lowest relative 11βHSD2 activity (highest THF+ αTHF:THE ratio) had the highest blood pressure. Those individuals with reduced liver 11βHSD1 activity will be more prone to type 2 diabetes (Valsamakis *et al.* 2004;Andrews *et al.* 2002). One would therefore anticipate that those with a reduced THF+ αTHF:THE ratio will have insulin resistance. In keeping with previous studies in adults this was the pattern we demonstrated. The initial apparently paradoxical results are anticipated given the tissue specific effects of 11βHSD. Low relative 11βHSD2 inactivation results in hypertension by exposing mineralocorticoid receptors in the kidney to cortisol and low relative 11βHSD1 (principally liver activity) leads to insulin resistance.

Initial examination of the results from the urinary steroid profiles showed no relationship between prematurity and the various steroid metabolite ratios suggesting no evidence of programming. However overall activity of 11βHSD1 is determined by both activity in adipose tissue and activity in the liver. Preterm infants, though, were significantly leaner than the term controls. It appears that this may have masked the relationship between prematurity and measures of cortisol metabolism. After adjustment for current fat mass there was a correlation between gestation and measures of cortisol metabolism. Total cortisol metabolites were highest in preterm infants compared to term controls. The ratio of cortisol:cortisone metabolites was lower in preterm individuals compared to term controls suggesting a relative reduction in activity of 11βHSD1 compared to 11βHSD2. This is similar to the patterns seen in obesity, the metabolic syndrome and in the polycystic ovary syndrome (PCOS) (Stewart *et al.* 1990;Rodin *et al.* 1994;Stewart *et al.* 1999;Andrew *et al.* 1998). These groups have high total cortisol and total androgen levels, a low THF+αTHF:THE ratio and raised 5α reductase activity (high THF:αTHF ratio). Obese individuals show subtle changes in cortisol activity and cortisol metabolism. There is an increase in 24-hour cortisol secretion with normal or even enhanced feedback sensitivity (Rask *et al.* 2001). However plasma cortisol concentrations are not increased suggesting there is increased peripheral metabolic clearance of cortisol as a consequence of reduced reactivation of cortisol by 11βHSD1 and enhanced 5α reductase activity (Strain *et al.* 1982;Andrew *et al.* 1998). Obese individuals with hypertension and insulin resistance have fasting or basal cortisol levels very similar to control populations but have evidence of raised cortisol metabolite excretion combined with a reduced cortisol:cortisone metabolite ratio (Andrew *et al.* 1998;Andrews *et al.* 2002).

Increased total androgens are also a feature of obesity and a PCOS picture. There is evidence of increased 5α reductase activity (reduced THF:αTHF ratio). Tissue specific changes in 11βHSD1 activity have been described with reduced reactivation of cortisol in the liver but enhanced reactivation in adipose tissue (Walker *et al.* 1998b). Given that the pattern of urinary cortisol metabolite excretion associated with prematurity is similar to that seen in obesity and polycystic ovary disease described above it is tempting to conclude that individuals born prematurely are likely to be at high risk of PCOS and the metabolic syndrome.

9.6.3 Prematurity, Antenatal steroids and 11βHSD

One of the key objectives of the study was to examine the effect of antenatal steroids on blood pressure and glucose metabolism. Administration of antenatal steroids had an adverse effect on both blood pressure and glucose metabolism. In animal models there is evidence of programming of 11βHSD following administration of antenatal steroids (Benediktsson *et al.* 1993). We looked at the effect on the urinary steroid profiles following administration of antenatal steroids. In order to differentiate and control for as many potential confounding variables as possible, particularly prematurity, we looked at a subgroup of singleton pregnancies born before 34 weeks gestation. There was a modest but significant increase in androgen production following the administration of antenatal steroids and this appeared to exhibit a dose-response effect. Those children who had received multiple doses had a greater increase in total androgen production compared to those who received only a single dose. Similar responses were seen with other measures of cortisol metabolism. There was a significant reduction in the cortisol:cortisone metabolite ratio. There was evidence of increased total cortisol metabolites but this did not quite reach statistical significance. Similarly there was a trend for increased 5α reductase activity, again in a dose response manner. Once again this pattern is seen in individuals with the metabolic syndrome and polycystic ovary syndrome (Stewart *et al.* 1990;Rodin *et al.* 1994). It suggests that in addition to the programming effect of prematurity itself antenatal steroids may exert an additional effect. In adults the various contributions of cortisol metabolism and tissue sensitivity in individuals with type 2 diabetes are difficult to establish as hypertension and obesity often coexist and may exert a confounding effect (Walker 2000). One weakness of our study is that we are not able to analyse tissue specific responses, only overall cortisol:cortisone metabolism. Measurement of urinary cortisone would have allowed us to make more specific comment about 11βHSD2 activity in the kidney (Palermo *et al.* 1996;Tomlinson *et al.* 2002). However an identical picture to the one we noted following administration of antenatal steroids has been documented in non obese normotensive men with type 2 diabetes compared to controls. There was evidence of impaired liver 11βHSD1 activity but normal tissue adipose 11βHSD1 activity. They had an increase in the relative excretion of A ring reduced metabolites of cortisol indicating enhanced 5α reductase activity (Andrews *et al.* 2002). This study group had few potential confounding variables and provide strong support for the hypothesis that 11βHSD is involved in programming and in particular may be the mechanism whereby antenatal steroids exert a programming effect. From studies of the conversion of administered cortisone to cortisol it appears that in obesity liver 11βHSD1 activity is reduced but adipose 11βHSD1 increased (Rask *et al.* 2001;Stewart *et al.* 1999). Overall levels of 11βHSD1 activity appear lower (reduced THF+αTHF:THE ratio). This is the pattern we observed associated with prematurity. A reduction in liver 11βHSD1 activity may be a protective response to try and shield the liver from increased cortisol production in fatty tissue.

9.6.4 Prematurity and Leptin

Leptin is another candidate by which programming may be mediated. As anticipated it was highly correlated with fat mass. After adjustment for fat mass higher levels of leptin were associated with raised blood pressure and reduced insulin sensitivity but not hyperlipidaemia. We found no link between gestational age at birth and leptin levels age 5 years despite it having been suggested that leptin may be influenced by prematurity with reduced cord blood levels in the neonatal period (Shekhawat *et al.* 2000). There is evidence that leptin concentrations are increased in preterm infants born to mothers with pregnancy induced hypertension – a risk factor for raised diastolic blood pressure (Hytinantti *et al.* 2000). In other studies whilst low birth weight was associated with raised leptin in later life it did not account for the hyperinsulinaenia seen (Phillips *et al.* 1999).

9.6.5 Interaction between mechanisms

It is likely that there is interaction between the various mechanistic pathways proposed even if under carefully controlled circumstances one or other may appear more prominent. For example there is evidence that IGF-I levels regulate activity of 11βHSD1 - but not 11βHSD2 (Moore *et al.* 1999). Growth hormone or IGF-I deficiency may effectively increase cortisol production in key target tissues including the liver and adipose tissue promoting insulin resistance and visceral adiposity. In a small study of patients on growth hormone replacement urinary cortisol metabolites fell as did the urinary THF+αTHF:THE ratio once growth hormone was started (Weaver *et al.* 1994). High levels of IGF-I inhibit 11βHSD1and may reduce effective levels of cortisol within tissues. IGF-1 may also mediate 5α reductase activity (Horton, Pasupuletti, and Antonipillai 1993). Transgenic mice with over expression of 11βHSD1 have evidence of leptin resistance (Masuzaki *et al.* 2003). In man increased adipose activity of 11βHSD1 is associated with insulin resistance and an increase in leptin over and above that normally seen for the degree of obesity (Wake *et al.* 2003). Leptin is related to growth hormone secretion and body fat in childhood (Fors *et al.* 1999).

9.7 Prematurity and Adrenarche

The urinary steroid profiles we obtained suggest children born prematurely are likely to be at increased risk of adrenarche and earlier onset of puberty. Others studies looking at the effect of low birth weight on androgens support this. A study of urinary steroid profiles in a group of 190 children born at term aged nine years (Clark *et al.* 1996) demonstrated higher output of urinary adrenal androgen metabolites in children who were lighter at birth. The relationship was linear. Urinary glucocorticoid metabolites were also elevated in children who were lighter at birth but the relationship was U-shaped (Clark *et al.* 1996). Studies from the ALSPAC cohort suggest that levels of the adrenal androgens, dehydroepiandrosterone sulfate and androstenedione, in the blood were inversely related to birth weight (Ong *et al.* 2004b). They were highest aged eight years in those children who were smallest at birth. High androgen levels were associated with higher levels of IGF-I, insulin resistance and central obesity (Ong *et al.* 2004b). A study of women in their forties suggested that prematurity may lead to the polycystic ovary syndrome (Cresswell *et al.* 1997). There is certainly evidence that low birthweight and thinness at birth are related to earlier menarche (Adair 2001;Ibanez *et al.* 2000;Neville and Walker 2005).

9.8 The Fetal Adrenal

Antenatal steroids appear to programme 11βHSD. It is not clear why simply being born prematurely (without antenatal steroid administration) should result in reduced activity and programming of 11βHSD. It is possible that the process by which the adrenal gland matures may be affected. The fetal adrenal cortex though has two distinct zones. The inner zone develops early during intrauterine life and comprises 80% of the weight of the adrenal gland at birth (Hingre *et al.* 1994). The outer permanent or adult zone of the fetal adrenal has limited growth and biosynthetic activity until the third trimester of pregnancy (Hingre *et al.* 1994). The fetal zone of the adrenal produces androgens and cortisone with relatively low levels of cortisol (Bolt *et al.* 2002). This suggests high levels of 11βHSD2 activity in the fetal cortex (Fujitaka *et al.* 1997). After birth cortisol production rises and the cortisol:cortisone ratio rises. Activity of the fetal zone persists to around 42 weeks post conceptual age in premature infants and then rapidly declines to levels similar to term infants at the same post conceptual age (Midgley *et al.* 1998). Others have suggested though that the fetal zone may persist for longer in preterm compared to term infants and that higher levels of 11βHSD 2 may persist in the fetal zone of the adrenal cortex in preterm infants (Fujitaka *et al.* 1997). There is some evidence that cortisone levels are higher in preterm infants born prior to 30 weeks compared to those born between 30-33 weeks suggesting altered 11βHSD activity particularly in infants born prematurely before 30 weeks gestation (Bolt *et al.* 2002). Cortisone levels were also noted to be highest in those infants with the lowest birthweight SDS (Bolt *et al.* 2002). It is tempting to suggest that this is a potential mechanism whereby the programming effect of prematurity may be affected. Premature delivery may have subtle longer-term effects on the maturation of the adrenal which might alter 11βHSD activity in later life.

Similarly antenatal steroids may have an effect of the outer adult zone of the developing fetal adrenal.

Chapter 10. Conclusions

Preterm birth programmes for subsequent hypertension and insulin resistance. Individuals born prematurely had significantly higher blood pressure, higher resting heart rate and evidence of insulin resistance compared to term controls. The greater the degree of prematurity the greater the magnitude of the effect on blood pressure, heart rate and insulin resistance. The systolic blood pressure in those born before 28 weeks gestation was 7 mmHg higher than in term controls. Almost 24% of children born prematurely had a systolic blood pressure greater than hundred and five mmHg compares to only 10% of term infants. It is likely that the elevated blood pressure and insulin resistance associated with prematurity will be amplified over time and that these risk factors will translate into a significantly increased risk of cardiovascular disease, cerebrovascular disease and type 2 diabetes in later life.

Preterm birth is associated with dramatic early growth failure and subsequent prolonged catch-up growth. The degree of growth failure is linked to the degree of prematurity. Again those born most prematurely exhibit the most marked growth failure and prolonged catch up growth over many years. Given the evidence from the work surrounding the influence of growth failure and catch-up growth on risk factors for the metabolic syndrome it seems likely that this pattern of growth is linked to the elevated blood pressure and insulin resistance demonstrated within the cohort. Whilst weight gain in childhood was important it was the failure of early linear growth that was the most important predictor of blood pressure and insulin resistance. We would not support the premise that relative under nutrition in the neonatal period is protective (Singhal *et al.* 2003).

Both the growth hormone-IGF-I axis and the pituitary adrenal axis look likely candidates for the mechanism by which the programming effect of prematurity is affected. There was a strong relationship between being born prematurely and raised levels of IGF-I. IGF-1 was closely correlated with raised blood pressure, raised resting heart rate and measures of insulin resistance. Preterm infants also exhibited patterns of urinary adrenal and cortisol metabolite excretion that are similar to those seen in the metabolic syndrome, type 2 diabetes and the polycystic ovary syndrome. There was evidence of increased adrenal metabolite excretion and down-regulation of 11β hydroxysteroid dehydrogenase type 1 as a consequence of prematurity. Such patterns of adrenal and cortisol metabolites significantly increase the concern that premature birth increases the risk of adrenarche and early puberty.

Antenatal steroids whilst clearly beneficial in terms of improved survival in prematurity have an adverse programming effect on blood pressure and glucose metabolism. Individuals born prematurely who had received antenatal steroids were more likely to have higher blood pressure and evidence of insulin resistance. This is consistent with the results of animal models investigating the effect of antenatal glucocorticoid exposure. A clear effect on cortisol metabolism of antenatal steroids is evident from urinary steroid metabolite profiles. This strongly suggests a programming effect of steroids on 11β hydroxysteroid dehydrogenase. A single dose of antenatal steroid appears to increase adrenal androgen production and reduce activity of 11β hydroxysteroid dehydrogenase type 1. Such changes in androgen and corticosteroid metabolism are associated with an increased risk of type 2 diabetes, hyperlipidaemia and polycystic ovary syndrome.

There appear to be significant long-term metabolic consequences of being born prematurely and interventions in the antenatal and neonatal period, in particular the administration of antenatal steroids, appear to have an adverse effect on the likelihood of long-term metabolic consequences. Individuals born particularly at the extremes of prematurity may need longer term surveillance to facilitate early detection of raised blood pressure, glucose intolerance and hyperlipidaemia to which they are at increased risk as a consequence of their prematurity.

10.1 Future study

Further work looking at patterns of growth in neonates is warranted particularly given that there is considerable variation in rates of growth between neonatal units and considerable variation in the achievement of nutritional targets. Improved growth rates may potentially negate some of the adverse effects on blood pressure and glucose metabolism of being born prematurely. Similarly it is important to establish whether the risk factors identified do track into adult life by examining post-pubertal cohorts. Finally, whilst antenatal steroids are vital in improving survival in preterm infants, studies examining different doses have never been undertaken. It is possible that the benefits of lung maturation may be obtained with lower doses and without the adverse metabolic effects we have demonstrated.

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Appendix

The following figures illustrate scatterplots of the raw growth data for weight, height and head circumference, expressed as standard deviation scores, at birth, 12 months and 5 years for the whole cohort.

Figure 17. Weight standard deviation score at birth plotted against gestational age at birth in weeks.



**Figure 18.** Weight standard deviation score at 1 year of age plotted against gestational age at birth in weeks.



Figure 19. Weight standard deviation score at 5 years of age plotted against gestational age at birth in weeks.



Figure 20. Height standard deviation score at birth plotted against gestational age at birth in weeks.



Figure 21. Height standard deviation score at 1 year plotted against gestational age at birth in weeks.



Figure 22. Height standard deviation score at 5 years of age plotted against gestational age at birth in weeks.



Figure 23. Head circumference standard deviation score at birth plotted against gestational age at birth in weeks.



Figure 24. Head circumference standard deviation score at 5 years plotted against gestational age at birth in weeks.



Figure 25. Birth Weight standard deviation score grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 weeks 34-37 weeks term

Figure 26. Weight standard deviation score at 1 year grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 27. Weight standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.

 2 23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 28. Length standard deviation score at birth grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 29. Length standard deviation score at 1 year of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 30. Height standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 31. Head circumference standard deviation score at birth grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

Figure 32. Head circumference standard deviation score at 5 years of age grouped by gestational age at birth (23-26 weeks, 27-30 weeks, 30-34 weeks, 34 – 37 weeks and Term) together with mean for each group.



23-26 weeks 27-30 weeks 30-34 week 34-37 weeks term

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