The effect of Intra Uterine Growth Restriction (IUGR) on heart rate and blood pressure in children

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Abstract

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Infants with Intra Uterine Growth Restriction (IUGR) are reported to be more prone to develop obesity and cardiovascular problems as adults. Research in this field is complicated due to use of different statistical models and inadequate cardiovascular measurements.

I intended to find out whether the difference in body size between IUGR and normal infants persisted through the childhood and whether there were any differences in cardiovascular parameters and maturation of circadian biorhythms between these groups. 75 nine year olds were recruited and forty-one of them were IUGR. All the children had their twenty-four hour heart rate variability, blood pressure and urinary Cortisol excretion measured.

IUGR children showed a greater increase in their weight between birth and nine years but the z scores for current weight, height and Body Mass Index (BMI) and incidence of obesity were higher in the control group.

Both groups showed similar diurnal variation of all cardiovascular parameters and no evidence of the cardiovascular system being dominated by the Sympathetic nervous system in the IUGR children, which could have been detrimental to their cardiovascular health.

The Systolic Blood Pressure (SBP) was significantly higher in the control group. Later weight was the better predictor of SBP. BMI was a significant predictor of SBP. Urinary cortisol/Creatinine ratio was not different between the two groups.

I conclude that IUGR children grow faster but remain shorter and lighter than their normal counterparts. IUGR children on average are physiologically indistinguishable from normal children and do not show any abnormality in the cardiovascular parameters, which could link them to future disease. Normal children have higher SBP than the IUGR children which is related to larger body mass. The current body size appears to have more impact on the level of blood pressure at the age of nine years compared to IUGR.

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Addendum:

One CD Rom containing individual socio-economic and medical data, individual serial anthropometric measurement data, individual ambulatory blood pressure measurement data, individual urinary Cortisol excretion data and individual heart rate variability data

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Finally, a big thank you to my family who has endured the bad as well as the good part of my candidature with endless patience and support. We look forward to a thesis-free life together.

Declaration

The work presented in this thesis was carried out between December 2003 and March 2006 at the Department of Health Sciences, University of Leicester under the supervision of Dr. Michael Wailoo. Financial assistance was obtained from Leicestershire Primary Care Research Alliance. It is wholly my own composition and contains no material that has been accepted for any degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the text. To the best of my knowledge and belief no material previously published or written by another person is included, except where due acknowledgment is made in the text of this thesis.

I performed the medical examination on the participants and fitted them with the ambulatory blood pressure monitors. The technicians at the Cardiac Investigations Department of Leicester Royal Infirmary performed the initial ECG on the children and fitted them with the ambulatory heart rate monitors. The technicians at the Chemical Pathology department of Leicester Royal Infirmary performed assays for urinary Cortisol excretion.

Abbreviations

ABP	Ambulatory Blood Pressure
ABPM	Ambulatory Blood Pressure Measurements
ANS	Autonomic Nervous System
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
DBP	Diastolic Blood Pressure
DF	Degree of Freedom
ECG	Electrocardiogram
F	F Value for the Statistical test
FFT	Fast Fourier Transformation
FOH	Foetal Origins Hypothesis
GLM	General Linear Model
HF	High Frequency
HF:LF	High Frequency Low Frequency Ratio
HRV	Heart Rate Variability
IGF	Insulin-like Growth Factor
IMD	Index of Multiple Deprivations
IUGR	Intra Uterine Growth Restriction
LF	Low Frequency
MDBP	Mean Diastolic Blood Pressure
MESOR	Midline Estimating Statistic of Rhythm
mmHg	Millimetre of Mercury
MSBP	Mean Systolic Blood Pressure
NEC	Necrotising Enterocolitis
P	Probability Value
PP	Pulse Pressure
RMSSD	Root Mean Square Standard Deviation
RTM	Regression to Mean
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDNN	Standard Deviation of NN Intervals
SGA	Small for Gestational Age
SUDI	Sudden Unexpected Death in Infants
Z SCORE	Standard Deviation Scores

FOREWORD

Children with Intra Uterine Growth Restriction (IUGR) are reported to suffer from increased incidence of perinatal mortality, sudden unexpected death in infancy, cognitive dysfunction during childhood, delayed maturation of Autonomic Nervous System (ANS) and increased incidence of obesity and cardiovascular morbidity during adulthood. I intended to find out whether there are any early signs of cardiovascular problems in IUGR children during their childhood and also to explore any possible link between "catch up" growth, current body size, ANS maturation and cardiovascular parameters in the IUGR children.

The IUGR and the normal children from an existing database are recalled at nine years of age for monitoring of twenty-four hour Ambulatory Blood Pressure, Heart Rate Variability and measurement of early morning urinary Cortisol Creatinine ratio. Information is also collected in relation to longitudinal anthropometric measurements between birth and nine years, current demographic, medical and socio-economic data. A number of variables is analysed for predicting cardiovascular outcomes. Particular emphasis is placed on use of regression models exploring the association between IUGR, birth weight and compensatory growth, current body size and current blood pressure.

The text comprises seven chapters. In the first chapter I review relevant literature in three sections. The first section refers to the difficulties in identifying children with IUGR, consequences of being IUGR, current view on the association between "catch up" growth and adult cardiovascular diseases and the importance of using appropriate statistical regression models in this field of research. The second section describes the various aspects of Heart Rate Variability and its development as a reliable, non-invasive tool for measuring the Autonomic Nervous system tone in adults and

children. The third section refers to technicalities of ambulatory blood pressure measurement, its advantage over casual blood pressure measurements and the scope of research exploring IUGR and ambulatory blood pressure.

The second chapter traces the formulation and development of the research proposal linked to the original study of physiological maturation of IUGR babies and my methodology to carry it out in the same population at the age of nine years.

The results are presented in the following way:

Chapter 3: Description of the subjects and comparison of the unadjusted socio-economic, demographic and anthropometric variables between the two groups as well as between the participants and the non-participants.

Chapter 4: Exploratory regression analysis of the longitudinal anthropometric data to find out whether the difference in body size between the two groups detected at birth remains significant at nine years.

Chapter 5: Description of twenty-four hour ambulatory blood pressure profile between two groups and comparison using Cosinor method.

Chapter 6: Exploratory regression analysis of association between Ambulatory blood pressure values, IUGR, compensatory growth and further testing of "Foetal Origins Hypothesis".

Chapter 7: Description and comparison of twenty-four hour Heart Rate Variability profile between two groups using Cosinor method and regression analysis.

Chapter 8: Comparison of urinary cortisol excretion between two groups.

The ninth chapter contains the discussion on my findings and the scope of future research and I also report the incidental finding of altered circadian biorhythm, detected in two children with neurodevelopmental delay and two children with autism.

By comparing the outcomes in the IUGR children with the control children, this research aims to find out whether there are any differences in the two groups in relation to cardiovascular parameters. Our findings would provide an insight into the effect of postnatal and environmental factors on physical growth and physiological maturation of IUGR children and any early indication of impending cardiovascular morbidity. This may give us opportunities for prevention of later cardiovascular disease, which is currently an important public health issue.

1: Introduction

1.1 Intrauterine Growth Restriction (IUGR):

Intrauterine growth restriction is an abnormality of foetal growth occurring at any point from conception to late pregnancy. It is a "continuum of conditions which ultimately result in the failure of the foetus to achieve its inherent growth potential" (Pollack, Divon 1992). It is likely that the timing, duration and the type of insult will result in differing levels and types of morbidity and mortality postnatally. In some cases the effects of the insult may be so severe that intrauterine death occurs whilst in other cases the effects may not become apparent until late in adult life.

1.1.1 Types of IUGR:

Two main patterns of foetal growth restriction are observed, namely symmetric and asymmetric. If foetal growth is impaired during the first or the second trimester, the infant will have symmetric growth restriction. In contrast, asymmetric growth, in which an infant has a smaller abdominal size compared to head size, will occur if the decrease in growth velocity happens in the last trimester. This head-sparing phenomenon is the most common form of IUGR (~ 70%-80%) and is attributed to the ability of the foetus to adapt, redistributing the cardiac output to the vital organs at the expense of the liver, muscle and fat. Although some overlap can occur, the timing of the growth delay is more important than the aetiology in determining the pattern of growth restriction.

1.1.2 Aetiology of IUGR:

While a large number of aetiologies are not identified, the known associations involve foetal, placental and/or maternal factors (**Table 1**). Specifically foetuses with

chromosomal disorders such as trisomy 13, 18 and 21 and other autosomal irregularities (e.g. deletions, ring chromosomes) often have impaired growth (Resnik 2002).

Less frequently IUGR may be due to first or second trimester foetal infections, including toxoplasmosis, cytomegalovirus, rubella, parvovirus and malaria. Chronic maternal vascular disease due to hypertension, diabetes mellitus, renal disease or collagen vascular disease is the most common cause of IUGR in developed countries (Lin, Santolaya-Forgas 1998). Hypercoagulable maternal conditions such as thrombophilia and antiphospholipid antibody syndromes also inhibit growth either by placental thrombosis formation or by secondary effects of maternal hypertension. (Martinelli, Grandone et al. 2001). Persistent maternal hypoxia due to high altitude, severe pulmonary or cardiac disease and /or severe chronic anaemia limits oxygen delivery to the foetus and attenuates foetal growth.

Periods of famine in the Netherlands, Germany and the former USSR have shown that severe maternal malnutrition can also impair foetal growth (Barker 1994, Roseboom, van der Meulen et al. 2001). Maternal toxins e.g. prolonged cigarette smoking and alcohol consumption and ingestion of other drugs (steroids, coumadin, hydantoin, cocaine and heroin) also contribute to IUGR (Haworth, Ellestad-Sayed et al. 1980, Lee, Chernausek et al. 2003).

Table 1 Maternal, Placental and Foetal aetiologies of IUGR

Ref: (Brodsky, Christou 2004)

Maternal	Placental	Foetal
A. Vascular disorders (~ 25%-30%): 1. Hypertension 2. Diabetes Mellitus 3. Renal disease 4. Collagen vascular disease **B. Hypercoagulable states:** 1. Thrombophilia 2. Antiphospholipid antibody syndrome **C. Persistent Hypoxia:** 1. High altitude 2. Pulmonary or cardiac disease 3. Severe anaemia **D. Undernutrition** **E. Toxins:** 1. Tobacco 2. Alcohol 3. Medications 4. Narcotic drugs **F. Uterine malformation or masses**	**A. Abnormal Trophoblast invasion** **B. Placental infarcts** **C. Placenta previa** **D. Circumvallate placenta** **E. Chorioangiomata** **F. Velamentous Umbilical cord insertion** **G. Umbilical-placental vascular anomalies**	**A. Genetic (~20%)** 1. Chromosomal abnormality 2. Syndromes 3. Congenital malformation **B. Multiple gestation (~ 5%)** **C. Intrauterine infections:** 1. Cytomegalovirus 2. Malaria 3. Parvovirus 4. Rubella 5. Toxoplasmosis 6. Herpes virus 7. HIV

1.1.3 Pathophysiology of IUGR:

These multifactorial causes of IUGR lead to 3 possible scenarios (**Figure 1.1**).

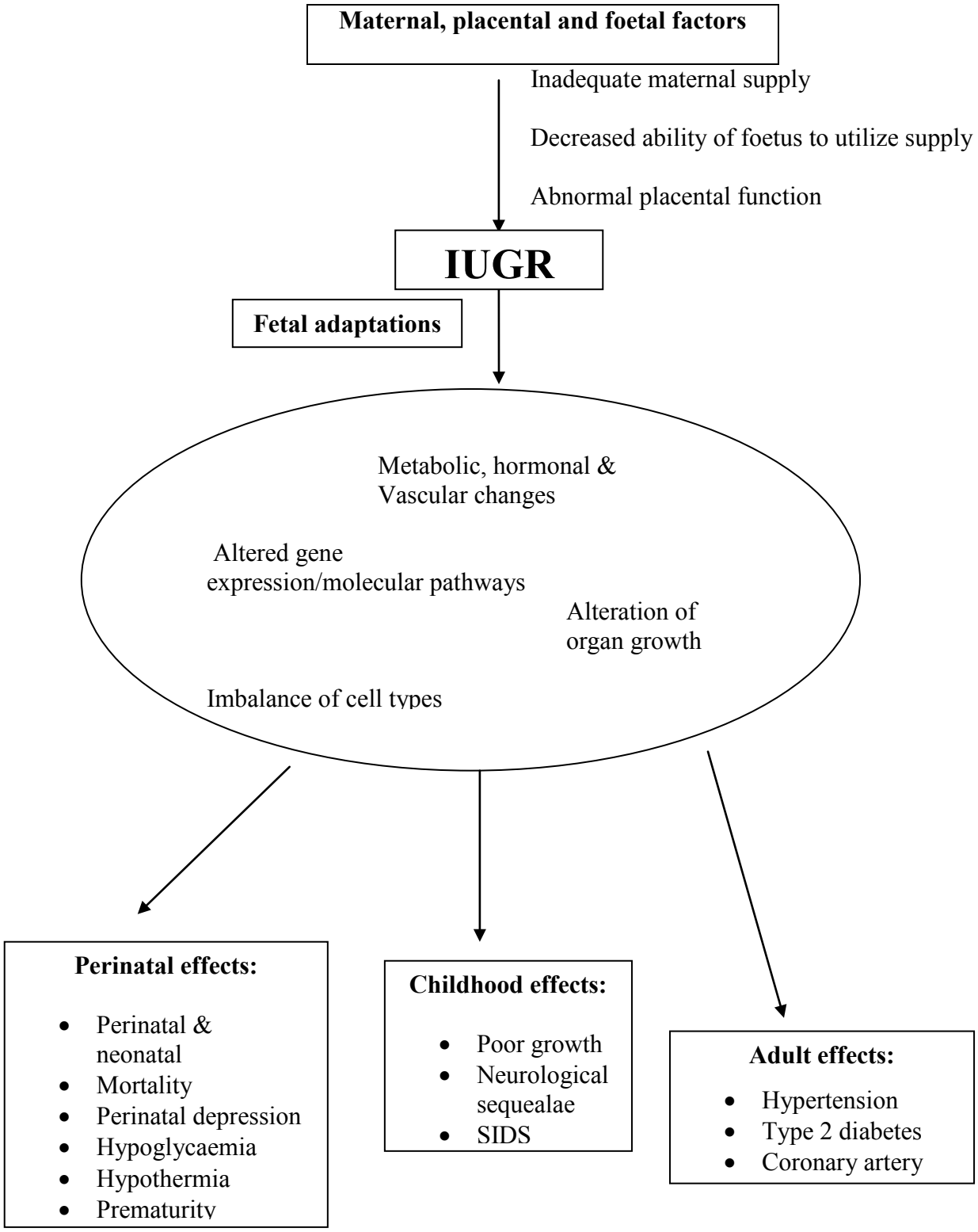
1. Abnormal placental function
2. Inadequate maternal supply of oxygen and/or nutrients, and/or
3. Decreased ability of the foetus to use the supply

The placenta plays an integral role in the first two categories. Abnormal development, inadequate perfusion, and dysfunction of the placental villi are often responsible for the development of IUGR, especially the early onset type. Oxidative stress, infarction, cytokine damage, and hypertension can cause further permanent damage to placental villi (Kingdom, Huppertz et al. 2000).

Recently, it has been postulated that insulin and its associated insulin-like growth factors (IGF) may play a critical role in the development of IUGR (Baker, Liu et al. 1993, Fowden 2003). Low levels of IGF-1 and IGF-1 receptor mutations have been observed in growth-restricted infants (Wang, Lim et al. 1991, Giudice, de Zegher et al. 1995, Woods, Camacho-Hubner et al. 1996, Abuzzahab, Schneider et al. 2003).

Other potential signalling pathways involved in placental causes of IUGR are the glial cell missing–1(GCM1) gene and leptin (Anson-Cartwright, Dawson et al. 2000, Christou, Serdy et al. 2002).

Figure 1: Pathophysiology of IUGR (Brodsky, Christou 2004)



NEC = necrotising enterocolitis. SIDS = sudden infant death syndrome

1.1.4 Use of birth weight as a "proxy" marker of IUGR

Intrauterine growth restriction is a confusing problem during pregnancy and to date there has not been a uniform and accepted definition of IUGR and Small for Gestational Age (SGA). References in the medical literature to underweight babies date back to 1919, when it was suggested that all newborns weighing less than 2500 grams should be classified as "premature" (Steven G. Gabbe, Jennifer R. Niebyl, Joe Leigh Simpson, Mikki Senkarik 1996). However it was not until 1961 that the world Health Organisation (WHO) acknowledged that many infants defined as "premature" were not born early but were simply "low birth weight" (Dunn 1985). The current WHO criterion for low birth weight is less than 2500 grams or below the 10^{th} percentile for gestational age. Similarly IUGR is often defined as that resulting in a birth weight below the 10^{th} percentile for gestational age using criteria specific to the population under study (Read, Catz et al. 1984). This definition has received much criticism not least because it incorporates one tenth of the normal population thereby including normal but constitutionally small infants, whilst making no allowance for infants who are above this percentile yet have failed to achieve their growth potential. This definition has also led to frequent erroneous use of IUGR as synonymous of SGA.

Other authors have suggested using the 5th or 3rd percentile for birth weight (equivalent to 2 standard deviations below mean) to define IUGR infants. The counter argument in favour of a strict definition is that birth weight is probably the single most important factor affecting neonatal morbidity and mortality and should be aggressively screened for (McCormick 1985).

1.1.5 Use of antenatal ultrasound scans to define IUGR:

Ultrasound biometry is now the gold standard for assessing the foetal growth. Measurements most commonly used are the biparietal diameter, head circumference, abdominal circumference and femur length. Percentiles have been established for each of these parameters and estimated foetal weight can be calculated. Accurate dating of pregnancy is essential in the use of any of these parameters. Abdominal circumference is the first measure to change and has a sensitivity of over 95 percent if the measurement is below 2.5th percentile (Vandenbosche, Kirchner 1998, Hadlock 1983, Brown, Miller et al. 1987). Also useful is the ratio of the head circumference to the abdominal circumference (HC/AC). Between 20 and 36 weeks of gestation, the HC/AC ratio normally drops almost linearly from 1.2 to 1.0. The ratio is normal in foetus with symmetric growth restriction and elevated in the foetus with asymmetric growth retardation (Peleg, Kennedy et al. 1998). It has been recommended that diminished growth velocity in foetus must be documented by at least 2 intrauterine growth assessments (Lee, Chernausek et al. 2003), **(Figures 2 & 3).**

Unfortunately valid assessment of duration of pregnancy and access to serial antenatal ultrasound scans are often very limited in developing countries. Therefore although we know that the size at birth is a function of two factors: **A)** the rate of foetal growth and **B)** the duration of gestation - the prevalence of low birth weight (< 2500 grams) is often used as a proxy to determine the magnitude of IUGR.

Figure 2 and Figure 3

Serial Abdominal Circumference measurements showing tapering of growth.

(Hadlock, Harrist et al. 1991)

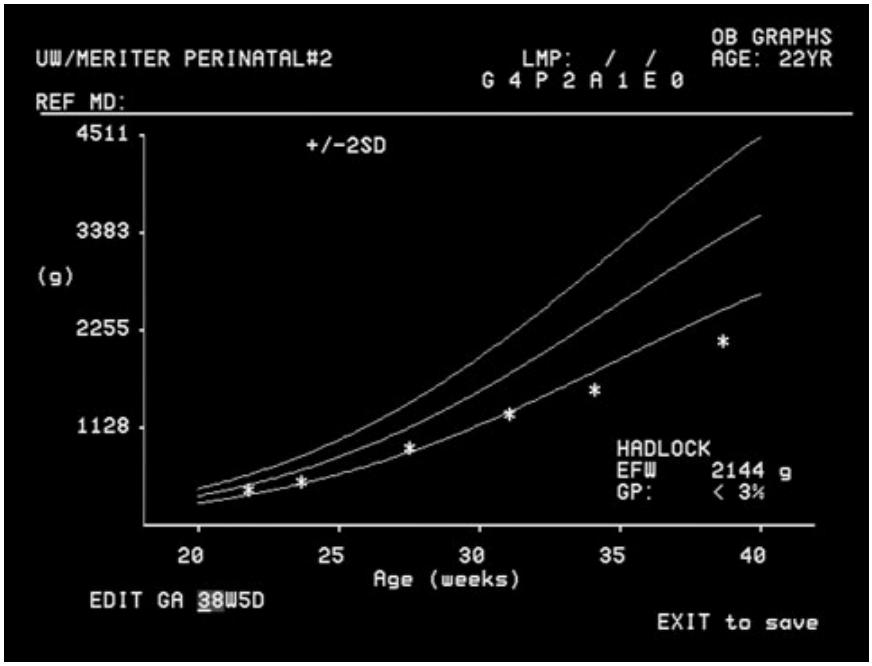


Figure 2

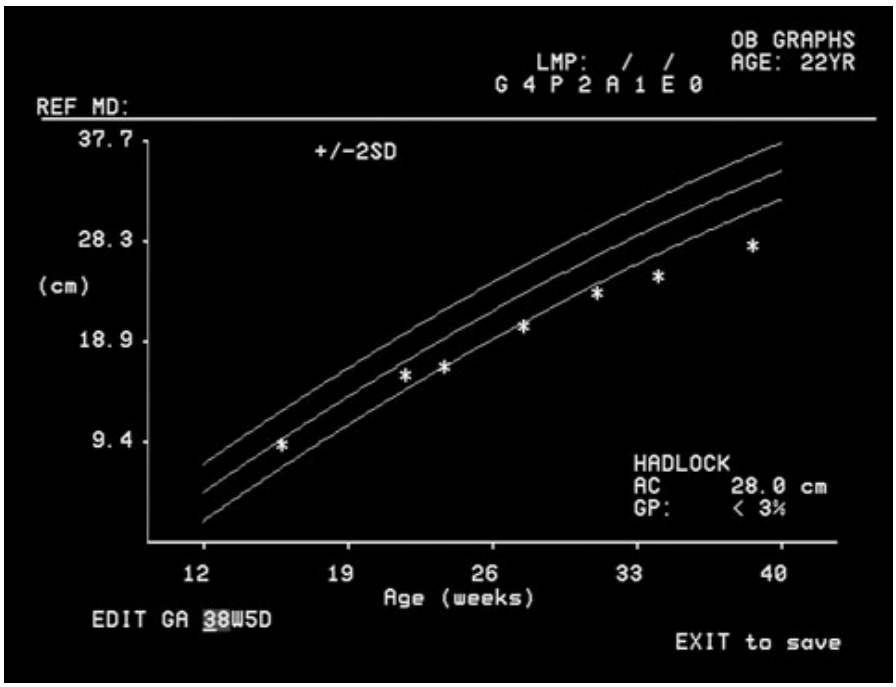


Figure 3

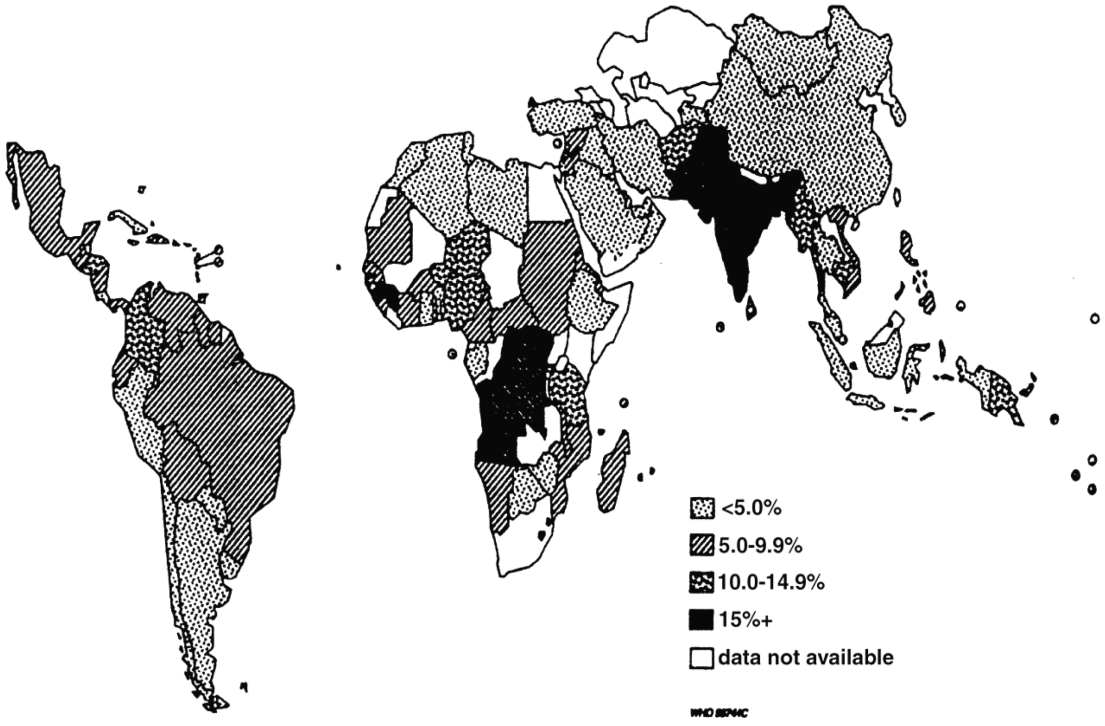
1.1.6 Epidemiology of IUGR:

The confusion over what constitutes an IUGR infant serves to hide the true incidence rate. The magnitude of the problem varies widely across countries (**Figure 4)**. Currently it is estimated that worldwide 30 million infants i.e. 23.8% of all deliveries, are born every year, who could be considered by the current classification (i.e. birth weight $< 10^{th}$ centile for gestational age reference curve) to have experienced IUGR. Nearly 75% of these infants are born in Asia, a further 20% in Africa and 5% in Latin America (de Onis, Blossner et al. 1998). If the low birth weight model is used as a proxy for identifying IUGR then 11% of all newborns in developing countries, or 13.7 million infants suffer from IUGR. This rate is approximately 6 times higher than developed countries (de Onis, Blossner et al. 1998).

Figure 4

Prevalence of intrauterine growth retardation in developing countries (birth weight less than 10th percentile for gestational age, WHO criteria).

(de Onis, Blossner et al. 1998)



1.1.7 Consequences of IUGR in the neonatal period and early infancy:

Numerous studies have shown that that IUGR foetus has a greater risk of foetal mortality, correlating directly with the severity of IUGR (Resnik 2002).

IUGR infants are prone to suffer from hypoglycaemia, hypothermia and polycythemia. The high incidence of premature delivery in IUGR infants leads to the characteristic postnatal risks of prematurity. Recent studies suggest that Respiratory Distress syndrome occurs at equal rates in growth-restricted and appropriately grown premature infants and perhaps may actually be more common in IUGR infants (Bernstein, Horbar et al. 2000). Some reports demonstrate that prenatal findings of abnormal flow in the foetal aorta as well as absent or reversed end-diastolic umbilical arterial flow predisposes the growth restricted foetus to develop Necrotising Enterocolitis (Bhatt, Tank et al. 2002) and there is an increased incidence of intraventricular haemorrhage in IUGR infants (Zaw, Gagnon et al. 2003).

Endocrinal abnormalities in the form of early hypothyroxinemia, reduced levels of IGF-1 and hyper secretion of growth hormone in IUGR babies have also been reported (Martin, Van Marter et al. 2001, Leger, Limoni et al. 1997). Increased rate of infection represents another risk (Vik, Vatten et al. 1996), partly as a result of lower levels of maternal IgG crossing the placenta and also because of decreased T and B lymphocytes at birth. IUGR infants are also at increased risk of Sudden Unexpected Death in Infant (SUDI) but the risk factors for IUGR are similar to those for SUDI, which suggests that factors causing IUGR may play a crucial role in SUDI (Cooke 1998).

Respiratory patterns of SGA newborns, closely dependant on maturing brain stem controls centre, differ significantly at 2 weeks of age, from that seen in normal newborns. SGA infants tend to experience more central respiratory pauses and an

absence of age related modification in respiratory patterns (Curzi-Dascalova 1995, Curzi-Dascalova, Peirano et al. 1996).

Researchers from Leicester have shown that IUGR infants develop temperature, heart rate and urinary cortisol patterns at a later postnatal age than non-IUGR infants (Jackson, Wailoo et al. 2004). These observations suggest that IUGR infants experience a delay in some aspect of ANS control and that possibly contribute to the slight increase in the incidence of SUDI in IUGR infants.

1.1.8 Consequences of IUGR during childhood and adolescence:

The effects of IUGR persist beyond the neonatal period and may have profound influences during childhood. There are a lot of potential confounders to be considered when assessing the available data regarding outcome of IUGR in childhood. Due to the nature of the development of IUGR, prospective cohort studies with clear denominator definition and randomized controlled trials of interventions are most likely to shed lights on this complex area.

Effect on childhood growth:

"Catch-up growth" has been defined as "the acceleration in growth that occurs when a period of growth retardation ends and favourable conditions are restored" (Ashworth, Millward 1986). Literature review indicates that IUGR newborns catch-up partially in growth relative to controls during the first one or two years of life (Villar, Smeriglio et al. 1984, Walther 1988, Fitzhardinge, Inwood 1989, Barros, Huttly et al. 1992, Albertsson-Wikland, Karlberg 1994). After about two years of age, IUGR subjects maintain their achieved place in the distribution and neither catch-up nor fall behind. Achieved size at 2 to 7 years was significantly different in cases compared to controls

in these studies. Longitudinal studies support a greater incidence of short stature in IUGR children born prematurely compared with full-term IUGR children (Leger, Limoni et al. 1997, Strauss, Dietz 1997). A large study of 40000 American children (Third National Health and Nutrition examination survey) between 1988 and 1994 found that despite catch-up growth, infants born SGA tend to remain shorter and lighter with smaller head circumferences through early childhood compared with AGA infants (Hediger, Overpeck et al. 1998).

Causes of poor postnatal growth in infants with IUGR:

Catch up growth is a poorly understood phenomenon. It has been postulated that insulin and its associated insulin-like growth factors (IGF 1 and IGF 2) may play a critical role in the development of IUGR (Baker, Liu et al. 1993, Fowden 2003). Alterations in the GH-IGF axis, IGF-1 receptor mutations and resulting low plasma levels of IGF-1 and IGF 2 have been observed in growth-restricted infants (Wang, Lim et al. 1991, Giudice, de Zegher et al. 1995, Woods, Camacho-Hubner et al. 1996, Abuzzahab, Schneider et al. 2003). Also higher basal levels of serum GH and a higher GH response to the Growth Hormone Releasing Hormone (GHRH) in IUGR infants suggest GH resistance or insensitivity (de Waal, Hokken-Koelega et al. 1994). A significant proportion of children (up to 60%) who fail to achieve catch-up growth have disturbed growth hormone secretion and low serum IGF-1 concentrations and in IUGR children who achieve catch up growth, levels of IGF 1 normalise (de Waal, Hokken-Koelega et al. 1994). It is thus likely that catch-up growth may result from increased production of growth factors. A number of randomized trials of GH treatment have demonstrated a short-term beneficial effect in IUGR children with poor

postnatal growth (de Zegher, Maes et al. 1996). However results of long-term outcome studies of final height are awaited.

Strauss & Dietz (Strauss, Dietz 1998) compared data from IUGR children who showed catch-up growth with those who did not. They found no difference in a number of perinatal risk factors. This would suggest that genetic factors rather than environmental events might account for the persistent effects of IUGR on postnatal growth. The discovery of single-gene mutations in IGF-1 in some infants with IUGR and the increased prevalence of IUGR in certain families support the hypothesis that genetic factors play an important role.

Neurological outcome of IUGR:

The literature is inconsistent about the influence of IUGR on long-term neurological outcome, ranging from minimal decreases in IQ (Sommerfelt, Andersson et al. 2000) or no effects (Paz, Laor et al. 2001, O'Keeffe, O'Callaghan et al. 2003) to spastic cerebral palsy (Blair, Stanley 1990, Uvebrant, Hagberg 1992, Topp, Langhoff-Roos et al. 1996). Most reports suggest that severe IUGR incurs subtle behavioural and learning disabilities during childhood (Scherjon, Oosting et al. 1998, Pryor, Silva et al. 1995, Goldenberg, Hack et al. 1998). The disparity of findings related to neurological outcome can be explained by the heterogeneity of the populations examined including differences in aetiology, definition of IUGR, age the children are examined, perinatal and neonatal complications, and distinct socio-demographic and postnatal environmental factors.

1.1.9 Long-term outcome of IUGR and Foetal Origins Hypothesis (FOH):

In the late 1980s David Barker and his colleagues from Southampton observed that the geographical distribution of heart disease in the UK was more closely related to a person's place of birth than where they currently lived (Barker, Osmond 1986). They suggested that early life events could cause permanent changes in physiology that, depending on the environment, may later predispose people to disease. Associations with birth weight and with growth in infancy suggested that early nutrition was an important component of these "programmed" effects. These first clues opened up a new area of research in a field now officially termed "the developmental origins of health and disease" (**DOHaD**).

In 1995 Barker wrote "The foetal origins hypothesis states that foetal under nutrition in middle to late gestation, which leads to disproportionate foetal growth programmes later coronary heart disease" (Barker 1995). He also described the "Thrifty Phenotype" which described the foetal response to inadequate nutrient supply and consisted of decreased muscle mass, insulin resistance, decreased capillary network and an increased stress response. In the setting of this phenotype, it was proposed that subsequent abundance of nutritional supplements might predispose an individual to the development of metabolic syndrome (**Syndrome X**), consisting of obesity, insulin resistance, glucose intolerance, hypertriglyceridemia and hypertension. This hypothesis now encompasses "catch up growth" as well to suggest that a combination of a low birth weight followed by a large increase in weight predisposes one to develop obesity, insulin resistance and cardiovascular diseases (Hales, Ozanne 2003).

1.1.10 Critical evaluation of Foetal Origins Hypothesis:

The evidence for FOH originated largely from retrospective cohort studies in which adult outcomes were correlated with birth weight records. The main controversies over the evidence have centred on four questions:

(1) Whether socioeconomic differences (which can affect both birth weight and later health) have been adequately controlled for in the analyses.

(2) The possibility that the correlations between low birth weight and later outcomes could arise from a common genetic factor that affected both (Hattersley, Tooke 1999).

(3) Statistical issues in relation to how to adjust for later changes in body size and fatness including the impact of random error, selective emphasis of particular results and publication bias (Huxley, Neil et al. 2002, Lucas, Fewtrell et al. 1999).

(4) Failure to replicate the observations in all studies.

Foetal Origins Hypothesis has been met with much scepticism (Metges 2001). Joseph, Kramer and Paneth (Joseph, Kramer 1996, Paneth, Susser 1995, Kramer 2000) found inconsistencies and conflicts within many studies and reported that proponents of this hypothesis have failed to explain temporal and international trends in coronary heart disease.

Two studies on contemporary British children and one study on aboriginal Australian children, who are increasingly being diagnosed with type 2 diabetes, found that insulin resistance was a function of current weight rather than low birth weight (Whincup, Cook et al. 1997, Wilkin, Metcalf et al. 2002). The concept of greater fatness in adults with poor prenatal growth and good postnatal growth has also been questioned by researchers looking into growth of children in Guatemala (Li, Barnhart et al. 2003). These researchers reported that children with IUGR, despite less growth retardation

post-natally, end up being shorter, lighter and weaker at adolescence compared to non-IUGR group.

It appears that maternal, foetal and infant nutrition is related to future health but adult factors also play an important role. A 22-year follow-up study in Great Britain found that cardiovascular diseases incidence and mortality of 62-81 year old males were predominantly related to factors in adult life (Wannamethee, Shaper et al. 2002).

A meta-analysis looking into the epidemiological studies reporting an inverse association between birth weight and subsequent blood pressure concluded that birth weight had little relevance to blood pressure in later life (Huxley, Neil et al. 2002).

Another more recent systematic review exploring infant size, growth relation and burden of adult diseases concluded that there was no single optimal pattern of infant growth that is associated with beneficial adult health outcomes and there was insufficient evidence to recommend prevention of adult disease through strategies to alter infant growth (Fisher, Baird et al. 2006).

1.1.11 Statistical issues for researchers looking into FOH:

To overcome the problems related to the interpretation of the association between postnatal growth and later blood pressure Lucas (Lucas, Fewtrell et al. 1999) suggested the use of four key regression models, which should include early size, early and later size, early and later size and their interactions and later size alone. Support for the "Foetal Origins Hypothesis" would require a significant negative relationship between birth weight and outcome in the early size model, possible augmented in the early and later size models; any interaction should be negative. Researchers using the recommended approach have reported the current weight to be the dominant variables in the regressions models.

Future research should be directed at prospective measurement of maternal nutrition during pregnancy, more refined indicators of foetal growth restriction, prospective measurements of growth parameters in children till adulthood, careful consideration of body composition, prospective measurement of cardiovascular parameters and appropriate modelling of postnatal influences as suggested by Lucas (Lucas, Fewtrell et al. 1999) for standardised comparative analysis.

1.2 Heart rate variability

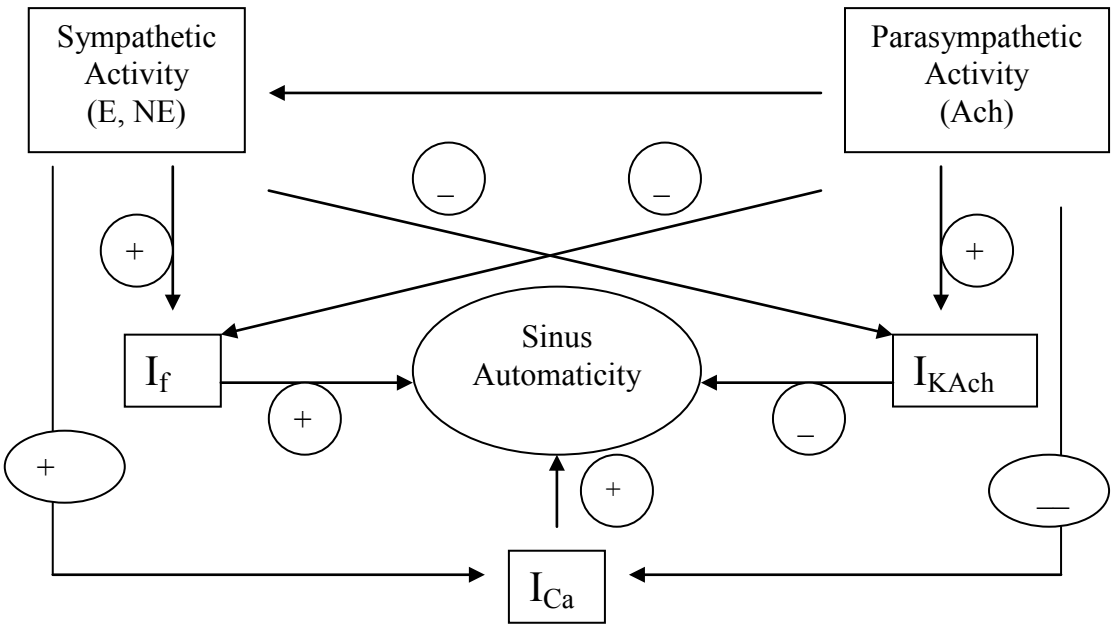
1.2.1 The heart and the Autonomic Nervous system (ANS)

The heart period, or time between two successive heartbeats, is based chiefly on two components: the intrinsic firing rate of the sinoatrial (SA) node of the heart and the modulation of the SA node firing by the collective inputs of the two ANS branches. The ANS regulation is effected through the interplay of the sympathetic and vagal outflows. Sympathetic stimulation gives rise to positive automaticity, inotropic and chronotropic effects. It also results in acceleration of the conductivity, hypertension, heart vessels dilation and constriction of the blood vessels in other tissues. Parasympathetic stimulation results in opposite effects **(Figure 5).**

Sympathetic influence is mediated by release of epinephrine and norepinephrine. Activation of beta-adrenergic receptors result in cyclic AMP mediated phosphorylation of membrane proteins and increases in ionised calcium and in hyperpolarisation-activated pacemaker current (If). The result is an acceleration of the slow diastolic depolarization. The parasympathetic influence on heart is mediated via release of acetylcholine by the vagus nerve. Muscarinic acetylcholine receptors respond to this release by an increase in cell membrane potassium conductance. Acetylcholine also inhibits the hyperpolarisation-activated pacemaker current. The Ik decay hypothesis

proposes that pacemaker depolarization results from slow deactivation of the delayed rectifier current, Ik, which, due to a time-independent background inward current, causes diastolic depolarization. Conversely, the If activation hypothesis suggest that following action potential termination, If provides a slowly activating inward current predominating over decaying Ik, thus initiating slow diastolic depolarization. Under resting conditions, vagal tone prevails and variations in heart period are largely dependent on vagal modulation. As the sinus node is rich in acetylcholinesterase, the effect of any vagal impulse is brief because the acetylcholine is rapidly hydrolysed. Parasympathetic influences exceed sympathetic effects probably via two independent mechanisms: a cholinergic induced reduction of norepinephrine release in response to sympathetic activity, and a cholinergic attenuation of the response to an adrenergic stimulus.

Figure 5. Effects of the autonomic regulation on ionic currents and the resulting changes of sinus automaticity (Sztajzel 2004)



E = epinephrine, NE = norepinephrine, Ach = acetylcholine
I_{Ca} = calcium current, I_f = hyperpolarisation activated "pacemaker" current, I_{KAch} = potassium current

In the course of the last two decades numerous studies have shown a significant relationship between ANS and cardiovascular morbidity. Perturbations of the ANS and its imbalance consisting of either increased sympathetic or reduced vagal activity may result in ventricular tachyarrhythmia and sudden cardiac death, which is nowadays one of the leading causes of cardiovascular mortality. Various methods are now available for assessing the status of the ANS. In recent years non-invasive techniques based on the ECG have been used as markers of autonomic modulation of the heart, these include Heart rate variability (HRV) (van Ravenswaaij-Arts, Kollee et al. 1993, Stein, Bosner et al. 1994) baroreflex sensitivity (BRS), QT interval and heart rate turbulence (Schmidt, Malik et al. 1999). Among these techniques analysis of HRV has emerged as a simple, non-invasive method to evaluate the sympatho-vagal balance at the sinoatrial level.

1.2.2 Definition and mechanisms of Heart Rate Variability (HRV)

HRV is a non-invasive measure of the ANS balance and imbalance. The index is the beat-to-beat variation (R-R) of the cardiac electrical signal expressed in normal sinus rhythm, sometimes called N-N. Typically the peak of the R wave is used as the reference point for analysis. HRV measures are of diagnostic value because these indices reflect the capacity of the individual's ANS and SA node to react to challenges in the internal and external environment. Therefore when the ANS and the SA node are dynamically responsive to change, there is adequate beat-to-beat variability and the heart is considered healthy. Reduced HRV is considered to reflect an ANS or SA node that is unable to respond or restricted in responsiveness.

HRV is not an absolute measure. Measures of HRV provide information on the relative inputs of the two ANS branches- the sympathetic and the parasympathetic nervous

system. Sustained sympathetic predominance is viewed as pathological and a contributor to essential hypertension. (Levine BS 2000) Conversely parasympathetic activity predominance is usually related to rest and restoration and is regarded as cardioprotective.

1.2.3 Techniques of measuring HRV:

Analysis of HRV consists of a series of measurements of successive RR interval variations of sinus origin, which provide information about autonomic tone. Different physiological factors may influence HRV such as gender, age, circadian rhythm, respiration and body position (Bonnemeier, Richardt et al. 2003). Measurements of HRV are non-invasive and highly reproducible. They may generally be performed on the basis of 24 hour Holter recordings or on shorter periods ranging from 0.5 to 5 minutes particularly in the field of dynamic electrocardiography (van Ravenswaaij-Arts, Kollee et al. 1993). Although computer analysis of tape recordings has improved, human intervention is required in most measurements of HRV parameters in order to detect erroneous beats, artefacts, and alterations in tape speed that may alter timing intervals. In 1996 a Task Force of the European Society of Cardiology (ESC) and the North American Society of Pacing and Electrophysiology (NASPE) defined and established standards of measurement, physiological interpretation and clinical use of HRV (Malik 1996).

1.2.4 Parameters of HRV used for Time Domain Analysis:

The basis of these methods is either the heart rate at any point in time or the intervals between successive complexes. In a continuous electrocardiographic record each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (i.e. intervals

between adjacent QRS complexes resulting from sinus node depolarisation), or the instantaneous heart rate is determined.

Statistical Methods

The measures obtained after analyses of a series of instantaneous heart rates or NN intervals can be divided into 2 classes:

A. Those derived from direct measurements of the instantaneous heart rate or NN intervals.

B. Those derived from the differences between NN intervals.

The variables to be obtained and the units of their measurements are the following:

1. Mean heart rate (**HR**, beats/min)
2. Mean NN interval (**MNN** or **MRR**, milliseconds)
3. Standard deviation of the NN interval (**SDNN** or **SDRR**, milliseconds) - the square root of variance between the NN intervals.

   Since variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording.
4. Standard deviation of the average NN interval calculated over 5-min periods within the 24 - hours recording (**SDANN**, milliseconds).

   This parameter is an estimate of the changes in heart rate due to cycles longer than 5 minutes
5. Mean of the standard deviations of all NN intervals for all 5 min segments of entire recording (**SDNN index**, milliseconds)

   This index reflects the variability due to cycles shorter than 5 minutes
6. The square root of the mean squared differences of successive NN intervals (**RMSSD**, milliseconds).

7. The number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording (**NN50**)
8. NN50 count divided by the total number of all NN intervals (**pNN50**, %)

SDNN is a global index of HRV and reflects all the long-term components and circadian rhythms responsible for variability in the recording period. SDANN is an index of the variability of the average of 5-minute intervals over 24 hours. Thus, it provides long-term information. It is a sensitive index of low frequencies like physical activity, changes in position and circadian rhythm. RMSSD and pNN50 are the most common parameters based on interval differences. These measurements correspond to short-term HRV changes and are not dependent on day/night variations (Malik 1996). They reflect alterations in autonomic tone that are predominantly vagally mediated. Compared to pNN50, RMSSD seems to be more stable and should be preferred for clinical use.

Geometric methods

Geometric methods are derived and constructed from the conversion of sequences of NN intervals. Different geometrical forms allowing assessment of HRV are available: the 24-hour histogram, the HRV triangular index, the triangular interpolation of NN interval histogram and the method based on Lorentz or Poincaré plots (Malik 1996). The 24-hour histogram assesses the relationship between the total number of RR intervals detected and the 24-hour RR interval variation. The triangular HRV index considers the major peak of the histogram as a triangle with its baseline width corresponding to the amount of RR interval variability, its height corresponds to

the most frequently observed duration of RR intervals, and its area corresponds to the total number of all RR intervals used to construct it. The triangular HRV index is an estimate of the overall HRV.

Geometrical methods are less affected by the quality of the recorded data and may provide an alternative to less easily obtainable statistical parameters. However, the time duration of recording should be at least 20 minutes, which means that short-term recordings cannot be assessed by geometric methods.

Among the various time domain and geometric methods available the Task Force of the ESC and the NASPE has recommended the use of four measures for HRV assessment: SDNN, SDANN, RMSSD and the HRV triangular index.

1.2.5 Parameters of HRV used for Frequency domain analysis

Frequency domain (power spectral density) analysis describes the periodic oscillations of the heart rate signal decomposed at different frequencies and amplitudes and provides information on the amount of their relative intensity (termed variance or power) in the heart's sinus rhythm (Sztajzel 2004, Eckberg 1997). Schematically, spectral analysis may be compared to the results obtained when white light passes through a prism, resulting in different lights of different colour and wavelength. Power spectral analysis can be performed in two ways:

1) Nonparametric method e.g. the fast Fourier transformation (FFT), which is characterized by discrete peaks for the several frequency components, and

2) Parametric method e.g. the autoregressive model estimation, resulting in a continuous smooth spectrum of activity.

While the FFT is a simple and rapid method, the parametric method is more complex and needs verification of the suitability of the chosen model. When using the FFT the

individual RR intervals stored in the computer are transformed into bands with different spectral frequencies. This process is similar to decomposing the sound of a symphony orchestra into the underlying notes. The results obtained can be transformed in Hertz (Hz) by dividing by the mean RR interval length. The total power of RR interval variability is the total variance and corresponds to the sum of the four spectral bands: the ultra low frequency band (ULF), the very low frequency band (VLF), the low frequency band (LF) and the high frequency band (HF) (Eckberg 1997, Malliani, Lombardi et al. 1994), **(Table 2 and Figure 6)**. Squared units are used for the absolute values expressed in milliseconds squared. LF and HF powers may be expressed in absolute values or in normalised values. Subtracting the VLF component from the total power performs the normalisation of LF and HF. It tends to reduce, on one hand, the effects of noise due to artefacts and, on the other hand, to minimize the effects of the changes in total power on the LF and HF components. It is useful when evaluating the effects of different interventions in the same subject or when comparing subjects with major differences in total power.

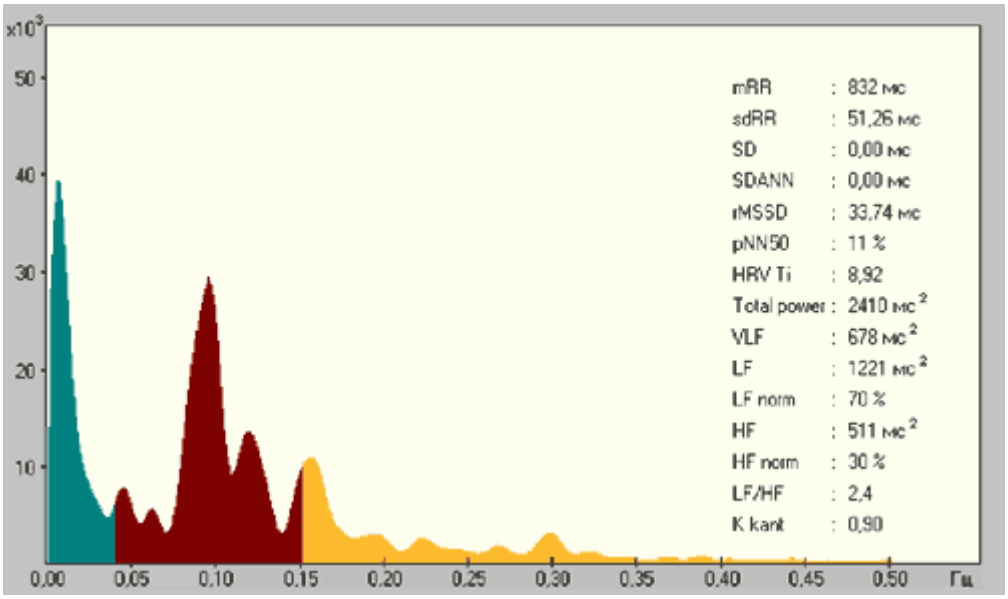
Normalised units are obtained as follows:

$$\text{LF or HF norm (nu)} = \frac{\text{LF or HF } (ms^2)}{\text{Total Power } (ms^2) - \text{VLF } (ms^2)} \times 100$$

Table 2 Range of frequencies for power spectral analysis of HRV

Variable (Description)	**Units**	**Frequency range** Hz
Total power (Variance of all NN intervals)	ms^2	<0.4
ULF (Ultra low frequency power)	ms^2	<0.003
VLF (Very low frequency power)	ms^2	<0.003–0.04
LF (Low frequency power)	ms^2	0.04–0.15
HF (High frequency power)	ms^2	0.15–0.4
LF/HF: Ratio of low-high frequency power	Numerical	

Figure 6 Frequency Distribution of power spectral analysis of HRV



Example of power spectral density obtained from 5-min recording. Blue zone reflects power of spectrum of RR interval in VLF range, red zone corresponds to LF power and the yellow zone stands for HF power.

1.2.6 Physiological significance of the different components of the spectrum:

The HF component is generally defined as a marker of vagal modulation. This component is respiration mediated and thus determined by the frequency of breathing. The LF component is modulated by both the sympathetic and parasympathetic nervous systems. In this sense, its interpretation is more controversial. Some authors consider LF power, particularly when expressed in normalised units, as a measure of sympathetic modulations; others interpret it as a combination of sympathetic and parasympathetic activity. The consensus is that it reflects a mixture of both autonomic inputs. In practical terms, an increase of the LF component has been generally considered to be a consequence of sympathetic activity.

The LF/HF ratio reflects the global sympatho-vagal balance and can be used as a measure of this balance. In a normal adult in resting conditions, the ratio is generally between 1 and 2. ULF and VLF are spectral components with very low oscillations. The ULF component might reflect circadian and neuroendocrine rhythms and the VLF component long period rhythms. The VLF component has been found to be a major determinant of physical activity and was proposed as a marker of sympathetic activity (Malik 1996).

Correlations between time and frequency domain indices:

There are established correlations between time domain and frequency domain parameters. pNN50 and RMSSD correlate between themselves and with HF power ($r = 0.96$), SDNN and SDANN indices correlate significantly with total power and the ULF component (Stein, Bosner et al. 1994).

Limitations of standard HRV measurements

Because HRV deals with RR interval variations its measurement is limited to patients in sinus rhythm and to those with a low number of ectopic beats. In this sense, approximately 20 to 30% of high-risk post-myocardial infarction patients are excluded from any HRV analysis due to frequent ectopy or episodes of atrial arrhythmias, particularly atrial fibrillation. The latter one may be observed in up to 15 to 30% of patients with heart failure, excluding these patients from any HRV analysis.

1.2.7 Nonlinear methods (Fractal Analysis) of HRV Measurement

Nonlinear methods are based on the chaos theory and fractals. Chaos has been defined as the study of multivariable, nonlinear and non-periodic systems (Goldberger 1996, Lombardi 2000). Chaos describes natural systems in a different way because it can account for nature's randomness and non-periodicity. Perhaps the theory of chaos may help in better understanding HR dynamics, taking into account that the healthy heartbeat is slightly irregular and to some extent chaotic. In the near future nonlinear fractal methods may give new insights into HR dynamics in the context of physiological changes and in high risk situations, particularly in patients after myocardial infarction or in the context of sudden death. Recent data suggest that fractal analysis in comparison to standard HRV measurements seems to detect abnormal patterns of RR fluctuations more efficiently (Lombardi 2000).

1.2.8 Heart rate variability and children:

Several studies have shown that HRV can be measured accurately in children from a very early age with the help of Holter ECG recorders (Finley, Nugent 1995, Massin, Maeyns et al. 2000, Kazuma, Otsuka et al. 2002). Researchers involved in "The

Bogalusa Heart Study", (Batten, Urbina et al. 2000) which was a long term epidemiologic study of cardiovascular disease risk factors with an age span from birth through early childhood, reported that all the time domain measures were highly reproducible in a sample of children, with an inter-observer variability of 1%. Frequency domain measures were also reproducible with an inter-observer variability of 4% overall. They also found that 24-hour indices were highly reproducible, stable and free of placebo effect thereby making them ideal for assessing intervention therapy.

Normal ranges of HRV in children and the influence of a developing autonomic nervous system on HRV indices:

Several researchers have tried to establish the normal ranges of HRV in children (Kazuma, Otsuka et al. 2002, Massin, von Bernuth 1997, Silvetti, Drago et al. 2001, Galeev, Igisheva et al. 2002). In the adult population it was shown that different HRV components decreased with increasing age due to physiological aging process. In childhood postnatal developments of all the components of the ANS are not parallel to chronological maturation. Data in literature, although sometimes conflicting, show that there is a progressive maturation of ANS after birth. In infancy sympathetic components develop faster than parasympathetic ones. In infants who subsequently succumbed to sudden infant death syndrome a delayed or deficient cardiovagal development was demonstrated (Antila, Valimaki et al. 1990). Some authors describe a gradual increase of parasympathetic relative to sympathetic mediation in first 6-10 years followed by a gradual decrease (Finley, Nugent 1995, Shannon, Carley et al. 1987, Pivik, Busby et al. 1996, Goto, Nagashima et al. 1997). Silvetti suggested that HRV indices were partially related to age and gender. Galeev reported that the values

of mean heart rate, SDNN, RMSSD, TF, VLF, HF in 6 to 16 year children increased with age wavelike changing from year to year (Galeev, Igisheva et al. 2002). Massin found a strong positive linear correlation between mean R-R interval (heart rate) and all parameters, which was confirmed by Rekawek (Massin, von Bernuth 1997, Rekawek, Miszczak-Knecht et al. 2003).

1.2.9 Circadian rhythm and HRV:

Circadian variation in HRV reflects circadian variations in ANS activity. This rhythm can reflect the changing sympathetic–parasympathetic balance. In adults, HRV indices decrease significantly during the day and increase during the night. (Yamasaki, Kodama et al. 1996, Korpelainen, Sotaniemi et al. 1997) The LF/HF ratio, believed to reflect the sympatho-vagal balance has an inverse rhythm, with a higher level during the day and lower value in the night. Massin reported that a significant circadian variation in HRV was present from late infancy or early childhood, characterised by a rise during sleep, except for the low to high frequency ratio that increased during daytime. (Massin, Maeyns et al. 2000) The appearance of these circadian rhythms was associated with sleep maturation and the very young infants did not show any significant diurnal rhythm because of the immaturity of the ANS and increased sleep time compared to those in older children.

1.3 Ambulatory Blood Pressure Measurement (ABPM)

Although the mercury sphygmomanometer has been the standard method for BP measurement for nearly a century, in the last few years there has been a rapid increase in the use of automated BP measuring devices. Ambulatory blood pressure monitoring (ABPM) has been developed to overcome some of the deficiencies and inaccuracies

related to classic BP measurements. ABPM provides information on an individual's BP from multiple BP readings obtained automatically during a 24-hour period with minimal intrusion into the daily activities of the person. The main advantage of ABPM includes: a) the lack of observer bias, b) superior correlation with end-organ damage and c) the determination of abnormal BP pattern over 24 hours (Portman, Yetman 1994).

1.3.1 Technique of ABPM:

Currently more than 20 different devices are present on market and have been subjected to the validation procedure by the British Hypertension Society (BHS) and/or Association for the Advancement of Medical Instrumentation (AAMI). However, only very few devices had undergone specific validation for children and generally failed to reach standards above the level at which a device can be recommended (O'Brien, Waeber et al. 2001).

The two major types of ambulatory monitors measure BP either by oscillometry or by auscultation with or without R wave gating. The latter technique provides accurate measurements but carries the risk of an increased frequency of error readings due to noise interference. Oscillometry accurately determines the SBP and mean arterial pressure, from which DBP is then derived, based on an algorithm. For children, who have a slightly hyperdynamic circulation, the oscillometric monitors are better choice (Yetman, Portman 1999).

Jacoby (Jacoby, Fixler et al. 1993) analyzed the limitation of the oscillometric ABPM method in physically active children of different ages and concluded that compared to oscillometric and auscultatory casual BP measurements, the ambulatory blood pressure monitor gave reasonably accurate readings. Nevertheless, validation of the ambulatory

readings would require simultaneous intra-arterial monitoring but such a study is unlikely to be performed in children.

O'Brien proposed that to validate the data collected from ABPM one should simultaneously measure 3 BP readings with a mercury sphygmomanometer, both before and after the data collection. The measurements should be within +/- 5 mmHg with a standard deviation of ≤ 8 mmHg (O'Brien E, Pickering T, Asmar R, et al 2002). The European society for hypertension has produced recommendations for ABPM (O'Brien E, et al: European Society of Hypertension Working Group on Blood Pressure Monitoring. 2003). The monitoring equipment is usually well tolerated with minimal disruption to subject's routine activities or sleep. To obtain an accurate BP measurement a cuff bladder width of approximately 40% of the upper arm circumference should be used. The bladder length should be at least 90% of arm circumference to avoid overestimation of BP. BP and heart rate are recorded automatically every 15-30 minutes during the daytime and 30-60 minutes during night time. Many monitors automatically repeat a BP measurement 2-3 minutes after a failed measurement. The subject is instructed to maintain a diary during the period of BP monitoring where he/she records the time of activities, times of going to sleep, waking up and any other relevant information.

1.3.2 Interpretation and analysis of ABPM:

After the monitoring is completed the data from the monitor is downloaded into a computer and with the help of software the BP results are analysed. The summary page typically presents the mean, range and standard deviation of BP and the systolic and diastolic BP loads for the entire monitoring. The recordings that are unphysiologic are edited, such as those with a pulse pressure ≤ 6 mmHg or ≥ 150 mmHg. Ideally

readings should not be edited unless independent information suggests that it is a machine error and not just an unusual value.

A successful monitoring is one in which < 25% of the readings are considered artefacts, > 80% of the proposed measurements are successfully obtained, or there are reliable data for at least 20 hours out of 24 hours (Portman, Yetman 1994, Harshfield, Alpert et al. 1994). European Society for Hypertension recommends that there should be at least 14 measurements of systolic and diastolic BP during day and at least seven such measurements at night and at least two-thirds of all attempted measurements should be successful. (O'Brien E, et al: European Society of Hypertension Working Group on Blood Pressure Monitoring. 2003)

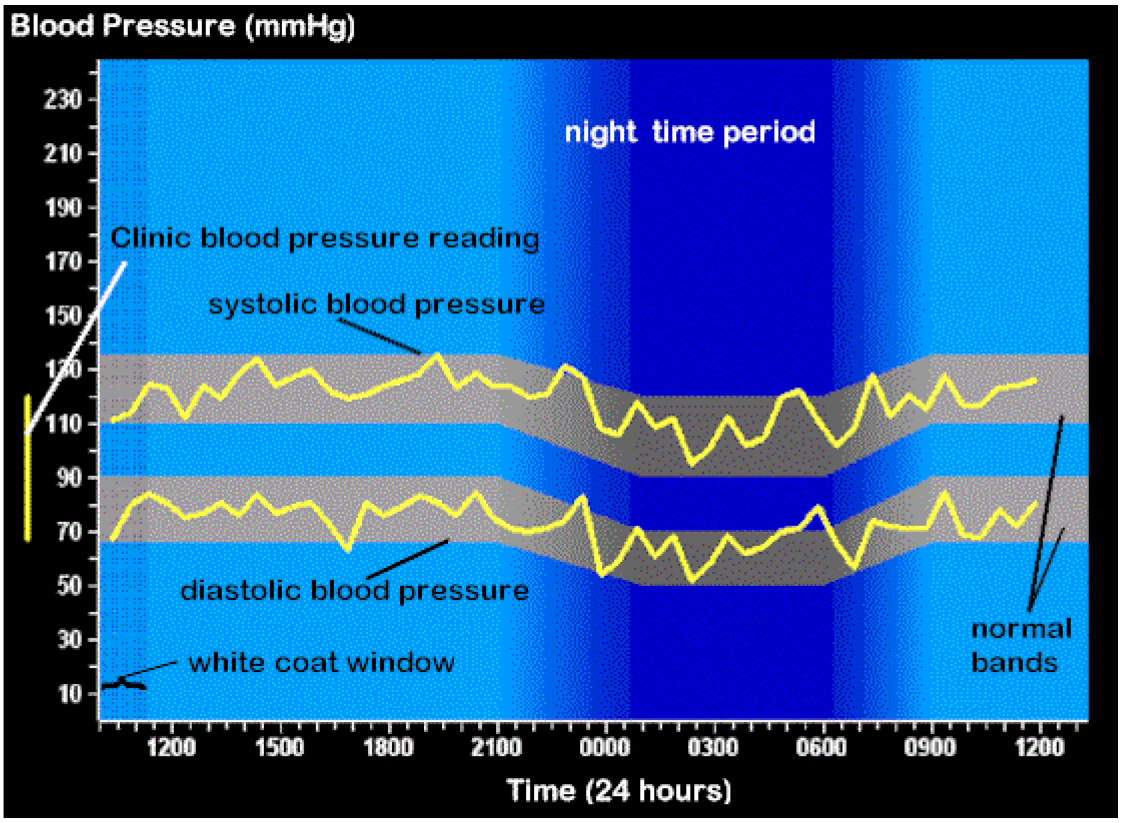
Lurbe showed that (Lurbe, Cremades et al. 1999) in children the quality of monitoring depends only in part on age and the ambulatory systolic BP and the pulse pressure amplitude have an important role in the number of erroneous measurements. Studies in children have shown that ABPM measurements are accurate and reproducible (Nicholson, Matthews et al. 1993).

Characteristic of a normal ABPM:

A characteristically normal ABPM recording demonstrates a bimodal peak, with a sharp early morning rise, followed by a plateau during the day and another peak in late afternoon **(Figure 7)**. There is a fall of BP by approximately 15-25% in the night. Lurbe reported (Lurbe, Redon 2000) that 83% of children had a significant systolic rhythm and 89% had a diastolic diurnal rhythm. Patients with decreases in sleep BP values < 10% than the daytime baseline have been defined as “Non-Dippers”. Racial differences in the diurnal pattern have been described by Harshfield (Harshfield,

Alpert et al. 1994): a higher night time BP in African-American compared to Anglo-American, despite similar day time BP.

Figure 7 Normal 24 hour ABPM profile obtained from commercial software



Each of the ABPM profiles in this package is laid out as follows:

- X axis: time (24 hour)
- Y axis: blood pressure (mmHg)
- The yellow line adjacent to the Y axis represents the clinic blood pressure obtained for that patient.
- The horizontal grey bars represent the accepted normal limits for systolic and diastolic blood pressure obtained by ABPM, including the nocturnal dip.
- The vertical bars on the left of the profile represent the "white coat" window, when the effect of medical staff on blood pressure may still be evident.
- The darker vertical band of shading represents night time.

1.3.3 Factors affecting accuracy of ABPM results:

1. Disturbed sleep pattern has not been found to be a significant problem especially in one study which looked into this problem specifically (Yetman, Portman 1999).
2. Age: in a large sample of 333 children (aged 3 to 18 years), Lurbe et al reported that ABPM recordings were highly successful. The success rate was directly related to the child's age, with older children having more successful readings (Lurbe, Cremades et al. 1999).
3. The lower SBP values give the poorest monitoring quality (Lurbe, Cremades et al. 1999).

1.3.4 Normative data for ABPM in children:

The 95th percentile values defined by the Task Force on High Blood Pressure In Children are the standard for assessment of the casual auscultatory BP throughout childhood. (National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents 1996) The validity of applying the threshold limits for the assessment of ambulatory oscillometric BP is uncertain. Harshfield showed that both daytime and night time SBP increase related to changes in body size and not to age, while DBP did not change significantly (Harshfield, Treiber 1999). Similar results were obtained from several other studies including the largest ABPM study in children performed by Soergel ML (Soergel, Kirschstein et al. 1997) which included ABPM recordings of 1141 children. This particular report from a European multicentre collaborative study group provides the most appropriate and complete reference data for ABPM in children.

1.3.5 Relation between ABPM and birth weight:

An inverse relationship between birth weight and BP has been suggested from a large number of studies which is one of the cornerstones of the "Foetal Origins" hypothesis proposed by Barker. Unfortunately there are very few studies investigating the link between ABPM and birth weight and the evidence is contradictory. Lurbe reported from a large study involving 630 children that after adjustment for gender, current age, weight and height children with lowest birth weight had the highest ambulatory BP values and lack of circadian variability (Lurbe, Torro et al. 2001). These findings are in contrast with the findings reported by Pearce (Pearce, O'Sullivan 2003) who found in 976 schoolchildren variation in SBP and DBP was not significantly associated with birth weight.

Rahiala compared ambulatory BP between two groups of healthy children aged 12 years, 50 children in each group (Rahiala, Tenhola et al. 2002a). The first group comprised of children born at term but who were SGA, and the second group comprised of children born at term with no evidence of IUGR. Birth weight had no direct association with blood pressure values. The blood pressure differences between two groups were more dependent on current body size.

1.4 Rationale for this study:

Intrauterine malnutrition is thought to have continuing influence on growth patterns into childhood but there are conflicting views as to:

- Whether growth restricted babies ever completely recover potential lost body mass and catch up in size with the normal population
- Whether IUGR children are more prone to develop obesity

- Whether the delay in ANS maturation and establishment of circadian biorhythms noted in IUGR infants persist during childhood
- Whether blood pressure values are associated with IUGR and Catch Up growth

Currently there have been few longitudinal studies on the physiological development of children who suffered from Intra Uterine Growth Restriction and often the definition of IUGR is based on birth weight, which is known to be a poor marker of intrauterine growth. The link between IUGR and obesity, if true, would give added significance to the later growth patterns of IUGR children and provide an opportunity for the early identification and possible prevention of serious illness in adulthood. There is currently no available data on the maturation of ANS in IUGR children. Although the ambulatory blood pressure measurement is vastly superior to casual one-off blood pressure measurements but very few researchers have looked into the relation between IUGR and ambulatory blood pressure values in childhood. We are fortunate to have an existing database of strictly defined IUGR children and access to their perinatal details as well as longitudinal anthropometric measurements from their developmental records (Red Books). The discovery of any association between body size and blood pressure values at a young age could be a focus of prevention in adolescence before the effects of hypertension cause long-term damage to various organs of the body.

This study addresses the following questions:

1. How do the Heart Rate Variability and ambulatory blood pressure parameters in nine-year-old children with IUGR compare with those of normal children from same age group?

2. Is there a difference in the maturation of circadian biorhythms controlled by the Autonomic Nervous System in the IUGR children compared to that of normal children?
3. Is there a difference in cortisol excretion in IUGR children compared to cortisol excretion in normal children?
4. Are there any continuing differences between the two groups in physical growth, which could be related to social and environmental conditions or to cardiovascular status?

Chapter 2: Methods of the study

2.1 Study Design:

Previous research undertaken in Leicester has shown that development of circadian rhythm in deep body temperature, heart rate and urinary cortisol excretion during the first few months of life is delayed in children with Intra Uterine Growth Restriction compared to children with normal intrauterine growth (Jackson. 2001, Jackson, 2004). It is also reported in current medical literature that children with IUGR are more likely to develop obesity, cardiovascular and metabolic diseases in later life (Barker 1999, Eriksson 2001, Horta 2003).

This study was designed to follow up the children who took part in the previous study to assess the current status of their cardiovascular system and urinary cortisol excretion and also to assess the maturation of their autonomic nervous system.

Children were fitted with 24 hour heart rate and blood pressure monitors on two separate occasions to assess heart rate variability and ambulatory blood pressure profile. Two urine samples were collected from each child to measure urinary cortisol excretion. Demographic and anthropometric data were collected from each child.

Full ethical permission for this study was obtained from the Leicestershire Local Research Ethics Committee.

2.2 Method of recruitment

Previous researchers from Leicester investigating development of circadian rhythm in deep body temperature, heart rate and cortisol excretion in IUGR infants had developed database of children in Leicestershire with and without IUGR. Within this database there were 69 IUGR children and 127 normal children without IUGR. Most

of the IUGR children were between the age of eight and ten years and only 5 were over the age of ten years at the beginning of this study. The IUGR children above the age of ten years were excluded from the study. Out of the 127 normal children, 64 were above the age of ten years and were excluded from the study. Therefore the final numbers of potential recruits were 64 from the IUGR group and 63 from the normal group.

2.2.1 Methods of identifying and recruiting IUGR and Normal infants as described in the previous study conducted by Jackson, Wailoo, Petersen et al

(Jackson, J.A. 2001, Jackson, J.A. 2004)**.**

Inclusion criteria:

- Caucasian
- Gestation ≥ 37 weeks
- No congenital or chromosomal anomaly
- No epilepsy or diabetes in mother
- No infants involved in child protection/adoption procedures
- Evidence of Intra Uterine Growth Restriction (IUGR)

Description of methods used to identify intrauterine growth retarded babies:

Serial antenatal ultrasound scans:

Contacts were made to the local maternity units where all the pregnant mothers underwent an initial routine ultrasound scan around 12-14 weeks of gestation to confirm the gestation of the foetus and to check for anomalies. Measurements of the Crown Rump length were taken at the initial scan and were plotted on a foetal growth chart used by the units. The measurement provided an ultrasound date of delivery

(UDD), which was compared, to the expected date of delivery (EDD) based on the last menstrual period. Follow up scans were only done if there was a discrepancy between the two dates or any abnormality detected.

Serial scanning was offered to all pregnant women who had previously delivered a small for gestation infant, who had maternal diseases such as epilepsy or diabetes, a history of previous intrauterine death or any previous abnormal pregnancy.

Several midwives trained to carry out this task performed most of the routine scanning. If the foetal growth was found to be slowing then the mother was routinely referred to an obstetrician who would check the foetal measurements and make a decision. Two health professionals therefore agreed upon diagnosis of IUGR.

At each scan measurements were made of foetal head circumference, biparietal diameter, abdominal circumference and femur length.

All measurements were plotted on the British Ultrasound percentile graphs. Foetuses were identified as having growth retardation when the foetal abdominal circumference was two standard deviations below the mean for gestation.

Identification of Control infants:

Previous studies carried out over a period of nine years had resulted in the existence of a large database containing measurements of the physiological development of normal babies. From this database matched controls were recruited.

Recruitment of IUGR and Control babies:

Parents of babies eligible to take part in the project were contacted shortly after the birth of their babies to discus the project in details and babies were recruited after obtaining full consent from the parents.

2.2.2 Recruitment of IUGR and Control children from the existing databases for the current study

Step 1: Invitation to take part in the current study

Names and date of births of potential recruits were submitted to the Child Health division of Leicester City West Primary Care Trust to obtain their current contact details. From updated details we found that several families had moved out of Leicestershire and they were excluded from the study.

The initial approach letter (**Appendix 1)** asking for permission to contact them to discuss more about the study was sent to 108 families. A reply slip (**Appendix 2)** and a stamped addressed envelope to return the reply slip were enclosed.

The reply slip requested the parents to confirm whether they were interested in the study and also to provide their telephone numbers to arrange a meeting with the researchers. If no reply was obtained after four weeks then a second approach letter with the reply slip and the prepaid envelope were sent.

Postal replies were reviewed and further contact was made with only those families who expressed an interest in the study.

Step 2: Home visit and demonstration of equipment

Subsequent contacts were made with the parents and a home visit was arranged to meet the child and the parents. Information leaflets for the parents and the children and

the consent form (**Appendix 3, 4 and 5**) were posted to them so that the parents and the children had opportunity to go through them before the meeting with the researcher. At these visits the study was explained in details, equipments were demonstrated and any questions were answered. Each visit took about 30-45 minutes and the parents and the children were reassured that there was no pressure to take part. All families were given time to think about the decision to take part.

Two to three days following the home visit parents were telephoned by the researcher to find out about their decision. If the parents did not wish to take part in the study then they were thanked for their time and no further contact was made. Reason for not taking part was almost always due to the child's anxiety over the equipment and occasionally due to anticipated inconvenience to the family. An appointment to come to the cardiac investigation department of Leicester Royal Infirmary (LRI) was arranged for the children who agreed to participate. The general practitioner was informed at this stage about the child's participation.

2.3 Step 3: First visit to LRI for physical examination, ECG and fitting with Holter ECG monitor:

Almost all the visits to the LRI were arranged on Tuesday or Thursday afternoon after school, as most of the parents did not want their children to miss school. Visit to LRI often involved travelling considerable distances for the families as children were participating from all over Leicestershire. The car-parking problem was another major issue for all the families. These issues were beyond our control and we tried our best to help the families by reimbursing the travel expenses and the parking fees as well as

keeping the appointment schedule as flexible as possible. Due to limited resources the Cardiac Investigation Department could only arrange two appointments per week. Consent forms signed by the parents/guardians and the researcher were obtained from the parents/guardians on arrival and a copy was given to them.

2.3.1 Following data were collected for each participating child (Appendix 6):

- Name, sex, date of birth, place of birth, birth weight and gestation of the child
- Full address with post code
- GP details
- Mother's name at the time of delivery and date of birth
- Maternal smoking status during pregnancy
- Placental weight from maternal record
- Whether the child was breast fed or not and if breast fed then duration of breast feeding
- Current smoking status in the family
- Presence of any significant medical condition in the child and family
- Any medication currently taken by the child
- Academic performance of the child
- Any developmental delay in the child
- If the "**RED BOOK**" (parent held record) was available then that was collected
- Index of Multiple Deprivation score (**IMD2004**) for each post code was obtained from Leicestershire and Rutland Health Informatics Service

2.3.2 Physical examination:

Complete physical examination including measurement of blood pressure was done on each child by me all the time. All children had their height and weight measured; the height by a portable Harpenden stadiometer to the nearest millimetre and the weight by a Marsden Professional Physician Scale to the nearest 100 grams. The heights of the mothers were also measured similarly. Paternal heights were mostly self-reported.

2.3.3 ECG and fitting of Holter ECG recorder and Urine collection:

Once I was satisfied with the physical examination then each child had a standard twelve lead ECG. If the ECG was within normal limits then each child was fitted with a Holter Ambulatory ECG recorder which he/she was instructed to carry for next 24 hours.

Two sterile preservative free plastic containers marked “A” and “B” were given to the parents at the time of their visit to the hospital. Parents were instructed to collect the first urine sample from the child next morning in the bottle marked “A” and a second urine sample half an hour after the first sample in bottle marked “B”. Once the urine samples were collected parents were instructed to store them in the freezer section of their refrigerator.

Originally the parents were instructed to bring the child back to LRI after 24 hours for removal of the ECG recorder. Subsequently it was felt that most of the parents were struggling to do so, as that again involved travelling to the hospital leading to further disruption to their routine. It was decided at this stage that the researchers would visit the children at home to collect the recorder and the urine samples.

2.3.4 Step 4: Fitting of Ambulatory Blood Pressure monitor:

Originally it was planned that the children would have to return to the hospital after a gap of two days to be fitted with the ambulatory blood pressure monitor. Although some of the parents did not object to that arrangement but it was felt that it would be a lot more convenient for the families if the blood pressure monitor could be fitted at home and collected from home by the researcher. The home visits were arranged at a convenient time for the family which gave us the opportunity to measure the heights of the fathers as well. A strict protocol was followed wherever the ambulatory blood pressure monitor was fitted.

2.4. Ambulatory ECG recording and initial screening:

LIFECARD CF Holter Ambulatory ECG recorder (Delmar-Reynolds Medical Limited, Hertford, UK) was used to record the ECG from each child. This equipment has been found to be safe, simple, effective and more importantly acceptable to most of the children. The LIFECARD CF allowed continuous recording of 3 channels of ECG over a period of twenty four hour whilst allowing the subject to carry on their normal activities. It needed one AAA battery and utilised a digital storage technique to store the ECG recording onto a Compact Flash (CF) Card.

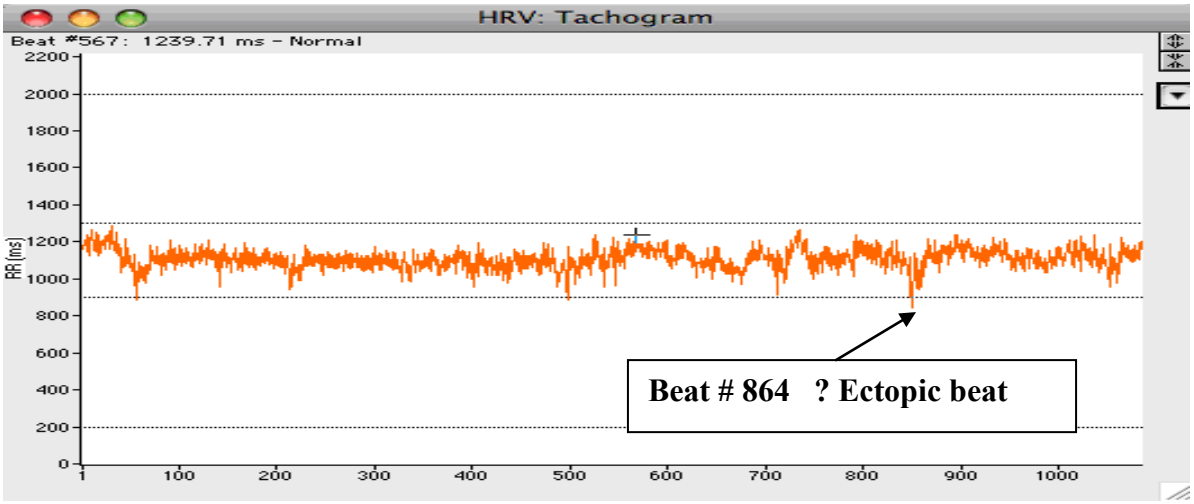
A CF card with the details of the child was initialised with the LIFECARD CF recorder at the beginning of recording. Three electrodes were attached to the chest of each child and after checking the quality of ECG the leads were secured with adhesive tapes. Date and time at the beginning of recording were noted. The recorder was either clipped to the belt of the child or put inside their trouser/pant pockets. It was explained to the children that they could follow their normal routine and only swimming or having a shower/bath were prohibited. All the children found this device quite interesting and most of them took great pride in showing that off to their peers. They

took part in all sorts of physical activities including playing football, rugby and practising dance lessons. The parents were also instructed to maintain a simple diary of their children's activities and sleep time, which was subsequently collected from them. After 24 hours the electrodes and the recorder were removed from the children and the CF card was taken out of the recorder. Then the ECG data from the CF card was analysed with the help of ECG screening software **LIFESCREEN (Delmar Reynolds Medical Limited, Hertford, UK)**. LIFESCREEN displayed continuous "Full Disclosure" ECG for visual inspection and also produced the following graphs:

1. Heart rate Graph: Heart rate in beats per minute, averaged over every whole minute of recording time. This graph helped us to verify the sleep time reported by the parents, as the heart rate is expected to slow down soon after falling off to sleep and to rise rapidly following awakening.

2. RR tachogram: This displayed every detected RR (beat-to-beat) interval. Lines joined adjacent points and possible ectopic intervals were readily observed as big "stick" in the graph. The "stick" could be cross-checked on the corresponding ECG.

Figure 8. RR Tachogram graph



3. RR histogram: This displayed a bar chart recording the number of RR intervals within a range. The "width" of each bar in the histogram was 50 milliseconds. From this display we could easily identify the longest RR intervals (slowest Heart rate) and the shortest RR intervals (highest heart rate). Any abnormally short or long RR interval could easily be crosschecked in the corresponding ECG strip to detect whether that particular interval was affected by motion artefact or not. Overall the quality of recordings was excellent with minimal artefact.

Thus the LIFESCREEN software was used to visually inspect the quality of the ECG and to identify suspected ectopics and artefacts to be excluded for subsequent Heart Rate Variability analysis.

2.4.1 Analysis of Heart Rate Variability (HRV):

Each CF card with the digitised ECG data was subsequently scanned on the commercially available Holter Scanning system **Pathfinder 700 HRV Analysis (Delmar Reynolds Medical limited, Hertford, UK**) for editing the raw ECG data. The effective sampling rate after interpolation was 128 Hz. All the recordings were scanned by one experienced technician and one researcher trained in arrhythmia analysis. The same Holter scanning settings and definitions were used for all recordings. Manual review was performed with attention to careful editing of noise and accurate beat definition.

The following exclusion criteria for the ECG recordings were used:

- Recording less than 23 hours
- Ectopic beats more than 1% of the total beats
- Presence of any degree of heart block

After the initial scanning with Pathfinder 700 system the ECG recordings were exported to a personal computer for Heart Rate Variability analysis with the commercially available software **HRV TOOLS (Delmar Reynolds Medical limited, Hertford, UK)**. This particular software was chosen for its flexibility to override automatic and default exclusion criteria and also for its ability to restrict analysis to certain defined time periods e.g. night or day. Exclusion criteria for each recording were individually defined after detailed analysis with LIFESCREEN and PATHFINDER.

Data segmentation:

All the RR (beat-to-beat) intervals in the ECG recording were divided into **96** contiguous, non-overlapping **Segments**, each having a segment length of **15** minutes. Each **15 minute Segment** was further divided into **three** contiguous, non-overlapping **5 minute Analysis Periods**. The final analysis results were obtained from **one 5 minute Analysis Period** with the highest percentage of valid RR data out of those three analysis periods. Any analysis periods with less than 90% valid data was excluded from the analysis.

Each recording was standardised to awake and sleep periods depending on the parents' diary and the child's mean heart rate graph and average values of all parameters were calculated for these two periods.

The 24 hour value for all the parameters were also calculated by adjusting the Segment and Period length i.e. setting the segment and period length to1440 minutes and performing a Single Segment analysis.

The following parameters were calculated for the Time Domain analysis of HRV:

- Mean RR interval i.e. mean heart rate
- SDNN: Standard deviation of all NN (normal beat-to-beat intervals) intervals
- RMSSD: The square root of the mean of the sum of the squares of differences between adjacent NN intervals
- SDNNi: Mean of the SD of all RR intervals for all five minute segments
- SDANN: Standard deviation of the averaged normal sinus RR intervals for all 5 minute segments.

For Spectral analysis (Frequency Domain) of HRV a Fast Fourier Transformation (FFT) was performed on the beat-to-beat fluctuations. The frequency bands were defined as:

- Ultra-Low Frequency (ULF): 0Hz to 0.003Hz
- Very-Low-Frequency (VLF): 0.003Hz to 0.04Hz
- Low-Frequency (LF): 0.04Hz to 015Hz
- High-Frequency (HF): 0.15Hz to 0.4Hz
- Total: 0Hz to Nyquist limit (i.e. all frequencies)

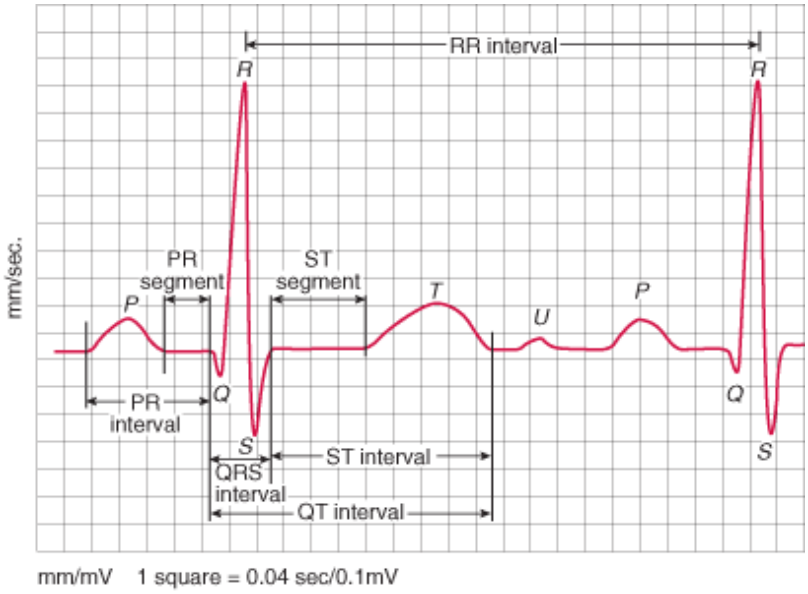
Also calculated were **normalised** values of LF and HF. The calculation used was:

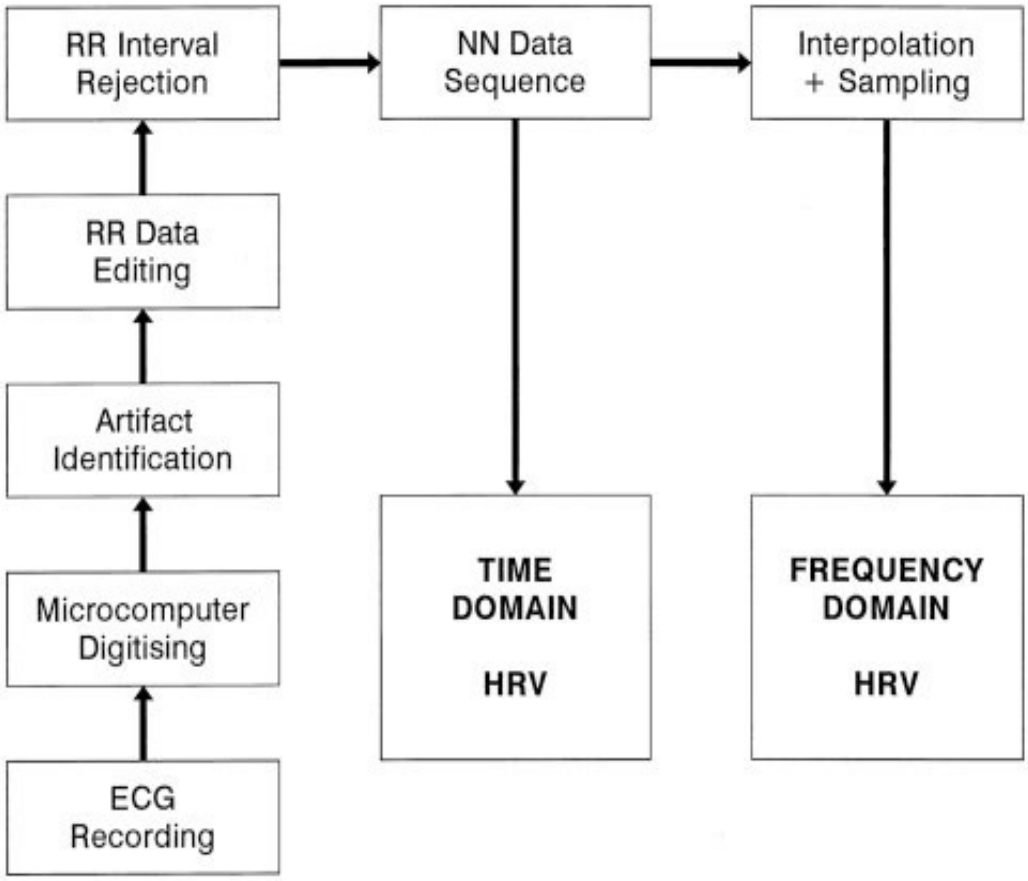
- Low-Frequency-Normalised (LFn) = (LF*1000) / (Total-VLF-ULF)
- High-Frequency-Normalised (HFn) = (HF*100) / (Total-VLF-ULF)

From the normalised values High-Frequency to Low Frequency power ratio (HF/LF) was calculated.

Figure 9

A normal ECG and summary of steps used in recording and processing ECG data for HRV analysis





2.5 Ambulatory Blood pressure measurement protocol:

Ambulatory blood pressure measurement was performed by **90217 ULTRALITE** oscillometric monitor from **Spacelabs Medical Inc WA, USA**. The monitor was chosen because it was a compact and light weight machine and has been widely used in clinical settings. The following protocol was followed for ABPM recording:

- If the child was coming to hospital then the child was allowed to relax in a quiet room for 10 minutes while the monitor was being initialised with child's details and the whole procedure was explained to the child and parents.
- If I was visiting the child at home then first ten minutes were spent in explaining the procedure to parents and the child. The monitor would have been already initialised before meeting the family.

The circumference of the non-dominant arm was measured in centimetres midway between the olecranon and acromion process. An appropriate cuff size with a bladder width of at least 40% of the arm circumference and the bladder length of at least 90% of the arm circumference was chosen from the following range:

a. Paediatric13-20 cm. **b**. Small Adult 17-26cm. **c**. Average adult 24-32cm.

The monitor was hung over shoulder with a strap and the child was instructed to relax the arm during the blood pressure measurement.

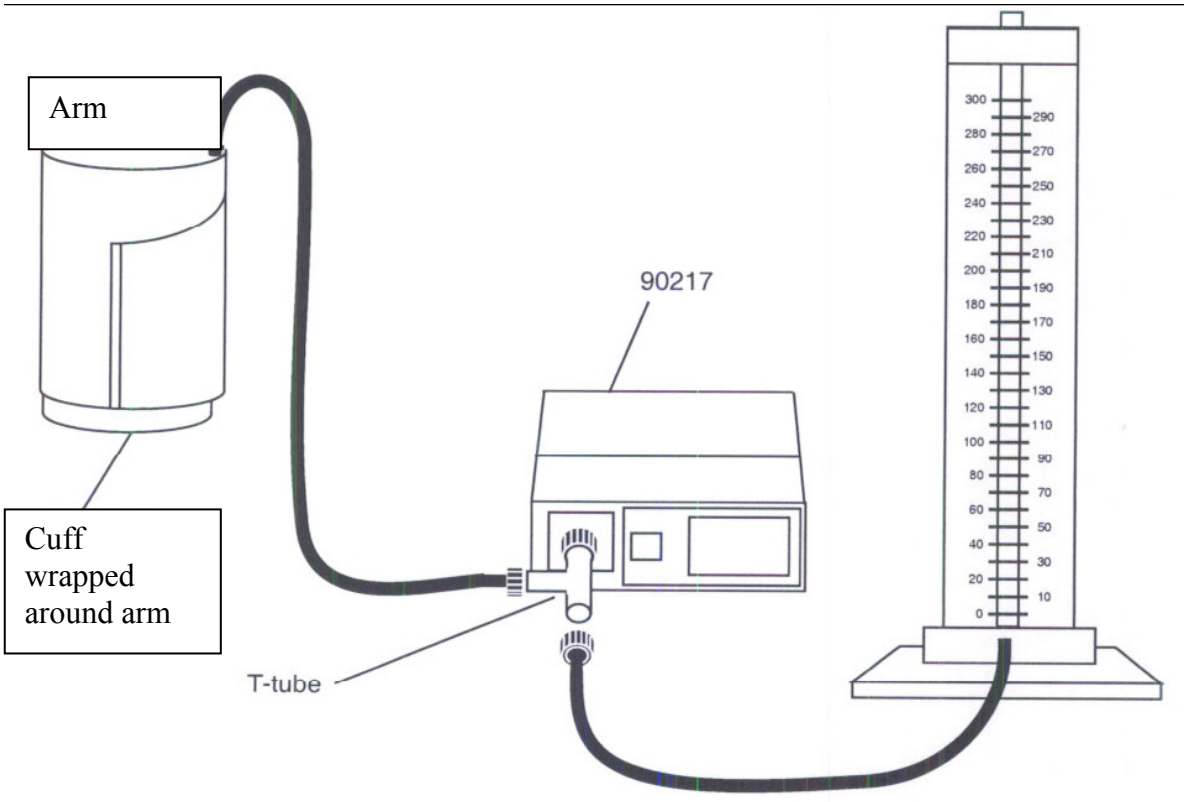
After 10 minutes blood pressure was measured on the non-dominant arm with the Spacelabs 90217 monitor and simultaneously validated via a T connector connected to a standard mercury sphygmomanometer **Accoson Decamet 300mmHG, Accoson Works, London, UK**. (**Figure 9**).

The blood pressure was rechecked again with the ABPM monitor and validated with the mercury manometer after 5 minutes. If the difference in the systolic or diastolic

blood pressure measured in these two different ways were more than 5 mmHg then the children were excluded.

Figure 10

Connections for the Spacelabs 90217 ABP monitor and simultaneous validation of blood pressure measurement with a mercury sphygmomanometer



To be considered a successful recording, an ABPM recording should contain at least 30 valid measurements during the whole 24 hour period, at least two-thirds of the systolic and the diastolic blood pressure measurements during the day time and the night time should be satisfactory and there should be no period more than one hour without a valid measurement. The children were instructed to follow their normal routine and the monitor and the activity diary were collected from their home by the researchers on completion of the recording.

2.5.1 Editing and analysis of the Ambulatory Blood Pressure (ABP) measurements:

The monitors were downloaded into a personal computer and the initial automatic editing was done by the software package provided by Spacelabs Medical Inc. The whole 24 hour periods were individually standardised into wake (day) and sleep (night) periods depending on parents' record of children's activities. All the measurements were checked again manually and accepted unless:

1. Systolic Blood Pressure (SBP) was ≥ 200 or ≤ 50 mmHg
2. Diastolic Blood Pressure (DBP) was ≥ 100 or ≤ 30 mmHg
3. Heart rate was ≥ 200 or ≤ 30 beats per minute

The following ABP parameters were calculated for each child:

1. Total time of scan
2. Successful readings
3. Percent successful
4. 24 hour mean Systolic and Diastolic BP
5. Day time mean Systolic and Diastolic BP
6. Night time mean Systolic and Diastolic BP
7. Day Night ratio of mean Systolic and Diastolic BP
8. Standard deviation of day time and night time Systolic and Diastolic BP
9. Standard deviation of systolic and diastolic BP over 24 hours

2.6 Urine collection and measurement of urinary cortisol creatinine ratio:

The researcher collected the urine samples from the participant's home. Each urine sample was labelled with name, date and time of collection and brought to the Biochemistry department of Leicester Royal Infirmary for analysis of Creatinine and

Cortisol. A preliminary screening test was done with Multistix 8 SG, Bayer Diagnostics, NY, USA to exclude any urinary infection. If no evidence of infection was detected then urine samples were placed in a 4° C store until analysed for creatinine and cortisol.

Creatinine estimation: Urinary Creatinine was measured using a kinetic colour test (Jaffè Method) on OLYMPUS analyser, Olympus Diagnostics, Hamburg, Germany. Creatinine formed a yellow-orange coloured compound in an alkaline medium. The rate of change in absorbance of 520/800nm was proportional to the creatinine concentration in the urine.

Cortisol estimation: Initial dichloromethane extraction of urine samples was followed by estimation of urine cortisol by IMMULITE 2500 CORTISOL solid-phase, competitive chemiluminescent enzyme immunoassay, available from Diagnostic Products Corporation, CA, USA.

Cortisol Creatinine ratio for two samples from each child was calculated manually.

Abnormal results:

If any abnormality was detected in any of the investigations then the parents and the child's general practitioner were informed and advice were sought from a paediatric cardiologist or a paediatric nephrologist.

2.7 Analysis of data obtained from the parent held developmental record (Red Book):

All the parents were requested to lend us their child's developmental record book commonly known as **'Red Book'**. From the Red Book all the available height and weight data were collected. Commercially available United Kingdom Growth Standards Data Analysis software (**LMS Research disc, Harlow Healthcare, Tyne**

and Wear, UK) was used to analyse the data. The LMS disk was designed to allow the researcher to access the weight, length/height, head circumference and BMI data supporting the UK growth reference charts. Using Microsoft Excel add-in macro sheets and worksheets it was possible to adjust measurements for age and sex and convert them to a standard deviation score (SD or Z score).

A Z score is defined as the number of standard deviations by which a measurement differs from the population mean. Z scores should be normally distributed with a mean of zero and a standard deviation of one (equivalent to a mean on the 50th centile with two thirds of the observations falling between the 16th and 84th centile). For the point estimate Z scores, a positive Z score implies a measurement above the mean and a negative Z score implies measurement below the mean. For changes in Z score over time a positive value denotes weight/height gain faster than normal between two point estimates and a negative value denotes weight/height gain slower than normal.

Z scores and corresponding centile values were calculated for all the available weight, height/length and BMI data. Z scores were also calculated for weight and height gain.

Statistical analysis:

Statistical analysis was carried out using the statistical software SAS version 9.1.3 for Windows (SAS Institute Inc, Cary, NC, USA). The use of various statistical methods including use of t test, Chi-Square test, Cosinor method, selection of candidate predictors and multivariable general linear models would be described in details in the relevant results sections.

Chapter 3: Results: Anthropometric measurements

3.1 The subjects

Analysis of the existing database of children who had participated in previous research projects identified 127 children suitable to participate in the current study and names and date of births of these potential recruits were submitted to the Leicester City PCT to obtain their current contact details. From updated details I found that 17 children had moved out of Leicestershire and they were excluded from the study. The initial approach letter asking for permission to contact them to discuss more about the study and a reply slip were sent to 108 families.

Ninety-two postal replies were returned. Three letters came back unopened as "Return to sender" as these families have moved out of their addresses and no further contact details were available. No reply was received from 13 families.

Out of 92 replies 7 families were not interested in the study and no further contact was made to these families.

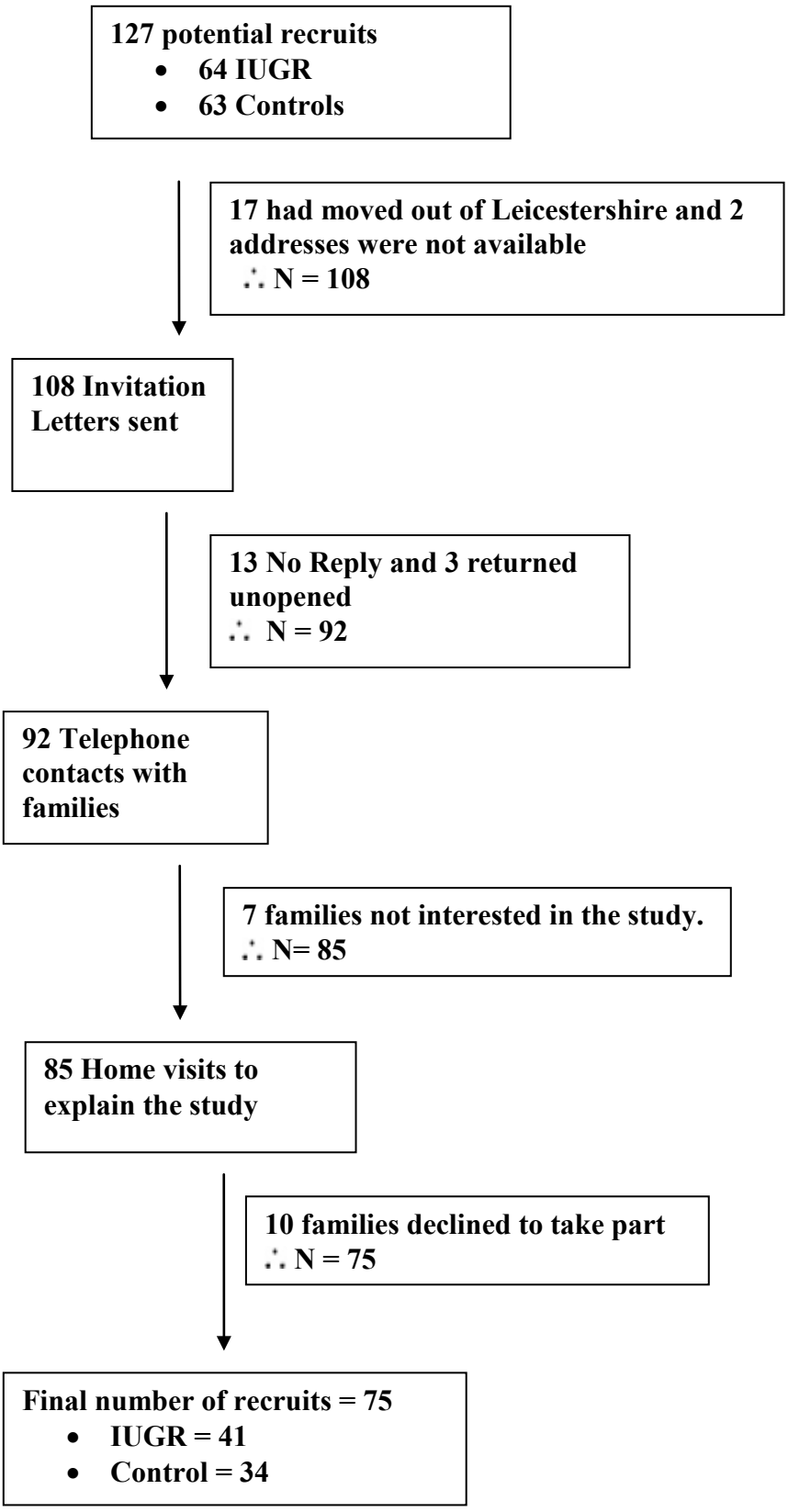
In 85 cases contacts were made with the parents and a home visit was arranged to meet the child and the parents.

Ten children declined to take part in the study. Reason for not taking part was almost always due to the child's anxiety over the equipment and occasionally due to anticipated inconvenience to the family.

The final numbers were 41 children in the IUGR group and 34 children in the control group. For these 75 children an appointment to come to the Leicester Royal Infirmary was arranged.

Figure **11** shows the recruitment process as a flow diagram.

Figure 11: Flow Chart of recruitment for current study with numbers



3.1.1 Comparison of the perinatal and socio-economic variables between the participants and the non-participants for the current study:

T tests and Chi-Square tests were used to compare the two groups with respect to, gender, birth weight, gestation and maternal smoking status in pregnancy. No differences were detected between the two groups with respect to the abovementioned variables (**Table 3**).

Table 3: Comparative analysis of perinatal and socio-economic variables between the participants and the non-participants

	Participants in two groups	**Non-participants in two groups**	**Probability values**
Number	IUGR: 41 Control: 34	IUGR: 23 Control: 29	
Gender*	IUGR: Male: 19 Female: 22	IUGR: Male: 11 Female: 12	p = 0.90
	Control: Male: 22 Female: 12	Control: Male: 14 Female: 15	p = 0.19
Mean birth weight, SD (grams)**	IUGR: 2563, 408	IUGR: 2688, 330	p = 0.21
	Control: 3529, 474	Control: 3637, 458	p = 0.37

Table 3 continued …..			
	Participants in two groups	**Non-participants in two groups**	**Probability values**
Gestation, SD (weeks)**	IUGR: 38.9, 1.3	IUGR: 38.6, 1.2	p = 0.34
	Control: 39.1, 0.9	Control: 39.4, 1.09	p = 0.23
Number of mothers smoking during pregnancy*	IUGR: Yes: 16 No: 25	IUGR: Yes = 10 No = 13	p =0.21
	Control: Yes: 9 No: 25	Control: Yes: 4 No: 25	p = 0.79
IMD Score, SD**	IUGR: 21.8, 16.72 Control: 12.94, 10.99	IUGR: 18.48, 9.93 Control: 10.27, 5.54	p = 0.24 P = 0.12

Probability values derived from t tests for continuous variables and from Chi-Square tests for categorical variables

* = Results obtained from Pearson's Chi Square Test

** = Results obtained from t tests

IMD = Index of Multiple Deprivations

3.1.2 Conversion of raw anthropometric data to standardised Z scores:

All the parents were requested to lend us their child's developmental record book commonly known as 'Red Book'. From the Red Book all the available height and weight data were collected. Commercially available United Kingdom Growth Standards Data Analysis software (LMS Research disc, Harlow Healthcare, Tyne and Wear, UK) was used to convert all the weight, height and BMI measurements into age and gender corrected corresponding standard deviation (z) scores with reference to 1990 British growth reference charts.

The Z score is a statistical measure of the distance (measured in standard deviations) from the mean of a data set in a Gaussian Population. A Z score of 0 is at the mean of the population and a z score of +1 or –1 means that the value is one standard deviation (SD) above or below the mean respectively. Z score values between +2 and –2 cover 95% of the values in a Gaussian population. By using z scores we aimed to standardise our data by reference to the1990 population mean. Changes of weight Z scores were calculated between birth and final weight. For changes in Z score over time a positive value denotes weight/height gain faster than normal between two point estimates and a negative value denotes weight/height gain slower than normal.

3.1.3 Comparison of the unadjusted perinatal, anthropometric and socio-economic variables between the IUGR and the Control group:

Unadjusted mean values and probability values were derived from t tests for continuous variables and from Chi-Square tests for categorical variables (**Table 4).** Placental weights are available for 35 children in IUGR group and for 32 children in the control group. Maternal and Paternal heights are available for 39 children in the IUGR group.

The participants in two groups were not different in gestation (IUGR 38.9 weeks v Control 39.1 weeks) and gender distribution. The IUGR infants had lower birth weight and placental weight and were less likely to be breast-fed. Maternal smoking in pregnancy, current smoking in the family and economic deprivation were commoner in the IUGR group. Maternal and paternal heights were not significantly different in two groups. The IUGR children are slightly older than the control children (IUGR 9.32 years v Control 9 years). IUGR children had slightly more medical conditions than the control group (29% vs.12%, p = 0.07). The commonest medical condition was asthma. In the IUGR group two children had autism, one had epilepsy, one had ADHD and two had unspecified developmental delay.

Two children in IUGR group had moderately delayed development and one of them attended a special school.

Table 4 Comparison of the unadjusted perinatal, anthropometric and socio-economic variables between the IUGR and the Control group

Continuous variables	**IUGR N=41 Mean (95% CI)**	**Control N =34 Mean (95% CI)**	**Difference of means (95% CI)**	**Probability value**
Gestation (Weeks)	38.98 (38.56, 39.40)	39.18 (38.86, 39.49)	-0.2 (-0.7,0.3)	0.45
Birth weight (Kg)	2.56 (2.43, 2.69)	3.53 (3.36, 3.69)	-1.0 (-1.2,-0.8)	<0.0001
Birth weight z score	-2.1 (-2.3, -1.8)	0.1 (-0.1, 0.4)	-2.2 (-2.6,-1.8)	<0.0001
Placental weight (Grams) [a] 488(448, 528)		631 (583, 679)	-143 (-203,-82)	<0.0001

Table 4 continued…

Continuous variables	IUGR N=41 Mean (95% CI)	Control N =34 Mean (95% CI)	Difference of means (95% CI)	Probability value
Breast feeding duration (Weeks)	4.2 (1.9,6.5)	10.5 (7.9,13)	-6.3 (-9.7,-2.8)	0.0006
Maternal height (cm) [b]	161.8 (159.2, 164.4)	161.7 (158.9, 164.4)	0.2 (-3.6,3.9)	0.93
Paternal height (cm) [b]	175.0 (172.5, 177.6)	178.2 (176.3, 180.1)	-3.2 (-6.3,0.03)	0.052
Current IMD score [c]	21.2 (15.9-26.5)	12.9 (9.1-16.8)	8.3 (1.6,14.9)	0.02
Current age (Years)	9.36 (9.12,9.54)	8.96 (8.78,9.23)	0.4 (0.09,0.7)	0.01
Final weight (Kg)	28.32 (26.78, 30.46)	32.64 (30.48, 34.80)	-3.8 (-6.5,-1.1)	0.007
Final weight z score	-0.4 (-0.7,-0.1)	0.6 (0.3,1.0)	-1 (-1.5,-0.5)	<0.0001
Change in weight (Kg)	26.26 (24.49, 28.03)	29.53 (26.95, 31.27)	-2.9 (-5.6,-0.1)	0.04
Change in weight z score	1.7 (1.3,2)	0.5 (0.1,1)	1.2 (0.6,1.8)	<0.0001
Final Height (cm)	131.4 (129.3, 133.4)	133.8 (132,135.5)	-2.4 (-5.1,0.3)	0.08
Final height z score	-0.6 (-0.9,-0.3)	0.16 (-0.1,0.5)	-0.8 (-1.2,-0.4)	0.0002
Final BMI	16.63 (15.83,17.42)	18.11 (17.22,19)	-1.5 (-2.7,-0.3)	0.01

Table 4 continued…

Continuous variables	**IUGR N=41 Mean (95% CI)**	**Control N =34 Mean (95% CI)**	**Difference of means (95% CI)**	**Probability value**
Final BMI z score	-0.1 (-0.4,0.3)	0.8 (0.4,1.1)	-0.8 (-1.3,-0.3)	0.002
Categorical variables	**IUGR N=41 Number (%)**	**Control N =34 Number (%)**	**Probability value**	
Gender			0.12	
Male	19 (46)	22 (64)		
Female	22 (54)	12 (36)		
Breast fed	19 (46)	25 (74)	0.02	
Maternal smoking in Pregnancy	16 (39)	9 (27)	0.25	
Current smoking	21 (51)	12 (35)	0.17	
Significant medical problem	12 (29)	4 (12)	0.07	
Currently on medication	11 (27)	4 (12)	0.16	
Normal development	37 (90)	34 (100)	0.11	
Mainstream schooling	40 (97)	34 (100)	0.36	

Probability values were derived from t tests for continuous variables and from Chi-Square tests for categorical variables.

a= Placental weights are available for 35 children in IUGR group and for 32 children in the control group

b= Maternal and Paternal heights are available for 39 children in the IUGR group

c = **IMD 2004** is a measure of multiple deprivations at the small area level defined as

Lower Layer of Super Output Area (SOA), which is developed from the Census 2001 to improve reporting of statistical data. The lower layer of SOA, on which IMD 2004 is based, typically represents an area with a minimum population of 1000 and mean population of 1500 and is more reflective of local population than a much larger electoral ward. The IMD 2004 contains seven Domains of deprivation: Income, Employment, Health and Disability, Education and Training, Barriers to Housing, Living Environment and Crime.

Chapter 4: Results: Exploratory regression analysis of Anthropometric data

4.1 Statistical methods:

To determine whether or not the difference in z scores between the two groups was significant at the various ages of measurement, multivariable general linear models were applied to the data. These models are an extension of multiple linear regression models, in which there is a continuous dependent variable, and a combination of continuous and categorical independent variables, the aim being to quantify the relationship between the predictors and the dependent variable, and to find the best predictors. Potential candidate predictors were: IUGR, duration of breast feeding, whether breast fed or not, maternal height, estimated paternal height, mean parental height, current smoking status in household, maternal smoking status in pregnancy, Indices of Multiple Deprivations (IMD 2004), gender, presence of a significant medical problem, and current use of medication.

Univariable analyses were used to narrow the selection process by eliminating variables with a probability >0.1 of predicting each dependent variable, and then the remaining variables were entered into a multivariable main effects model using a backward stepwise procedure and significant interactions were then explored. This method was used owing to the small number of subjects per candidate predictor variable.

4.2 Comparison of the z score data for weight at birth, final weight, final height and final BMI between the two groups

Multivariable regression models using Standard Deviation (z) Scores confirm that IUGR children are born lighter and they are shorter and remain lighter at 9 years compared to the control group. **Table 5** shows the comparison of the adjusted z scores of the weights, heights and BMIs of the two groups at birth and 9 years. The average birth weight in the IUGR group is more than 2 standard deviations below the mean (z = -2.1) whereas the average birth weight of the control group is just above the mean (z = 0.2). At the age of 9 years the average weight of the IUGR is 0.4 SD below the mean (z = -0.4) and the average weight of the control group is 0.6 SD above the mean (z = 0.6). The average height at the age of 9 years for the IUGR group is half a SD below the mean (z = -0.5) whereas the corresponding value for the control group is on the mean (z = 0.0). The average BMI z-score for the IUGR group at 9 years of age is just below the mean (z = -0.2) whereas the corresponding value for the control group is 0.7 SD (z = 0.7) above the mean. All these differences are highly significant.

Table 5 Adjusted means for birth weight z score, weight z score, height z score and BMI z score at 9 years for two groups

Z score	IUGR Adjusted Mean (95% CI)	Control Adjusted Mean (95% CI)	Mean Difference (95% CI)	Probability value
Birth Weight	-2.1 (-2.3, -1.8)	0.2 (-0.2, 0.5)	2.3 (-2.6, -1.8)	< 0.0001
Final Weight	-0.4 (-0.7, -0.1)	0.6 (0.3, 1.0)	1.0 (-1.5, -0.5)	< 0.0001
Final Height	-0.5 (-0.7, -0.2)	0.0 (-0.3, 0.2)	-0.5 (-0.8, -0.1)	0.002
Final BMI	-0.2 (-0.5, 0.1)	0.7 (0.3, 1.1)	-0.9 (-1.4, -0.4)	0.002

Probability values are for general linear model analysis after adjusting for any significant covariates.

4.3 Comparison of change in weight z score between birth and 9 years between the two groups

The change in weight z score variable measures changes in the position of weight between birth and 9 years relative to the mean weight of the 1990 reference population. A positive value indicates that the weight has moved upwards in the centile chart whereas a negative value means that the weight has fallen down in the centile chart.

Table 6 shows the comparison between the two groups in relation to change of weight z score between birth and 9 years. At the age of one year the average weight of the IUGR group has increased by 0.9 SD compared to an increase of 0.3 SD for the control group. At the age of two years the average weight of the IUGR group has increased by 1.2 SD compared to an increase of 0.3 SD for the control group. At the age of final measurement the average weight of the IUGR group has increased since birth by 1.5 SD compared to an increase of 0.4 SD for the control group.

Although the available weight measurements were fewer, the trend of rapid weight gain in IUGR children was present in the early years. Fifty-three children had weight measurements at 1 year and at 2 years of age. The rate of weight gain appears to be more pronounced during the second year of life for the IUGR children. Thereafter the increase in z score changes slows down considerably.

The children in the IUGR group are lighter at birth and despite having a higher rate of weight gain they remain lighter than the control group by the age of 9. Between birth and 9 years of age the IUGR group increased their z score by 1 standard deviation more than the control group, but at 9 years of age they remained 1 standard deviation behind the control group.

Within each of the 2 groups there was no correlation between initial score and final score, so Regression to Mean (RTM) had already occurred within each group. When the data for birth weight z scores are ranked within each group, it becomes apparent that the initial z score has no significant predictive value concerning the final z score. The change of weight z score for each group exceeds that expected under RTM and was present even after adjustment for birth weight z score.

Table 6 Change of weight z score from birth to 9 years between two groups

Group	**Change in weight z score between birth and one year Mean (95% CI)***	**Change in weight z score between birth and two year Mean (95% CI)***	**Change in weight z score between birth and nine years Mean (95% CI)**	**Correlation between birth weight z score and weight z score at nine years (pr)**
IUGR	0.9 (0.3,1.4)	1.2 (0.7,1.6)	1.5(1.2, 1.9)	0.10 (0.52)
Control	0.3(-0.1,0.7)	0.3(-0.2,0.8)	0.4 (-0.01, 0.8)	0.13 (0.48)
Difference between changes in mean z scores between two groups	0.6	0.9	1.1	
Probability	p = 0.1	p = 0.01	p = 0.001	

Probability values are for general linear model analyses after adjusting for any significant covariates.

* Measurements were available for 53 children

4.4 Determinants of Birth weight z score, Final weight z score, Final height z score, Final BMI z score and Change of weight z score obtained by Multivariable statistical analysis

Univariable analyses were used to narrow the selection process by eliminating variables with a probability >0.1 of predicting each dependent variable, and then the remaining variables were entered into a multivariable main effects model, and significant interactions were then explored. The stepwise method was also used to reach the final model.

Results of the multivariable analysis in **Table 7** shows that IUGR is the only common significant predictor for all the outcome measurements. Paternal and maternal heights are additional significant predictors for final height z score. For the change of weight z score there is a main effect of IUGR, and interactions between using medication and maternal height and interaction between using medication and having been breast-fed are also significant. The number of children involved in some of the interactions are relatively small thus making the interpretation difficult. Birth weight is not a significant predictor of final weight z score.

For change in weight z score competing models were set up, comparing birth weight versus IUGR. The birth weight model accounted for 59.54 % of the variability in the data, considerably more than the model which features IUGR rather than birth weight (37.7%). However when they are included in the same model IUGR does not significantly predict change in weight z score between birth and nine years over and above birth weight (for IUGR, $p = 0.2048$) but birth weight remains significant ($p < 0.001$). Thus it may be concluded that the best predictor of change in weight z score between birth and 9 years in the current sample is weight at birth rather than membership or not of the Intra Uterine Growth Retardation group. However we

include the results from a model including IUGR and not birth weight because the principal aim of the research was to compare the IUGR group with the Control group. IUGR probably predicts change in weight z score because of its high correlation with birth weight ($r = 0.74$, $p < 0.0001$, tolerance 0.45).

Table 7 Multivariable analysis for Birth weight z score, Final weight z score, Final height z score, Final BMI z score and Change of weight z score

Significant predictor(s)	**Birth weight z score**	**Final weight z score**	**Final BMI z score**	**Final height z score**	**Change of weight z score between birth and 9 years**
IUGR	Yes ($p< 0.0001$)	Yes ($p< 0.0001$)	Yes ($p = 0.0167$)	Yes ($p<0.0016$)	Yes ($p =0.0002$)
Birth weight	No	No	No	No	Yes ($p = 0.001$)
Paternal height	No	No	No	Yes ($p =0.017$)	No
Maternal height	No	No	No	Yes ($p=0.04$)	No

4.5 BMI Centile data

The overall comparison of body size is shown in **Table 8**, where at least half of the control group are above the 85th centile for BMI and 23% above the 95th centile, i.e. there is a greater tendency to obesity in the control group of children.

Table 8: BMI centile data at the age of 9 years for both groups

	IUGR (n = 41)	**Control (n = 34)**
BMI Centile (mean, median)	47.32, 47.94	71.18, 83.44
Number of children above the 85th Centile for BMI (percentage)	5 (12%)	17 (50%)
Number of children above the 95th centile for BMI (percentage)	3 (7%)	8 (23.5%)

4.6 Summary and Conclusion:

IUGR children were smaller at birth but showed a greater increase in their weight between birth and nine years. At nine years the weight, height and BMI z scores were lower in children than the control children. The predictors of these differences were IUGR, birth weight and maternal and paternal heights. This analysis shows that IUGR infants grow faster but remain shorter and lighter than their normal counterparts, that is, they fail to fully catch up by 9 years of age.

Chapter 5: Results: Ambulatory Blood Pressure

5.1 Description of twenty-four hour ambulatory blood pressure profile in IUGR and control children:

Of seventy-five participants six ABP recordings were excluded because they failed to meet the inclusion criteria resulting in thirty-eight participants in IUGR group and thirty-one participants in the control group. Average values for sleep time (9 hours), number of blood pressure readings in 24 hours (44), percentage of successful readings (81%) and the other blood pressure parameters were not statistically different between the two groups when analysed using independent samples t-test (**Table 9)**.

Table 9 Unadjusted mean values for ABP parameters for two groups:

Category	**Mean (Standard Deviation)**
Mean percentage of successful readings	IUGR = 80.3 (5.7)
	Control = 81.7 (7.8)
Mean Number of BP readings	IUGR = 44.4 (6.3)
	Control = 44.8 (6.8)
Mean duration of sleep(hours)	IUGR = 9.80 (0.56)
	Control = 9.88 (0.50)
Mean Day SBP (mmHg)	IUGR = 108.26 (7.32)
	Control = 109.77 (6.56)
Mean Day DBP (mmHg)	IUGR = 68.66 (4.05)
	Control = 68.84 (5.04)
Mean Day pulse pressure (mmHg)	IUGR = 39.61 (6.11)
	Control = 40.94 (4.23)

Table 9 Unadjusted mean values for ABP parameters for two groups (Continued….)	
Category	**Mean (Standard Deviation)**
Mean Night SBP (mmHg)	IUGR = 95.68 (8.77)
	Control = 97.13 (6.58)
Mean Night DBP (mmHg)	IUGR = 55.76 (4.69)
	Control = 56.61 (5.30)
Mean Night Pulse pressure (mmHg)	IUGR = 39.92(6.88)
	Control = 40.52 (4.61)
Mean 24 hour SBP (mmHg)	IUGR = 103.69 (7.32)
	Control = 104.84 (6.06)
Mean 24 HR DBP (mmHg)	IUGR = 64.22 (3.87)
	Control = 63.90 (4.40)
Mean Day Night SBP ratio	IUGR = 1.12 (0.07)
	Control = 1.12 (0.06)
Mean Day Night DBP ratio	IUGR = 1.23 (0.10)
	Control = 1.21 (0.10)
Mean Standard deviation SBP 24 hr	IUGR = 10.97 (1.73)
	Control = 10.91 (2.03)
Mean Standard deviation DBP 24 hr	IUGR = 10.71 (1.6)
	Control = 10.08 (2.07)

SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, PP = Pulse Pressure

During each 24 hour period blood pressure varies by day and also by night, and there is a diurnal biorhythm with clearly demarcated day versus night activity patterns. The

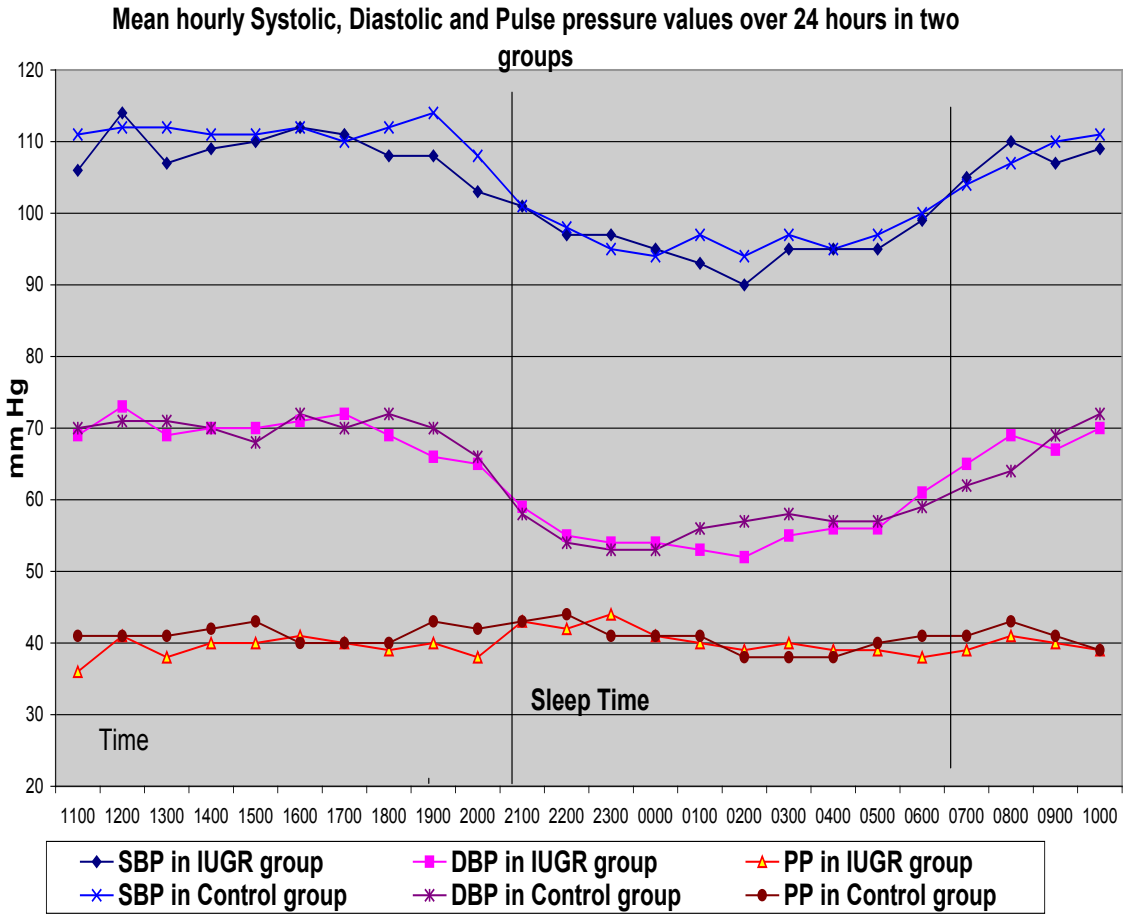
marked fall in SBP and DBP and heart rate with sleep is symmetrical and similar in nine-year-old normal and IUGR children. In spite of the day/night change in blood pressure, the pulse pressure remains constant at around 40 mmHg throughout day and night. During the day with wakefulness the mean SBP and DBP are 108 and 69 mmHg for the IUGR group and 109 and 69 mmHg for the control group. With sleep there is a gradual fall in SBP to reach a mean minimum of 96 mmHg in the IUGR group and 97 mmHg in the control group after two to three hours of sleep. The corresponding DBP values are 55 and 56 mmHg for the IUGR and the control group respectively. This is followed by a period of stability and then a gradual rise to normal daytime levels prior to waking (**Figure 12**).

Proportionately the SBP mean nocturnal fall was 11.1% (range -1.8% to 25%) for the IUGR children and 11.8% (range 0.9% to 20%) for the control group while it was 17.6% (range 3.1% to 31.9%) for the DBP in the IUGR group and 17.4% (range 3.1% to 34%) for the control group.

In order to assess blood pressure variability for the 24 hour period we calculated the respective standard deviations. For SBP it was 10.97 mmHg for the IUGR group and 10.91 mmHg for the control group. Similarly the standard deviation of the DBP was 10.71 mmHg for the IUGR group and 10.08 mmHg for the control group.

Sub-analysis of ambulatory blood pressure values based on gender showed that the average values of 24 hour, daytime and night-time values of SBP and DBP were the same for each gender.

Figure 12 Twenty four hour ambulatory blood pressure profile for IUGR and Control group



5.2 Comparison of Ambulatory Blood Pressure profile in two groups:

Statistical analysis was carried out using the statistical software SAS version 9.1 for Windows (SAS Institute Inc, Cary, NC, USA). We used two statistical methods for comparing Mean Hourly Systolic, Diastolic and Pulse Pressure values between the two groups over 24 hours.

1. Mean scores between two groups at each data point were compared using f tests.
2. The **Cosinor** method, which estimates three parameters describing the pattern of changes in responses over a 24 hour period.

Cosinor analysis represents the mathematical best fit of the data to a cosine curve defined by the equation: $\mathbf{F(t) = M + A\ cos(\omega t + \phi)}$, where

- **M** = the mean level over the period (termed **Mesor:** midline estimating statistic of rhythm)
- **A** = the **Amplitude** of the cosine curve, which is equal to half the difference between the highest and lowest value
- **ø** = phase angle of the maximum value or the timing of the highest point (also termed **Acrophase**)
- **ω** = the **frequency**
- **t** = **time**

Multiple regression can be used to fit a standard cosine model to the data for each subject. The following model was fitted using ordinary least squares:

$$\mathbf{Y_{it} = \beta_{0j} + \beta_{1i}\ sin(2\pi\ t / 24) + \beta_{2i}\ cos(2\pi\ t / 24) + \epsilon_{it}}$$

For each subject the Acrophase and Amplitude can then be calculated using the formulae below:

1. $\mathbf{AMP_i = \sqrt{(\beta^2_{1i} + \beta^2_{2i})}}$
2. $\mathbf{ACR_i = 24/(2\ \pi)\ arctan\ (\beta_{1i} / \beta_{2i})}$

Group differences for the three parameters (Mesor, Amplitude and Acrophase) can then be calculated using analysis of variance.

Tables **10, 11 and 12** show comparisons between the IUGR and Control groups with respect to blood pressure parameters using the Cosinor method. None of the differences between the groups were significant. Comparison of the means for two groups at each time point with f tests, detected significant differences at two time points, which were not uniformly recurring and are quite possibly type I errors.

Table 10 Mesor values for Blood Pressure variables in the two groups

Variable	IUGR mean	Control mean	Diff	t	P (differences)
SBP (mmHg)	103	105	1.59	0.92	0.36
DBP (mmHg)	63	64	0.42	0.45	0.65
PP (mmHg)	40	41	1.15	0.9	0.37

Table 11 Amplitude values for Blood Pressure variables in the two groups

Variable	IUGR mean	Control mean	Diff	t	P (differences)
SBP (mmHg)	10.2	10.0	0.28	0.29	0.77
DBP (mmHg)	10.1	9.3	0.78	0.9	0.35
PP (mmHg)	3.6	3.2	1.15	0.9	0.57

Table 12 Acrophase values for Blood Pressure variables in the two groups

Variable	IUGR mean	Control mean	Diff	t	P (differences)
SBP (mmHg)	-0.15	-0.24	0.09	0.75	0.46
DBP (mmHg)	-0.13	-0.17	0.04	0.32	0.75
PP (mmHg)	0.04	-0.02	0.06	0.25	0.80

SBP = Systolic Blood Pressure DBP = Diastolic Blood Pressure

PP = Pulse Pressure P = Probability values for differences between means

Diff = differences in mean t = Test Statistic

5.3 Summary and Conclusion:

Both groups of children showed similar diurnal variation in blood pressure. At night mean SBP fell from 108 to 96 mmHg (11.1% decrease) for the IUGR group and from 110 to 97 mmHg (11.8% decrease) for the controls. Mean DBP fell from 68 mmHg (17.6% decrease) for the IUGR and from 69 to 57 mmHg (decrease of 17.4%) in the control group. The pulse pressure stayed steady at 40 mmHg during the twenty four hour period. It appears that at the age of nine years the ambulatory blood pressure profile related to biological circadian of the IUGR children rhythm is indistinguishable from that of the control children.

Results : Chapter 6 : Exploratory analysis of relationship between Ambulatory Blood Pressure, current body size and "Catch Up" growth and further testing of "Foetal Origins Hypothesis" for the study population

6.1 Statistical Methods:

Blood Pressure outcomes were investigated for their associations with a pool of candidate predictor variables: weight at birth, final weight, IUGR or Control, maternal height, paternal height, mid parental height, use of medication, type of medication, breast fed or not, duration of breast feeding, maternal cigarette smoking during pregnancy, current smoking status of parents, IMD (Index of Multiple Deprivation), sex, change in weight z score between birth and final measurement, current BMI, current BMI z score and final Height z score.

The modelling procedure was the same for each outcome: Univariable analysis for each of the candidate predictors was used to select variables preceding multivariable stepwise regressions, and a final model was selected based on these multivariable regressions for each BP outcome. The aim was to find the best set of predictors for each variable.

For further analyses to test the "Foetal Origins Hypothesis", the approach of Lucas (Lucas, Fewtrell et al. 1999) was followed and slightly expanded.

1. To explore the association between current BP and birth weight before and after adjusting for current weight or change in weight z score:

BP is modelled as a function of:

- Early weight only

- Early and Late weight
- Early and Late weight plus their Interaction
- Late weight only
- Z score for change in weight between Early and Late periods

The aim is to see whether early weight or late weight is the more important indicator of later BP. If early weight is related to later BP then this should be evident in the following ways:

- The early model should be significant and have a negative coefficient
- The coefficient for early weight should be more negative in the combined model
- When the interaction is significant, it should have a negative coefficient.

2. To explore the association between current BP and IUGR before and after adjusting for current weight or for change in weight z score:

If the IUGR group has higher Blood Pressure than the Normal group then intra-uterine growth retardation may be responsible for this, and there may be a foetal aetiology. The approach suggested by Lucas is used again.

6.2 Outcome measurements and predictors:

Analysis (**Table 13)** showed similar sets of predictors for 24 hour mean SBP (change in z score and IUGR) and daytime mean SBP (change in z score and IUGR and gender). The significant predictors for daytime mean DBP were birth weight, final weight and change in weight z score. The only significant predictor for night time mean SBP was final weight. No significant predictors were found for the following 6 blood pressure measurements: 24 hour mean DBP, night time mean DBP, day night ratio of SBP, day night ratio of DBP, variability of SBP over 24 hours, variability of

DBP over 24 hours. It shows similar sets of predictors for 24 hr mean SBP (change in z score and IUGR) and daytime mean SBP (change in z score and IUGR and gender). The adjusted mean values for the 24 hour SBP and day time SBP were significantly higher in the control group (**Table 14**)

It was noted that BMI was a highly significant univariable predictor for 24 hour MSBP, MSBP day and MSBP night and it was also significant in some multivariable models. This is further explored in the next section.

Table 13 Main blood pressure variables and their predictors

	24 hour Mean SBP	**Mean SBP day**	**Mean SBP night**	**Mean DBP day**
Predictors				
Change in weight z score	p = 0.001	p = 0.001		p = 0.045
IUGR	p = 0.043	p = 0.0055		
Final weight			p = 0.033	p = 0.006
Birth weight				p = 0.0155
Interaction between gender and change in weight z score		p = 0.058		
Gender		p = 0.015		
Model r^2	0.155	0.265	0.066	0.146

Table 14 Least Square means for the blood pressure variables for the two groups after adjustment for significant covariates

Blood Pressure Outcome	IUGR	Control	P values	f
24 hour MSBP (mmHg)	102.66	106.10	0.04*	4.22
MSBP day (mmHg)	105.99	113.03	0.01*	8.24
MSBP night (mmHg)	96.30	96.37	0.97	0.01
MDBP day (mmHg)	68.03	69.61	0.32	1.01

p = Probability value f = Test Statistic value

6.3 BMI and Ambulatory Blood Pressure:

In Univariable analysis, out of ten BP variables analysed, only Daytime MSBP, Night time MSBP and 24 hour MSBP were significantly related to BMI or BMI z score. All the associations were positive, so the higher the BMI the higher the blood pressure. After adjusting for birth weight the associations were stronger. After adjusting for change in weight z score the associations were weaker, but this is because the correlation between BMI and change in weight is highly significant (0.89; pr < 0.0001). However even after adjusting for change in weight z score, age and sex, BMI remains a significant predictor of 24 hour MSBP [p = 0.0250], night time MSBP [p =0.0420] and daytime SBP [p = 0.0282]). Final BMI z score no longer had any predictive power after adjustment for change of weight z score. **(Table 15)**

In the analyses looking into the association between the three blood pressure variables and BMI, change in weight z score was always a weaker predictor of blood pressure than BMI and was not a significant predictor in any of these analyses.

The relationship between 24 hour SBP and BMI was significant (p= 0.0016) overall (**Figure 13)** and for the control group (p = 0.0097) **(Figure 14**), but borderline for the

IUGR group (p = 0.0616) (**Figure 15**). Analysis of the relationship between current weight and Systolic BP gives very similar results.

Table 15 Relationship between SBP and BMI

	BMI Univariable	**BMI Adjusted for only Change in Weight z score**	**BMI Adjusted for only Birth Weight**
Day SBP	p = 0.0013 r = 0.38	p = 0.0289 r = 0.27	p = 0.0003 r = 0.43
Night SBP	p = 0.0151 r = 0.29	p = 0.0477 r = 0.24	p = 0.0092 r = 0.31
SBP 24hr	p = 0.0016 r = 0.37	p = 0.0273 r = 0.27	p = 0.0004 r = 0.42

p = Probability value r = Correlation/partial Correlation coefficient value

Figure 13 BMI and 24 hour Mean SBP in all children

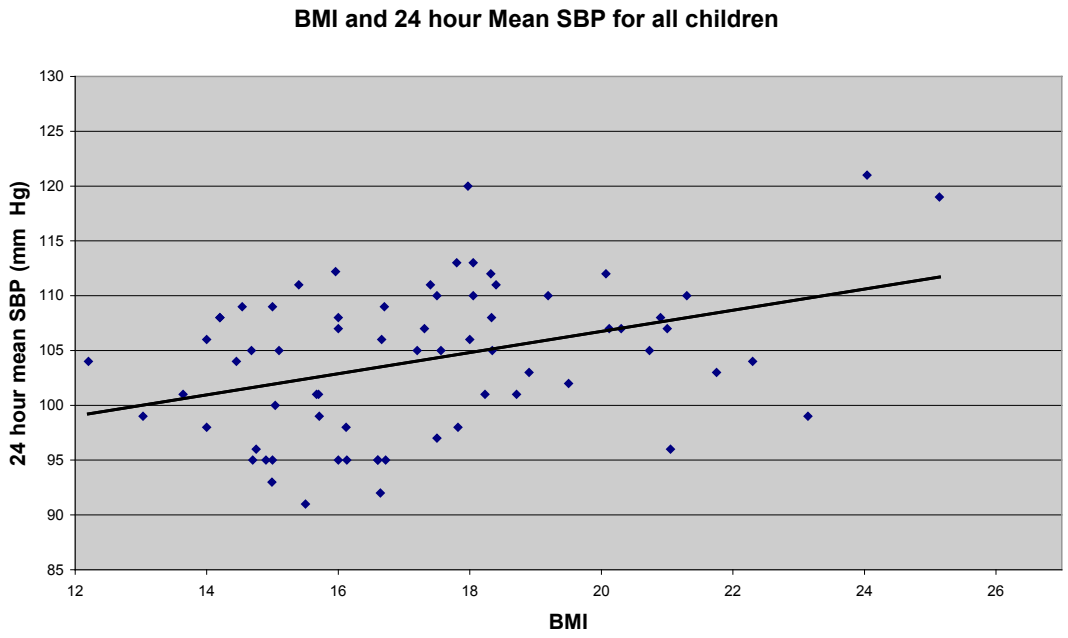


Figure 14 BMI and 24 hour Mean SBP in the Control group

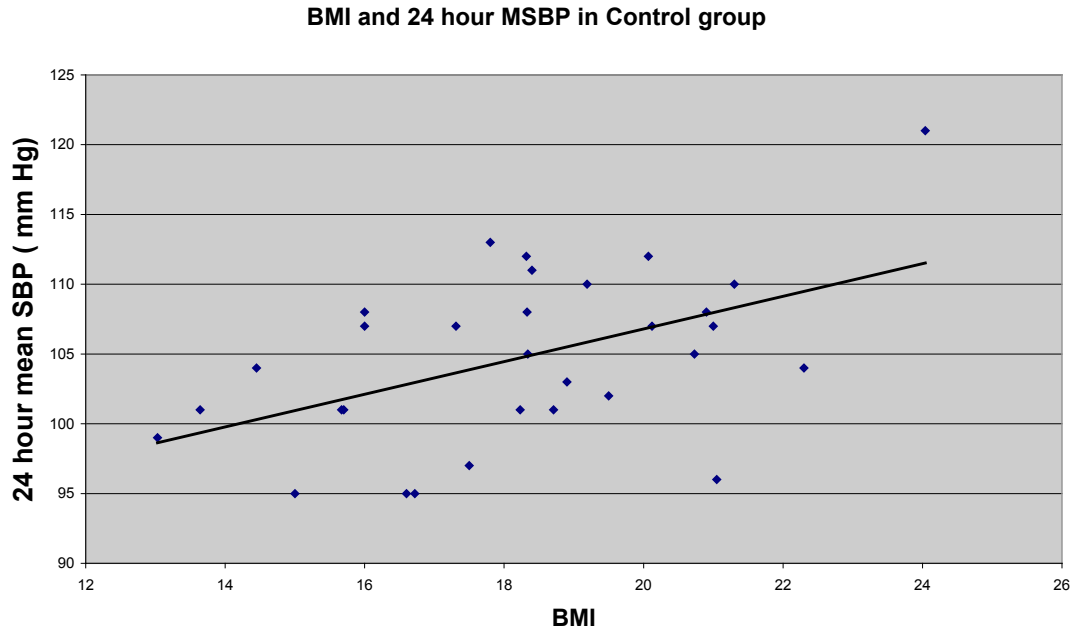
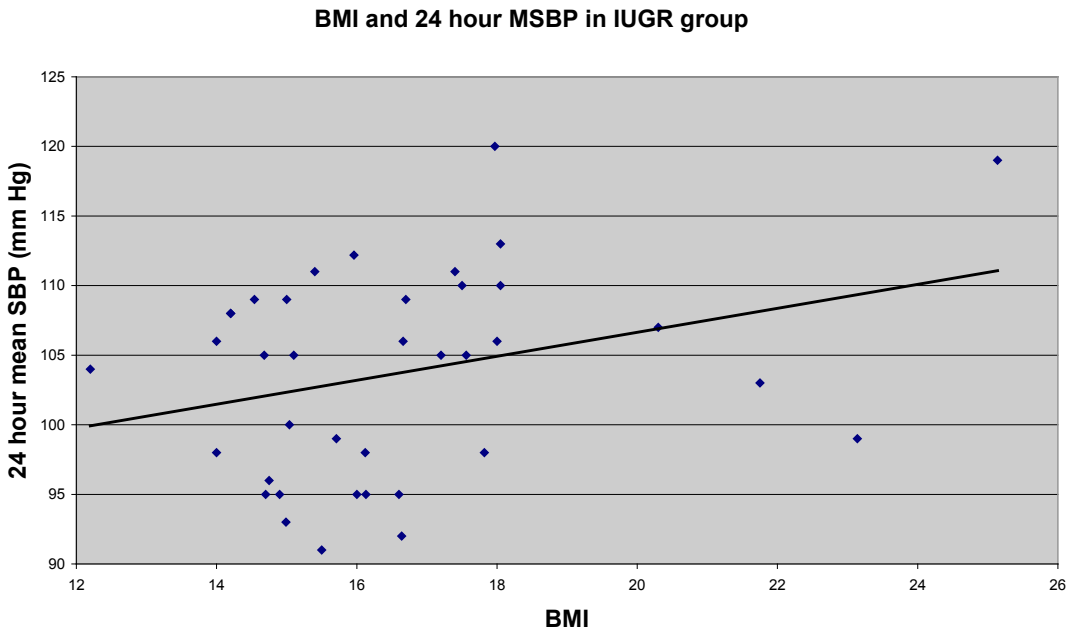


Figure 15 BMI and 24 hour Mean SBP in the IUGR group



6.4 Analysis of blood pressure data exploring the Foetal Origins hypothesis:

Each of the ten Ambulatory Blood Pressure Variables was tested for the Foetal Origins Hypothesis in the way described earlier in this chapter. The Blood Pressure variables are:

- 24 hour mean Systolic and Diastolic blood pressure
- Day time mean Systolic and diastolic blood pressure
- Night time mean Systolic and diastolic blood pressure
- Day Night ratio of mean Systolic and Diastolic blood pressure
- Standard deviation of day time and night time Systolic and diastolic blood pressure
- Standard deviation of systolic and diastolic blood pressure over 24 hours

Table **16** and **17** show the results based on the models suggested by Lucas et al for **24 hour Mean SBP**. The Birth Weight models (**Table 16**) show that early weight is not as important in determining later systolic BP as later weight, and change in weight. If the foetal origins hypothesis holds then the early model should be significant and the slope negative, and while the slope is indeed negative, the model is not significant. (p = 0.3416) The slope is more negative in the combined model than in the early model, which provides some weak evidence for the foetal origins hypothesis. The interaction though is close to zero and slightly positive. After adjusting for change in weight z score, there is a significant association between birth weight and blood pressure. The IUGR models (**Table 17**) suggest more importance for the later weight and also change in weight rather than IUGR / birth weight in predicting mean systolic Blood Pressure.

Table 16 Regression models for Birth Weight and 24hour MSBP to test Foetal Origins Hypothesis

Effect	**Model**	**df**	**Estimate**	**F**	**P**
Birth Weight	Early	1, 68	-1.21	0.92	0.3416
Birth Weight adj[1]	Combined	1, 68	-2.46	4.06	0.0479 *
Current Weight			0.45	12.92	0.0006 *
Interaction Birth Weight X Current Weight	Interaction	1, 68	-2.96	0.17	0.6840
Current Weight	Late	1, 68	0.38	9.50	0.0030 *
Birth weight adj[2]	Change	1, 68	1.64	1.06	0.3074
Change in weight z score			2.05	7.58	0.0076 *

adj[1] = birth weight adjusting for current weight only

adj[2] = birth weight adjusting for change in z score for weight only

Table 17 Regression models for IUGR and 24 hour MSBP to test the Foetal Origin Hypothesis

Effect	Model	df	LSMeans	F	P
IUGR	Early	1, 68	IUGR=103.7 Norm=104.8	0.49	0.4878
IUGR adj[1] **Current Weight**	Combined	1, 68	IUGR=104.4 Norm=104.0	0.08 8.90	0.7832 0.0040 *
Interaction IUGR X Current Weight	Interaction	1, 68	IUGR=104.0 Norm=103.8	0.80	0.3739
Current Weight	Late	1, 68		9.50	0.0030 *
IUGR adj[2] **Change in weight z score**	Change	1, 68	IUGR=102.7 Norm=106.1	4.22 11.55	0.0439 * 0.0012 *

adj[1] = iugr adjusting for current weight only; adj[2] = iugr adjusting for change in z score for weight only

The rest of the analysis for the other **nine** ABP parameters is shown in **Table 18** to **Table 35**. The analysis suggested that on the whole, later weight and change in weight are more important than early weight in predicting later BP, although neither early nor later weight were significant in predicting blood pressure variability, day-night ratio and night time DBP. In predicting twenty four hour mean DBP early and late weight were equally important, and both more so than change in z score for weight between early and later periods.

There was thus only limited support for the foetal origins hypothesis since later weight was usually the better predictor of later BP, and all of the interactions between early and later weight were non-significant. In most cases however the coefficients for early weight were negative and were more negative for the combined models, which provide some support for the foetal origins hypothesis.

Furthermore when similar models were used to explore the association between BP and IUGR, there was even less evidence for the foetal origins hypothesis than when birth weight was used as the comparator variable.

Table 18 Regression models for Birth Weight and 24hour MDBP to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-1.17	2.39	0.1272
Birth Weight adj[1]	Combined	1, 68	-1.64	4.53	0.0371 *
Current Weight			0.17	4.60	0.0357 *
Interaction Birth Weight X Current Weight	Interaction	1, 68	-0.02	0.02	0.8879
Current Weight	Late	1, 68	0.12	2.45	0.1222
Birth weight adj[2]	Change	1, 68	0.51	0.21	0.6458
Change in weight z score			-0.46	1.20	0.2769

Table 19 Regression models for IUGR and 24hour MDBP to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=64.2 Norm=63.9	0.01	0.7555
IUGR adj1	Combined	1, 68	IUGR=64.5	0.77	0.3845
Current Weight			Norm=63.6	3.11	0.0825
Interaction IUGR X Current Weight	Interaction	1, 68	IUGR=64.4 Norm=63.5	0.25	0.6191
Current Weight	Late	1, 68		2.45	0.1222
IUGR adj2	Change	1, 68	IUGR=63.9	0.21	0.6466
Change in weight z score			Norm=64.3	3.49	0.0661

adj[1] = iugr adjusting for current weight only; adj[2] = iugr adjusting for change in z score for weight only

Table 20 Regression models for Birth Weight and SBP Day: Night to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.003	0.07	0.7879
Birth Weight adj[1]	Combined	1, 68	-0.004	0.07	0.7926
Current Weight			0.000	0.00	0.9808
Interaction Birth Weight	Interaction	1, 68	0.003	1.36	0.2474
X Current Weight					
Current Weight	Late	1, 68	0.000	0.00	0.9573
Birth weight adj[2]	Change	1, 68	-0.0016	0.01	0.9249
Change in weight z score			0.0013	0.03	0.8674

Table 21 Regression models for IUGR and SBP Day: Night to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=1.13	0.03	0.8744
			Norm=1.13		
IUGR adj[1]	Combined	1, 68	IUGR=1.13	0.03	0.8539
Current Weight			Norm=1.13	0.01	0.9126
Interaction IUGR	Interaction	1, 68	IUGR=1.12	1.14	0.2902
X Current Weight			Norm=1.13		
Current Weight	Late	1, 68		0.00	0.9572
IUGR adj[2]	Change	1, 68	IUGR=1.12	0.09	0.7613
Change in weight z score			Norm=1.13	0.15	0.6911

adj[1] = iugr adjusting for current weight only;

adj[2] = iugr adjusting for change in z score for weight only

Table 22 Regression models for Birth Weight and DBP Day: Night to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.01	0.20	0.6545
Birth Weight adj[1]	Combined	1, 68	-0.009	0.21	0.6478
Current Weight			0.000	0.01	0.9130
Interaction Birth Weight	Interaction	1, 68	-0.0014	0.12	0.7285
X Current Weight					
Current Weight	Late	1, 68	0.000	0.00	0.9829
Birth weight adj[2]	Change	1, 68	-0.016	0.38	0.5375
Change in weight z score			-0.005	0.19	0.6678

These results suggest that neither early nor late weight, nor the interaction, nor the change in weight z score, are important in determining later day night ratio for SBP.

Table 23 Regression models for IUGR and DBP Day: Night to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=1.23	0.38	0.5413
			Norm=1.22		
IUGR adj[1]	Combined	1, 68	IUGR=1.23	0.41	0.5260
Current Weight			Norm=1.22	0.04	0.8516
Interaction IUGR	Interaction	1, 68	IUGR=1.23	0.06	0.8144
X Current Weight			Norm=1.21		
Current Weight	Late	1, 68		0.00	0.9829
IUGR adj[2]	Change	1, 68	IUGR=1.23	0.47	0.4974
Change in weight z score			Norm=1.22	0.10	0.7587

adj[1] = iugr adjusting for current weight only

adj[2] = iugr adjusting for change in z score for weight only

Table 24 Regression models for Birth Weight and 24 hour SBP variability to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.395	1.30	0.2587
Birth Weight adj[1]	Combined	1, 68	-0.531	2.18	0.1446
Current Weight			0.049	1.77	0.1874
Interaction Birth Weight X Current Weight	Interaction	1, 68	0.096	2.07	0.1542
Current Weight	Late	1, 68	0.033	0.89	0.3484
Birth weight adj[2]	Change	1, 68	-0.086	0.04	0.8516
Change in weight z score			0.222	1.09	0.3010

Table 25 Regression models for IUGR and 24 hour SBP variability to test FOH

Effect	Model	Df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=11.0 Norm=10.9	0.02	0.9002
IUGR adj[1]	Combined	1, 68	IUGR=11.0	0.21	0.6490
Current Weight			Norm=10.9	1.07	0.3037
Interaction IUGR X Current Weight	Interaction	1, 68	IUGR=11.0 Norm=10.7	1.31	0.2569
Current Weight	Late	1, 68		0.89	0.3484
IUGR adj[2]	Change	1, 68	IUGR=10.8	0.29	0.5936
Change in weight z score			Norm=11.1	2.63	0.1096

adj[1] = iugr adjusting for current weight only

adj[2] = iugr adjusting for change in z score for weight only

Table 26 Regression models for Birth Weight and 24 hour DBP variability to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.602	3.14	0.0809
Birth Weight adj[1]	Combined	1, 68	-0.634	3.15	0.0803
Current Weight			0.011	0.10	0.7561
Interaction Birth Weight X Current Weight	Interaction	1, 68	0.040	0.35	0.5554
Current Weight	Late	1, 68	-0.007	0.04	0.8446
Birth weight adj[2]	Change	1, 68	-0.637	2.00	0.1621
Change in weight z score			-0.025	0.01	0.9051

Table 27 Regression models for IUGR and 24 hour DBP variability to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=10.7 Norm=10.1	1.98	0.1642
IUGR adj[1]	Combined	1, 68	IUGR=10.7	1.99	0.1634
Current Weight			Norm=10.1	0.07	0.7861
Interaction IUGR X Current Weight	Interaction	1, 68	IUGR=10.69 Norm=10.01	0.35	0.5578
Current Weight	Late	1, 68		0.04	0.8446
IUGR adj[2]	Change	1, 68	IUGR=10.7	1.14	0.2893
Change in weight z score			Norm=10.1	0.28	0.5992

adj[1] = iugr adjusting for current weight only

adj[2] = iugr adjusting for change in z score for weight only

Table 28 Regression models for Birth Weight and Day time MSBP to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-1.400	1.15	0.2872
Birth Weight adj[1]	Combined	1, 68	-2.763	5.00	0.0287 *
Current Weight			0.490	15.16	0.0002 *
Interaction Birth Weight	Interaction	1, 68	0.131	0.31	0.5774
X Current Weight					
Current Weight	Late	1, 68	0.410	10.90	0.0015 *
Birth weight adj[2]	Change	1, 68	1.762	1.18	0.2817
Change in weight z score			2.272	8.96	0.0039 *

Table 29 Regression models for IUGR and Day time MSBP to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=108.3	0.80	0.3755
			Norm=109.8		
IUGR adj[1]	Combined	1, 68	IUGR=109.0	0.02	0.8993
Current Weight			Norm=108.8		0.0025 *
Interaction IUGR	Interaction	1, 68	IUGR=108.7	1.80	0.1839
X Current Weight			Norm=108.4		
Current Weight	Late	1, 68		10.90	0.0015 *
IUGR adj[2]	Change	1, 68	IUGR=107.1	5.95	0.0174 *
Change in weight z score			Norm=111.2	14.71	0.0003 *

adj[1] = iugr adjusting for current weight only; adj[2] = iugr adjusting for change in z score for weight only

There is weak evidence here of an effect of IUGR on late SBP during the day (IUGR after adjusting for change in z score). There is some evidence for an effect of later weight and also of change in weight.

Table 30 Regression models for Birth Weight and Day time MDBP to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.788	0.88	0.3511
Birth Weight adj[1]	Combined	1, 68	-1.307	2.35	0.1300
Current Weight			0.186	4.61	0.0355 *
Interaction Birth Weight	Interaction	1, 68	-0.086	0.29	0.5945
X Current Weight					
Current Weight	Late	1, 68	0.149	3.13	0.0816
Birth weight adj[2]	Change	1, 68	-0.072	0.00	0.9482
Change in weight z score			0.515	0.99	0.3228

Table 31 Regression models for IUGR and Day time MDBP to test FOH

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=68.7	0.03	0.8692
			Norm=68.8		
IUGR adj[1]	Combined	1, 68	IUGR=69.0	0.18	0.6695
Current Weight			Norm=68.5	3.24	0.0763
Interaction IUGR	Interaction	1, 68	IUGR=68.9	0.02	0.8813
X Current Weight			Norm=68.4		
Current Weight	Late	1, 68		3.13	0.0816
IUGR adj[2]	Change	1, 68	IUGR=68.3	0.62	0.4344
Change in weight z score			Norm=69.3	2.48	0.1203

adj[1] = iugr adjusting for current weight only; adj[2] = iugr adjusting for change in z score for weight only

Table 32 Regression models for Birth Weight and Night time MSBP to test FOH

Effect	Model	df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.595	0.16	0.6879
Birth Weight adj[1]	Combined	1, 68	-1.61	1.17	0.2840
Current Weight			0.36	5.75	0.0193 *
Interaction Birth Weight	Interaction	1, 68	-0.129	0.21	0.6478
X Current Weight					
Current Weight	Late	1, 68	0.317	4.75	0.0329 *
Birth weight adj[2]	Change	1, 68	1.360	0.50	0.4819
Change in weight z score			1.405	2.44	0.1227

Table 33 Regression models for IUGR and Night time MSBP to test FOH

Effect	Model	Df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=95.7	0.57	0.4510
			Norm=97.1		
IUGR adj[1]	Combined	1, 68	IUGR=96.3	0.01	0.9413
Current Weight			Norm=96.4	4.08	0.0475 *
Interaction IUGR	Interaction	1, 68	IUGR=96.3	0.00	0.9763
X Current Weight			Norm=96.4		
Current Weight	Late	1, 68		4.75	0.0329 *
IUGR adj[2]	Change	1, 68	IUGR=95.0	2.23	0.1404
Change in weight z score			Norm=98.0	3.79	0.0557

adj[1] = iugr adjusting for current weight only

adj[2] = iugr adjusting for change in z score for weight only

Table 34 Regression models for Birth Weight and Night time MDBP to test Foetal Origins Hypothesis

Effect	Model	Df	Estimate	F	Pr
Birth Weight	Early	1, 68	-0.419	0.20	0.6543
Birth Weight adj[1]	Combined	1, 68	-0.863	0.81	0.3721
Current Weight			0.159	2.65	0.1080
Interaction Birth Weight X Current Weight	Interaction	1, 68	-0.004	0.00	0.9811
Current Weight	Late	1, 68	0.134	2.06	0.1558
Birth weight adj[2]	Change	1, 68	0.484	0.16	0.6938
Change in weight z score			0.649	1.29	0.2607

Table 35 Regression models for IUGR and Night time MDBP to test Foetal Origins Hypothesis

Effect	Model	df	LSMeans	F	Pr
IUGR	Early	1, 68	IUGR=55.8 Norm=56.6	0.50	0.4828
IUGR adj[1]	Combined	1, 68	IUGR=56.0	0.07	0.7973
Current Weight			Norm=56.3	1.60	0.2110
Interaction IUGR X Current Weight	Interaction	1, 68	IUGR=55.9 Norm=56.2	0.20	0.6533
Current Weight	Late	1, 68		2.06	0.1558
IUGR adj[2]	Change	1, 68	IUGR=55.4	1.67	0.2004
Change in weight z score			Norm=57.1	2.53	0.1167

adj[1] = iugr adjusting for current weight only

adj[2] = iugr adjusting for change in z score for weight only

6.5 Summary and conclusion

The adjusted mean values for 24 hour mean SBP (106.10 mmHg v 102.66 mmHg, p = 0.04) and daytime mean SBP (113.03 mmHg v 105.99 mmHg, p = 0.01) were significantly higher in the control group. There was no support for Foetal Origins Hypothesis as later weight was the better predictor of later SBP. BMI was a significant predictor of SBP even after adjusting for gender, age and change of weight z score. At 9 years of age, normal children have higher ambulatory SBP than the children who were growth restricted in utero and the higher SBP is related to larger body mass.

Chapter 7 Results: Heart Rate Variability

Out of seventy-five ambulatory ECG recordings two were rejected because they failed to meet the inclusion criteria. Heart rate in beats per minute was derived from the formula: 60000/RR interval in Milliseconds.

7.1 Description of HRV parameters in two groups

Table 36 shows the mean values, Standard Deviations and comparative analysis for the following HRV parameters using t-test:

1. Day time and night time Heart Rate (beats per minute)
2. Day time, night time and twenty four hour mean SDNN (Standard Deviation of NN intervals)
3. Day time and night time mean HF and LF ratio (HF: LF)
4. Day time and night time mean of the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD)
5. SDNNi: Mean of the SD of all RR intervals for all five minute segments
6. SDANN: Standard deviation of the averaged normal sinus RR intervals for all 5 minute segments.

Table 36 Mean values and comparison of HRV parameters between two groups

Variable	Mean (SD)		t	Pr
	IUGR (n= 39)	Control (n= 34)		
Mean Day Time Heart Rate (BPM)	94.95 (9.77)	98.04 (8.95)	-1.40	0.1656
Mean Night Time Heart Rate (BPM)	72.78 (7.37)	72.78 (7.27)	-0.00	0.9984

Table 36 Mean values and comparison of HRV parameters between two groups **Continued from page 115**				
Variable	**Mean (SD)**		**t**	**Pr**
	IUGR (n= 39)	**Control (n= 34)**		
Mean SDNN (ms) Night	90.7 (34.5)	96.5 (29.8)	-0.96	0.3388
Mean RMSSD (ms) Day *	51.7 (25.8)	48.1 (24.4)	0.61	0.6601
Mean RMSSD (ms) Night *	89.3 (51.0)	92.3 (42.9)	-0.61	0.5433
Mean HF:LF Day *	0.83 (0.54)	0.79 (0.40)	0.02	0.9817
Mean HF:LF Night *	1.81 (1.15)	1.84 (0.77)	-0.41	0.6829
SDNN 24 (ms)	152.9 (36.7)	160.1 (28.6)	-0.93	0.3544
SDNNI 24 (ms)	82.8 (26.6)	84.0 (22.6)	-0.21	0.8371
SDANN 24 (ms)	124.5 (30.5)	133.4 (24.5)	-1.37	0.1756

* t-test carried out on log transformed data BPM = Beats per minute ms = milliseconds

7.2 Description of the diurnal HRV Profile in two groups

The HRV parameters do not differ between the two groups but they clearly show a diurnal biorhythm with day/night activity patterns. Sleep time is associated with significantly lower heart rate and significantly higher SDNN, HF: LF and RMSSD. The changes in all the HRV parameters with sleep are symmetrical and similar in control and IUGR group. The average daytime heart rate is 94 beats and 98 beats per minute for the IUGR and the control groups respectively. The average night-time heart rate is 72 beats per minute for each group. Synchronous with the fall in blood pressure is a fall in heart rate with sleep. The heart rate gradually falls to a minimum of 70 beats

per minute after two to three hours of sleep. This follows a period of stabilisation before returning to the pre-sleep level at the time of waking up.

The average daytime SDNN is 74 milliseconds and 72 milliseconds respectively for the IUGR and the control group, whereas the average night time SDNN is 91 milliseconds and 97 milliseconds. The SDNN increases to a maximum 130 milliseconds just prior to waking up between 6 and 7 am followed by a fall to the pre-sleep level, just as the heart rate is increasing (**Figure 16**). For children in both groups the HF/LF is much higher at night compared to the daytime (1.8 v 0.8) suggesting a significant parasympathetic dominance on the heart during sleep (**Figure 17**). The parasympathetic dominance on the heart starts shortly after the onset of sleep followed by a rise to the maximum level within a couple of hours. The parasympathetic dominance then continues through the night falling to the pre-sleep level at the time of waking up.

Figure 16 Heart Rate and SDNN profile in two groups

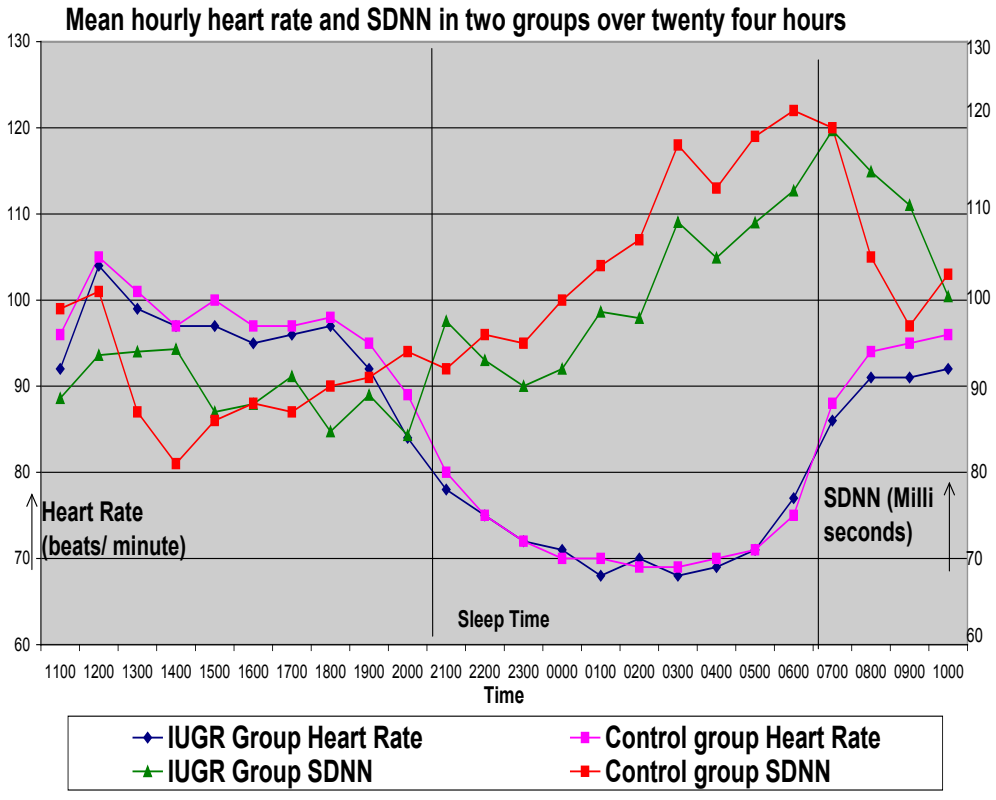
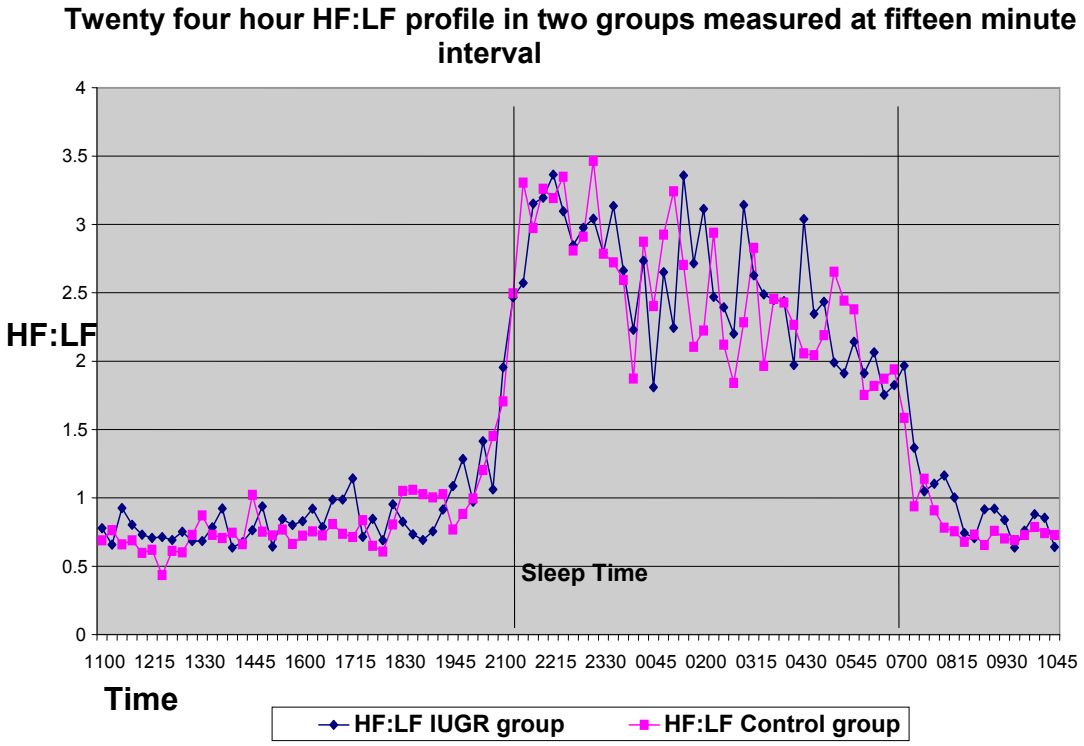


Figure 17 HF:LF profile in two groups



7.3 Comparison of 24 hour HRV profile in two groups:

I used similar statistical methods for comparing RR intervals, SDNN and HF: LF ratio values between the two groups over 24 hours as described in the section on blood pressure parameters. These are:

1. Mean scores between two groups at each data point were compared using f tests.
2. The Cosinor method, which estimates three parameters describing the pattern of changes in responses over a 24 hour period.

The tables **37, 38 and 39** show comparisons between the IUGR and Control groups using the Cosinor method for HRV variables. None of the differences between the groups were significant. Comparison of the means for two groups at each time point with f tests, detected significant differences in two time points, which were not uniformly recurring and are quite possibly type 1 errors.

Table 37 Mesor values for HRV variables in the two groups

Variable	IUGR mean	Control mean	Pr (differences)
RR interval (ms)	723	702	0.18
HF:LF	1.6	1.5	0.70
SDNN (ms)	99	102	0.66

Table 38 Amplitude values for HRV variables in the two groups

Variable	IUGR mean	Control mean	Pr (differences)
RR interval (ms)	146	146	.99
HF:LF	1.3	1.2	.72
SDNN (ms)	20.3	22.7	.43

Table 39 Acrophase values for HRV variables in the two groups

Variable	IUGR mean	Control mean	Pr (differences)
RR interval (ms)	-0.47	-0.46	.77
HF:LF	-0.34	-0.33	.92
SDNN (ms)	0.28	0.53	.23

7.4 Regression analysis for HRV variables:

The modelling procedure and the candidate predictor variables were similar to the method used for blood pressure variables. Univariable analysis for each of the candidate predictors was used to select variables preceding multivariable stepwise regressions, and a final model was selected based on these multivariable regressions for each BP outcome. No differences were detected between the two groups in relation to all the HRV parameters (**Table 40)**.

The dominant predictor variable overall was paternal height, which was significant for five Heart Rate variables. However these heart rate variables are highly correlated with each other. Cortisol production in the morning, Cortisol production in the evening and gender were all significant for one variable each.

Table 40 Regression analysis and significant predictors for HRV parameters

HRV Parameter	**Predictor 1**	**Predictor 2**	**LS Means**		**Pr**
			IUGR	Control	
Mean Day Time Heart Rate (BPM)	Morning Cortisol: Creatinine (6.34, 0.014*)	Gender (3.40, 0.0698)	94.8	98.9	0.0593
Mean Night Time Heart Rate (BPM)	Gender (4.87, 0.03*)		72.6	73.5	0.6171
Mean SDNN(ms) Day +	Paternal height (3.99, 0.05)		71.5	74.4	0.5526
Mean SDNN(ms) Night +	Paternal height (3.78, 0.05)		87.6	99.0	0.1265

Table 40 Regression analysis and significant predictors for HRV parameters : continued from page 120

HRV Parameter	Predictor 1	Predictor 2	LS Means		Pr
			IUGR	Control	
Mean RMSSD (ms) Day +	Paternal height (5.30, 0.02*)	Night time Cortisol: Creatinine (4.24,0.04*)	48.6	50.8	0.6986
Mean RMSSD (ms) Night +	Paternal height (4.20, 0.04*)		84.7	95.7	0.3183
Mean HF:LF Day +	Paternal height (5.65, 0.02*)		0.80	0.85	0.6812
Mean HF:LF Night +	Paternal height (5.28, 0.02*)		1.67	1.81	0.5423
SDNN 24 (ms)			153.3	159.8	0.4101
SDNNI 24 (ms)	Paternal height (6.01, 0.017*)		79.9	86.5	0.2448
SDANN 24 (ms)			125.5	132.5	0.2894

Selection criterion for entry into model = 0.2 Selection criterion for staying in model = 0.1

Variables marked with a '+' were logged before analysis due to striking non-normality combined with a low sample size.

* These predictors were significant at the 0.05 level.

Chapter 8 Results: Urinary Cortisol excretion

8.1 Analysis of Urinary Cortisol Creatinine ratio:

Morning urinary cortisol creatinine ratio is higher than the corresponding night-time value for children in both groups confirming the surge in cortisol production before waking up. Comparison of the unadjusted and adjusted mean values between the two groups using independent samples t-test and general linear model did not show any significant difference **(Table 42)**.

Table 41 Night time and Early morning Cortisol Creatinine ratio in two groups

	IUGR		Control	
	Night time	**Early morning**	**Night time**	**Early morning**
Maximum	40.22	94.68	22.78	81.43
Minimum	3.27	6.79	2.66	7.68
Unadjusted Mean	6.73	21.77	4.16	18.72
SD	9.35	37.14	9.42	31.38

Table 42 General Linear Models comparing Morning and Night time Cortisol Creatinine ratio between the two groups

Period	**Estimates from GLM**				
	LS Mean Cortisol Creatinine ratio	**SD**	**F**	**df**	**P**
Morning	IUGR = 36.13 Control = 30.38	21.69 18.35	1.50	1	0.2251
Night	IUGR = 9.12 Control = 9.55	6.55 4.22	0.11	1	0.7389

8.2 Cardiovascular outcomes and urinary Cortisol excretion

Blood Pressure:

No significant difference was detected between IUGR and Control groups regarding mean 24hour SBP either before or after adjusting for morning cortisol or night cortisol (**Table 43**).

Table 43 General Linear Models exploring relationship between mean 24-hour SBP and Cortisol Creatinine ratio in the two groups.

	Estimates from GLM			
	Mean SBP 24hr	**F**	**df**	**P**
Before Adjustment	IUGR = 103.7 Control = 104.8	0.49	1	0.4878
After Adjustment	IUGR = 103.6 Control = 105.0	0.76	1	0.3880

Night time cortisol was significantly related to 24hour MDBP and 24 hr variability in DBP. The associations for night time cortisol and day-night ratio for both SBP and DBP approached significance. Morning cortisol was significantly related to variability in 24 hr SBP, and the association with 24 hr MDBP also approached significance (**Table 44)**.

Table 44 Univariable analysis of Cortisol Creatinine ratio and Blood Pressure variables

BP Variable	Morning Cortisol: Creatinine	Night time Cortisol: Creatinine
MSBP 24 hr	p = 0.3209	p = 0.8048
MDBP 24 hr	p = 0.0532 +	p = 0.0035 *
MSBP day-night ratio	p = 0.1656	p = 0.0503 +
MDBP day-night ratio	p = 0.1885	p = 0.0572 +
SBP SD 24hr	p = 0.0486 *	p = 0.0719
DBP SD 24hr	p = 0.2791	p = 0.0398 *
MSBP day	p = 0.1422	p = 0.7638
MDBP day	p = 0.2678	p = 0.1888
MSBP night	p = 0.7408	p = 0.3966
MDBP night	p = 0.6143	p = 0.5682

* = Significant at 5% level.

+ = Approaching significance at 5% level.

Heart rate variability:

Early morning cortisol and night time mean cortisol were noted to be significant predictors of mean day time heart rate and mean day time RMSSD respectively but adjusted mean values were not different.

Chapter 9 Discussion

9.1 IUGR, compensatory growth and current body size

Intrauterine malnutrition leads to reduction in body size and weight at birth and is thought to have continuing influence on growth patterns into childhood (Barker 2003). However, there are conflicting theories about the degree to which the catch up growth occurs. Barker suggested that poor prenatal growth may lead to obesity later, a view that is not universally supported (Martorell, Khan et al. 1994, Martorell, Ramakrishnan et al. 1998, Martorell, Stein et al. 2001, Li, Stein et al. 2003).

The different outcomes of these studies are influenced by the following factors:

- Confusing terminology and a lack of uniform diagnostic criteria for IUGR
- Failure to differentiate between babies born with a low birth weight which is actually appropriate for their gestational age and true intra uterine growth restriction where the babies may have low or normal birth weight but have failed to reach their genetic potential
- Statistical analyses used to detect catch up growth (McCormick 1985, Cameron, Preece et al. 2005)

If as it has been suggested that intrauterine growth retardation followed by catch up growth in children, may together predispose to cardiovascular and metabolic diseases in adulthood (Barker 1995, Eriksson, Forsen et al. 2001, Barker 1999) then catch up growth has more far-reaching significance than has been previously thought. Comparisons using only raw anthropometric data or the z scores risk underestimating the level of catch up growth. “Regression to Mean” is a naturally occurring phenomenon where small babies move up the weight centiles and the bigger babies move down the weight centiles towards the 50^{th} centile as they grow older. Its impact

on measurement of catch up growth is often overlooked in epidemiological studies (Cameron, Preece et al. 2005). The IUGR children in my study population were all diagnosed antenatally using serial ultrasound scans and very strict criteria. This identified only the children with genuine IUGR due to antenatal insults and eliminated the ambiguity of using Birth Weight as a surrogate marker for IUGR. I used both raw data and z scores and accounting for "Regression to Mean", as suggested by Cameron, I obtained a closer comparison with the most appropriate norms, which can change over time.

My analysis shows that IUGR children were smaller at birth but showed a greater increase in their weight between birth and nine years. Despite growing at a significantly faster rate the IUGR group have lower weight, height and BMI z scores at the age of nine years. The predictors of these differences are IUGR, birth weight and maternal and paternal heights.

This demonstrates that by any criterion, growth restricted infants do not fully 'catch up' with the control group of infants in height, weight or body stature by the age of nine years. Children with IUGR are substantially smaller and lighter compared to the control children at the age of nine years and may well remain so throughout life. I also compared both the IUGR and control group with another control group, namely the 1990 British Growth reference group, used to produce z score data. This confirmed that the complete catch up growth had not occurred.

Clearly the impact of malnutrition in utero extends at least into early childhood and beyond and is a most powerful influence on the growth process. Birth weight on its own has a weak influence on later body size or weight, although it is a good predictor of change in z score for weight. If it can be construed that changes in weight z scores imply rate of growth, then the growth retarded infants grow faster than normal

children, while remaining smaller in actual weight throughout. It remains to be seen whether at puberty the difference in size can be reduced by the growth spurt. From the evidence of this work that would seem unlikely.

I analysed the impact of a wide range of perinatal, socio-economic and demographic variables on the growth of the children. In this study there are generally more illnesses in the IUGR group, mainly wheezing and asthmatic illnesses, and surprisingly two cases of autism. All the usual factors reported to be related to smallness at birth are overwhelmingly present in these IUGR infants, e.g. low socio-economic status and maternal smoking and they continue to be present during the remainder of early childhood. So great are the influences of intrauterine conditions on the developing foetus that they are not easily reversed when normal nutrition is restored. Although the parental heights of the IUGR children do not differ from those of the parents of our control group, there is an overall relationship between parental heights and heights of 9 year olds generally. This suggests that final height is largely genetically determined and the environmental factors, unless extreme, are perhaps not as important.

Similar smallness and stunting in IUGR children have been shown in Guatemalan children, Finnish children and South African children, in spite of the use of different measuring techniques and analytical methods (Martorell, Ramakrishnan et al. 1998, Martorell, Stein et al. 2001, Li, Stein et al. 2003, Cameron, Preece et al. 2005). However, Adair demonstrated significant compensatory growth in Filipino children, utilising change of height z scores. (Adair 1999) The similarity of growth pattern in groups so diverse in culture, nutrition and socio-economic status is testimony to the widespread nature of intrauterine malnutrition and the irreversibility of its impact.

Obesity has been predicted in IUGR infants as a consequence of early overfeeding and may be the basis of a general increase in morbidity and particularly premature

cardiovascular diseases in adults (Eriksson, Forsen et al. 2001, Barker 1999). I have found that the tendency to obesity is more likely in the normal group of children which is in keeping with widely reported increase in the incidence of obesity in young children in rich affluent Western societies, undoubtedly nutritionally related (Chinn, Rona 2001, Martorell, Kettel, Khan et al. 2000, Ogden, Flegal et al. 2002).

That obesity can be detected in normal nine year olds may be a sign of poor early nutrition, coupled with a predisposition from early childhood or even in utero, an effect opposite to that of foetal growth restriction. The importance of the detection of obesity in early childhood can now be measured by its impact on the cardiovascular system.

I managed to recruit seventy five children, out of the original population of 127 subjects, losing forty one percent due to attrition over nine years. This is an unfortunate but common occurrence in epidemiologic studies. Serial weight measurements were not available for all the children for the first two years but the trend of rapid weight gain was clearly present in the IUGR group from an early age. Body size is a product of weight and height and if I had access to serial height measurements from the early childhood then that would have provided a better reflection of change in body size.

Future Direction:

These findings call for a larger long-term prospective study comparing the pattern of growth in antenatally diagnosed IUGR children with children without any evidence of IUGR. A greater understanding of the cellular and molecular process underlying the compensatory growth is required. The greatest need for research is in the developing countries where IUGR is much more widespread and its multifactorial nature more

diverse and less well understood. Careful consideration of body composition is essential and estimation of fat free mass in children would provide a superior reflection of the nutritional status. Children should be targeted at an earlier age to prevent the development of obesity, as children are getting overweight within first decade of their lives. As the incidence of cardiovascular problems and obesity are more common in South Asian populations in Britain, it would be interesting to compare the growth pattern of children in different ethnic groups in Britain.

9.2 Analysis of the circadian cardiovascular rhythms

The 24 hour physiological biorhythms by which all living creatures exist in equilibrium with the environment, are not present in human infants at birth, but develop under suprachiasmatic control by the age of four months (Petersen, Pratt et al. 2001, Lodemore, Petersen et al. 1991, Tappin, Ford et al. 1996). Infants who have suffered foetal malnutrition are late in acquiring mature biorhythms in relation to changes in deep body temperature, heart rate and cortisol secretion (Jackson, Wailoo et al. 2004, Jackson, Wailoo et al. 2001) and also appear to have the potential for developing premature cardiovascular diseases in adult life (Barker 2003). Whether these events are in any way related, is not clear. But, if there is any likelihood that premature cardiovascular disease in adults is preceded by detectable changes in the cardiovascular status in children, then those who have been growth restricted infants would be the most likely affected.

In assessing cardiovascular status, no single parameter is entirely representative of total function. By using 24 hour measurements of several parameters including blood pressure and HRV, I have shown that the many aspects of cardiovascular activity are not only functionally integrated over a period of time, but will adapt to the needs of the

child throughout day time activity and night time sleep. For the purpose of statistical analysis of the circadian rhythm I considered several methods including separate linear models fitted to the data of each subject, periodic spline models and cumulative sums. Eventually I used the method of Cosinor fitting by least squares as developed by Halberg, which is a well-established method in Chronobiology to compare circadian rhythms between patient groups (Guo, Stein 2003).

My analysis shows that by the age of nine years the children in the study population have well-defined cardiovascular 24 hour biorhythms, in which both blood pressure and heart rate fall appreciably with sleep and are elevated again with wakefulness. There is a clearly fully matured and functionally integrated autonomic nervous system which maintains a steady pulse pressure during day and night, irrespective of the variation in systolic and diastolic blood pressure. Also during sleep at night, heart rate slows while at the same time variability of heart rate increases, all under autonomic control. Fast Fourier Transformation of the heart rate signal and Power Spectral analysis does not show any evidence to suggest that the IUGR children have higher sympathetic dominance of their cardiovascular system compared to their normal counterparts, which could predispose them to premature cardiovascular morbidity. The degree of maturation of ANS is similar in both groups. Similar findings have been reported on ex-preterm children suggesting a faster ANS maturation during first two years of life compared to children born at term, with a higher speed of recovery for the parasympathetic arm (De Rogalski Landrot, Roche et al. 2007). To my knowledge this the first study looking into the ANS maturation in nine year old children with IUGR. The changes in heart rate and blood pressure during sleep are almost certainly in harmony with changes in deep body temperature, respiratory rate, cortisol secretion and that of other biomeasures, all orchestrated by biological clock in the

suprachiasmatic nuclei and mediated by melatonin (Petersen, Pratt et al. 2001, Lodemore, Petersen et al. 1991, Tappin, Ford et al. 1996).

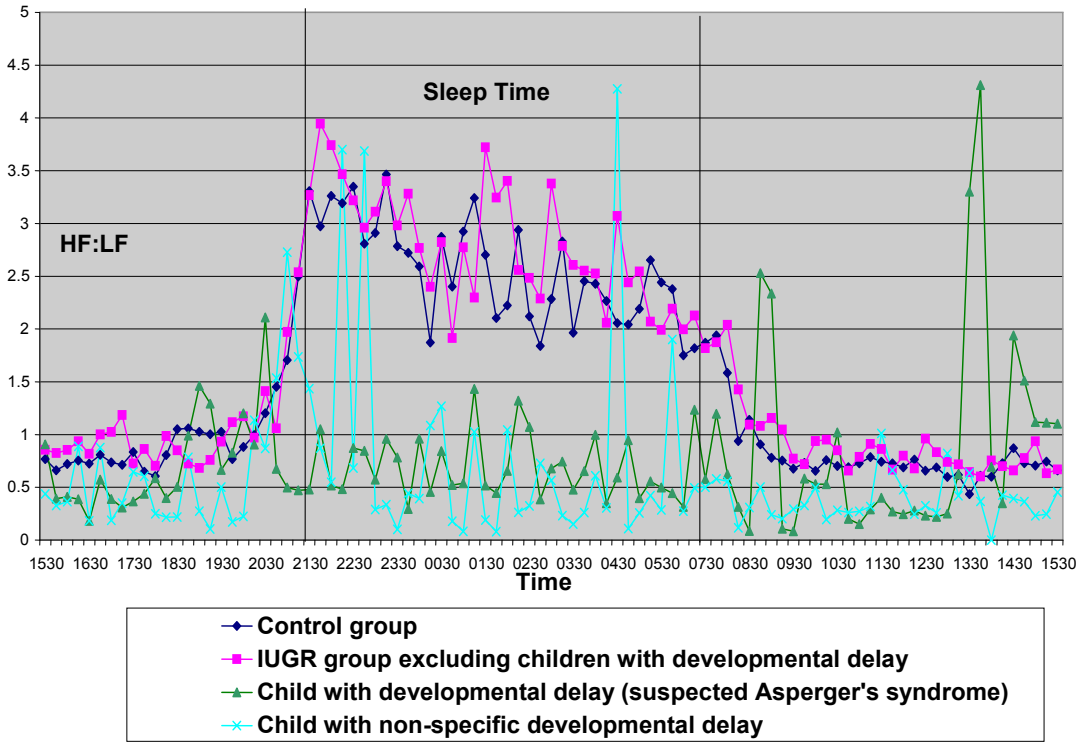
The similarities between normal nine year olds and those children, who had suffered from intra uterine growth restriction, are in contrast with the difference in the physiological status that occurred when these children were infants (Jackson, Wailoo et al. 2004). Then the IUGR children were clearly delayed physiologically. However by the age of nine years the IUGR children are physiologically indistinguishable from the normals, although they remain smaller and shorter than their normal counterparts. (Chakraborty, Joseph et al. 2007)The absence of data does not allow us to ascertain at what age physiological catch up occurs in growth restricted infants, although there are suggestions that it occurs in late infancy (Finley, Nugent 1995, Massin, Maeyns et al. 2000, Massin, von Bernuth 1997, Silvetti, Drago et al. 2001b), albeit on sparse data.

The impact of sleep, presumably melatonin mediated, is to slow the heart rate and lower the blood pressure while the heart rate variability increases to peak prior to waking, coinciding with the period of increased sympathetic activity when acute cardiovascular events are commoner (Muller, Tofler et al. 1989) and most sudden infant deaths are discovered (Blair, Platt et al. 2006).

For children in both groups HF/LF is much higher at night compared to the daytime suggesting a significant parasympathetic dominance on the heart during sleep.

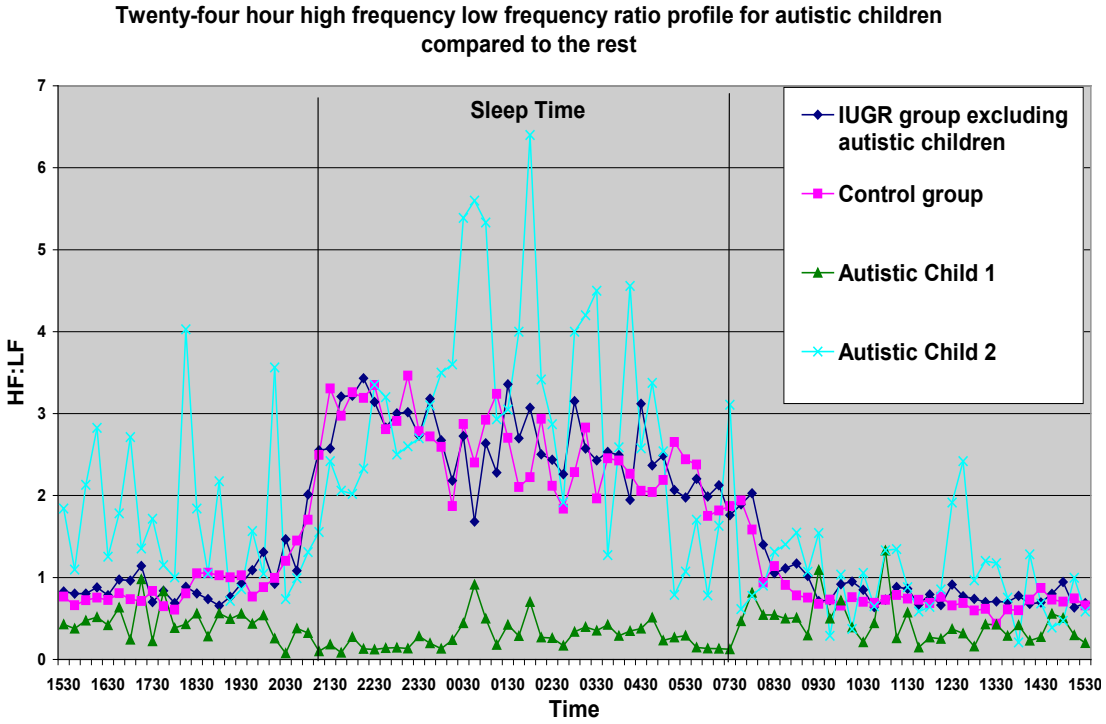
Within the growth restricted group, some children with neurological deficits had lower blood pressure and pulse rate during sleep but had reduced cardiac parasympathetic activity during sleep (**Figure 18**).

Figure 18: **Diurnal HF: LF profile in children with Neurodevelopmental disorders**



This may be an indication of dysfunction of the biological clock and could explain the erratic sleep pattern and behaviour of these children. Of particular interest are the few children with Autistic Spectrum disorder where one child shows an absence of any day/night variation in the sympatho-vagal balance, while at best the others are erratic and probably unstable (**Figure 19**). By contrast children with well controlled asthma showed a normal 24 pattern of cardiovascular change including sympatho-vagal balance. Similar findings suggesting ANS imbalance in Autistic children have been reported by other researches (Ming, Julu et al. 2005, Tordjman, Anderson et al. 2005). Further studies are needed with larger number of autistic children for better understanding of autonomic arousal, mechanisms underlying the lower melatonin production and exploration of therapeutic interventions using ANS manipulation.

Figure 19: HF:LF profile for children with Autism



By every criterion, children aged nine, whether normal or growth restricted in utero are similar physiologically during sleep and wakefulness. By this age the well established 24 hour biorhythm by which all creatures exist is integrated functionally by melatonin in relation to sleep.

9.3 Exploration of the relationship between IUGR, compensatory growth, current body size and testing of "Foetal Origins Hypothesis"

The use of continuous ambulatory monitoring enabled me to demonstrate the full nature of physiological circadian blood pressure changes, which would not have been possible with one-off measurements. My analysis also takes into account an extensive range of potentially confounding variables and utilises an expanded form of the regression analysis with four models as suggested by Lucas et al (Lucas, Fewtrell et al. 1999). Although the Foetal Origins Hypothesis relates to morbidity in adulthood but there have been reports to suggest metabolic and cardiovascular changes related to the

hypothesis in children as young as eight years (Adair 1999, Bavdekar, Yajnik et al. 1999). The expectation based on Barker's theory (FOH) that blood pressure in IUGR children might be raised relative to controls is not confirmed by our findings. Later weight was usually the better predictor of later blood pressure and all of the interactions between early and later weights were non-significant. Substituting birth weight with IUGR also failed to provide any support for the Foetal Origins Hypothesis. Surprisingly the association between current body size and systolic blood pressure is significantly positive only in the normal children within the study population. This, in combination with our finding that ambulatory blood pressure at the age of 9 years is not influenced by the birth weight or IUGR; suggest that postnatal and therefore more remediable factors are more significant than intrauterine events. This is consistent with several other studies suggesting postnatal change in size as the major determinant of subsequent blood pressure and there is insufficient evidence to recommend prevention of adult diseases through strategies to alter infant growth (Huxley, Neil et al. 2002, Fisher, Baird et al. 2006, Stein, Bosner et al. 1994, Falkner, Hulman et al. 2004, Williams, Poulton 2002). Rahiala et al found no difference in similar ambulatory blood pressure values between growth restricted and normal babies at 12 years of age until they adjusted for their current body size (Rahiala, Tenhola et al. 2002b). In our study, adjustment for similar variables, as well as change in weight z score over time, shows that the control group have a significantly higher systolic blood pressure than the IUGR group.

A recent study of Danish children has demonstrated that the association between childhood weight between the age of 7 and 13 and coronary heart disease in adulthood is linear, and even a small increase in BMI at the age of 7 years elevates the risk of coronary heart disease in adulthood (Baker, Olsen et al. 2007). Our findings

concerning the association of body size with raised ambulatory systolic blood pressure as early as age 9 may well signify that there is an association between early events and cardiovascular disease and obesity in adults - a scourge of overeating and lack of physical activities in affluent Western countries. The public health implications of these findings are clear and highlight the need for early lifestyle modification to prevent an epidemic of obesity and heart disease.

The possible role of cortisol in the causation of high blood pressure is given only slight credence. A recent meta-analysis looking into the association between cortisol and birth weight reported that although an inverse association between birth weight and circulating cortisol was detected, the majority of the studies were underpowered and the strength of the overall association was weak (van Montfoort, Finken et al. 2005). Although we carried out few significance tests and did not adjust for Type 1 Errors, it appears from our study that cortisol production is linked to variability of systolic blood pressure and diastolic blood pressure and may be an indication of a potential causative pathway. During infancy the night-times cortisol in our study population remained elevated in the growth restricted group compared to the control infants and could have been a potential hypertensive source. However, at the age of 9 years, the night-time cortisol is no longer elevated in the IUGR group. Whether cortisol contributes to raised blood pressure in childhood is debatable but is unlikely to be a continuation of the pattern seen in infancy.

9.4 Regression analysis of the HRV parameters

The Autonomic Nervous system goes through maturational changes and it has been shown that IUGR infants are delayed in ANS maturation compared to healthy infants (Jackson, Wailoo et al. 2004, Jackson, Wailoo et al. 2001). It has also been suggested

that IUGR infants retain the high Sympathetic dominance on the heart (Galland, Taylor et al. 2006), which is reported to have a detrimental effect on cardiovascular health and may be related to the long term metabolic and cardiovascular disturbances that are reported to be commoner in this population. The objective of this study was to quantify autonomic tone in children with IUGR through different measures of HRV. There was no evidence of persisting sympathetic dominance in the IUGR group and after regression analysis I did not find any differences between the two groups in relation to all the HRV parameters. The dominant predictor for several HRV parameters was the paternal height but its significance is unclear at present. Therefore my findings fail to support an encoding influence on autonomic control early in life, which could link with an association between foetal growth restriction and a greater risk of cardiovascular morbidity at the age of nine years. Further longitudinal studies are needed to determine the exact nature of this relationship.

References

ABUZZAHAB, M.J., SCHNEIDER, A., GODDARD, A., GRIGORESCU, F., LAUTIER, C., KELLER, E., KIESS, W., KLAMMT, J., KRATZSCH, J., OSGOOD, D., PFAFFLE, R., RAILE, K., SEIDEL, B., SMITH, R.J., CHERNAUSEK, S.D. and INTRAUTERINE GROWTH RETARDATION (IUGR) STUDY GROUP., 2003. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *The New England journal of medicine,* **349**(23), pp. 2211-2222.

ADAIR, L.S., 1999. Filipino children exhibit catch-Up growth from age 2 to 12 years. *The Journal of nutrition,* **129**(6), pp. 1140-1148.

ALBERTSSON-WIKLAND, K. and KARLBERG, J., 1994. Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatrica (Oslo, Norway : 1992). Supplement,* **399**, pp. 64-70; discussion 71.

ANSON-CARTWRIGHT, L., DAWSON, K., HOLMYARD, D., FISHER, S.J., LAZZARINI, R.A. and CROSS, J.C., 2000. The glial cells missing-1 protein is essential for branching morphogenesis in the chorioallantoic placenta. *Nature genetics,* **25**(3), pp. 311-314.

ANTILA, K.J., VALIMAKI, I.A., MAKELA, M., TUOMINEN, J., WILSON, A.J. and SOUTHALL, D.P., 1990. Heart rate variability in infants subsequently suffering sudden infant death syndrome (SIDS). *Early human development,* **22**(2), pp. 57-72.

ASHWORTH, A. and MILLWARD, D.J., 1986. Catch-up growth in children. *Nutrition reviews,* **44**(5), pp. 157-163.

BAKER, J., LIU, J.P., ROBERTSON, E.J. and EFSTRATIADIS, A., 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell,* **75**(1), pp. 73-82.

BAKER, J.L., OLSEN, L.W. and SORENSEN, T.I., 2007. Childhood body-mass index and the risk of coronary heart disease in adulthood. *The New England journal of medicine,* **357**(23), pp. 2329-2337.

BARKER, D.J., 1995. Fetal origins of coronary heart disease. *BMJ (Clinical research ed.),* **311**(6998), pp. 171-174.

BARKER, D.J. and OSMOND, C., 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet,* **1**(8489), pp. 1077-1081.

BARKER, D.J.P., 2003. The developmental origins of adult disease. *European Journal of Epidemiology,* **18**(8), pp. 733-736.

BARKER, D.J.P., 1999. Early growth and cardiovascular disease. *Archives of Disease in Childhood,* **80**(4), pp. 305-306.

BARKER, D.J.P., 1994. Mothers, Babies and disease in later life. First edn. London: B.M.J. Publishing.

BARROS, F.C., HUTTLY, S.R., VICTORA, C.G., KIRKWOOD, B.R. and VAUGHAN, J.P., 1992. Comparison of the causes and consequences of prematurity and intrauterine growth retardation: a longitudinal study in southern Brazil. *Pediatrics,* **90**(2 Pt 1), pp. 238-244.

BATTEN, L.A., URBINA, E.M. and BERENSON, G.S., 2000. Interobserver reproducibility of heart rate variability in children (the Bogalusa Heart Study). *The American Journal of Cardiology,* **86**(11), pp. 1264-6, A9.

BAVDEKAR, A., YAJNIK, C.S., FALL, C.H., BAPAT, S., PANDIT, A.N., DESHPANDE, V., BHAVE, S., KELLINGRAY, S.D. and JOGLEKAR, C., 1999. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? *Diabetes,* **48**(12), pp. 2422-2429.

BERNSTEIN, I.M., HORBAR, J.D., BADGER, G.J., OHLSSON, A. and GOLAN, A., 2000. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. *American Journal of Obstetrics and Gynecology,* **182**(1 Pt 1), pp. 198-206.

BHATT, A.B., TANK, P.D., BARMADE, K.B. and DAMANIA, K.R., 2002. Abnormal Doppler flow velocimetry in the growth restricted foetus as a predictor for necrotising enterocolitis. *Journal of postgraduate medicine,* **48**(3), pp. 182-5; discussion 185.

BLAIR, E. and STANLEY, F., 1990. Intrauterine growth and spastic cerebral palsy. I. Association with birth weight for gestational age. *American Journal of Obstetrics and Gynecology,* **162**(1), pp. 229-237.

BLAIR, P.S., PLATT, M.W., SMITH, I.J., FLEMING, P.J. and SESDI SUDI RESEARCH GROUP, 2006. Sudden Infant Death Syndrome and the time of death: factors associated with night-time and day-time deaths. *International journal of epidemiology,* **35**(6), pp. 1563-1569.

BONNEMEIER, H., RICHARDT, G., POTRATZ, J., WIEGAND, U.K., BRANDES, A., KLUGE, N. and KATUS, H.A., 2003. Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. *Journal of cardiovascular electrophysiology,* **14**(8), pp. 791-799.

BRODSKY, D. and CHRISTOU, H., 2004. Current concepts in intrauterine growth restriction. *Journal of intensive care medicine,* **19**(6), pp. 307-319.

BROWN, H.L., MILLER, J.M.,JR, GABERT, H.A. and KISSLING, G., 1987. Ultrasonic recognition of the small-for-gestational-age fetus. *Obstetrics and gynecology,* **69**(4), pp. 631-635.

CAMERON, N., PREECE, M.A. and COLE, T.J., 2005. Catch-up growth or regression to the mean? Recovery from stunting revisited. *American Journal of Human Biology,* **17**(4), pp. 412-417.

CHAKRABORTY, S., JOSEPH, D.V., BANKART, M.J., PETERSEN, S.A. and WAILOO, M.P., 2007. Fetal growth restriction: relation to growth and obesity at the age of 9 years. *Archives of disease in childhood.Fetal and neonatal edition,* **92**(6), pp. F479-83.

CHINN, S. and RONA, R.J., 2001. Prevalence and trends in overweight and obesity in three cross sectional studies of British Children, 1974-94. *BMJ (Clinical research ed.),* **322**(7277), pp. 24-26.

CHRISTOU, H., SERDY, S. and MANTZOROS, C.S., 2002. Leptin in relation to growth and developmental processes in the fetus. *Seminars in reproductive medicine,* **20**(2), pp. 123-130.

COOKE, R.W., 1998. Smoking, intra-uterine growth retardation and sudden infant death syndrome. *International journal of epidemiology,* **27**(2), pp. 238-241.

CURZI-DASCALOVA, L., 1995. Development of the sleep and autonomic nervous system control in premature and full-term newborn infants. *Archives de Pediatrie : Organe Officiel de la Societe Francaise de Pediatrie,* **2**(3), pp. 255-262.

CURZI-DASCALOVA, L., PEIRANO, P. and CHRISTOVA, E., 1996. Respiratory characteristics during sleep in healthy small-for-gestational age newborns. *Pediatrics,* **97**(4), pp. 554-559.

DE ONIS, M., BLOSSNER, M. and VILLAR, J., 1998. Levels and patterns of intrauterine growth retardation in developing countries. *European journal of clinical nutrition,* **52 Suppl 1**, pp. S5-15.

DE ROGALSKI LANDROT, I., ROCHE, F., PICHOT, V., TEYSSIER, G., GASPOZ, J.M., BARTHELEMY, J.C. and PATURAL, H., 2007. Autonomic nervous system activity in premature and full-term infants from theoretical term to 7 years. *Autonomic Neuroscience: Basic & Clinical,* **136**(1-2), pp. 105-109.

DE WAAL, W.J., HOKKEN-KOELEGA, A.C., STIJNEN, T., DE MUINCK KEIZER-SCHRAMA, S.M. and DROP, S.L., 1994. Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. *Clinical endocrinology,* **41**(5), pp. 621-630.

DE ZEGHER, F., MAES, M., GARGOSKY, S.E., HEINRICHS, C., DU CAJU, M.V., THIRY, G., DE SCHEPPER, J., CRAEN, M., BREYSEM, L., LOFSTROM, A., JONSSON, P., BOURGUIGNON, J.P., MALVAUX, P. and ROSENFELD, R.G., 1996. High-dose growth hormone treatment of short children born small for gestational age. *The Journal of clinical endocrinology and metabolism,* **81**(5), pp. 1887-1892.

DUNN, P.M., 1985. The search for perinatal definitions and standards. *Acta Paediatrica Scandinavica. Supplement,* **319**, pp. 7-16.

ECKBERG, D.L., 1997. Sympathovagal balance: a critical appraisal. *Circulation,* **96**(9), pp. 3224-3232.

ERIKSSON, J.G., FORSEN, T., TUOMILEHTO, J., OSMOND, C. and BARKER, D.J., 2001. Early growth and coronary heart disease in later life: longitudinal study. *BMJ (Clinical research ed.),* **322**(7292), pp. 949-953.

FALKNER, B., HULMAN, S. and KUSHNER, H., 2004. Effect of birth weight on blood pressure and body size in early adolescence. *Hypertension,* **43**(2), pp. 203-207.

FINLEY, J.P. and NUGENT, S.T., 1995. Heart rate variability in infants, children and young adults. *Journal of the Autonomic Nervous System,* **51**(2), pp. 103-108.

FISHER, D., BAIRD, J., PAYNE, L., LUCAS, P., KLEIJNEN, J., ROBERTS, H. and LAW, C., 2006. Are infant size and growth related to burden of disease in adulthood? A systematic review of literature. *International journal of epidemiology,* **35**(5), pp. 1196-1210.

FITZHARDINGE, P.M. and INWOOD, S., 1989. Long-term growth in small-for-date children. *Acta Paediatrica Scandinavica. Supplement,* **349**, pp. 27-33; discussion 34.

FOWDEN, A.L., 2003. The insulin-like growth factors and feto-placental growth. *Placenta,* **24**(8-9), pp. 803-812.

GALEEV, A.R., IGISHEVA, L.N. and KAZIN, E.M., 2002. Heart rate variability in healthy six- to sixteen year old children. *Fiziologiia cheloveka,* **28**(4), pp. 54-58.

GALLAND, B.C., TAYLOR, B.J., BOLTON, D.P. and SAYERS, R.M., 2006. Heart rate variability and cardiac reflexes in small for gestational age infants. *Journal of applied physiology (Bethesda, Md.: 1985),* **100**(3), pp. 933-939.

GIUDICE, L.C., DE ZEGHER, F., GARGOSKY, S.E., DSUPIN, B.A., DE LAS FUENTES, L., CRYSTAL, R.A., HINTZ, R.L. and ROSENFELD, R.G., 1995. Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *The Journal of clinical endocrinology and metabolism,* **80**(5), pp. 1548-1555.

GOLDBERGER, A.L., 1996. Non-linear dynamics for clinicians: chaos theory, fractals, and complexity at the bedside. *Lancet,* **347**(9011), pp. 1312-1314.

GOLDENBERG, R., HACK, M., GRANTHAM-MCGREGOR and SCHURCH, B., 1998. Report of the IDECG/IUNS Working Group on IUGR effects on neurological, sensory, cognitive, and behavioral function. *European journal of clinical nutrition,* **52 Suppl 1**, pp. S100-1.

GOTO, M., NAGASHIMA, M., BABA, R., NAGANO, Y., YOKOTA, M., NISHIBATA, K. and TSUJI, A., 1997. Analysis of heart rate variability demonstrates effects of development on vagal modulation of heart rate in healthy children. *The Journal of pediatrics,* **130**(5), pp. 725-729.

GUO, Y.F. and STEIN, P.K., 2003. Circadian rhythm in the cardiovascular system: chronocardiology. *American Heart Journal,* **145**(5), pp. 779-786.

HADLOCK, F.P., 1983. The cephalic index as a predictor of impending fetal demise. *Radiology,* **149**(1), pp. 325-326.

HADLOCK, F.P., HARRIST, R.B. and MARTINEZ-POYER, J., 1991. In utero analysis of fetal growth: a sonographic weight standard. *Radiology,* **181**(1), pp. 129-133.

HALES, C.N. and OZANNE, S.E., 2003. The dangerous road of catch-up growth. *The Journal of physiology,* **547**(Pt 1), pp. 5-10.

HARSHFIELD, G.A., ALPERT, B.S., PULLIAM, D.A., SOMES, G.W. and WILSON, D.K., 1994. Ambulatory blood pressure recordings in children and adolescents. *Pediatrics,* **94**(2 Pt 1), pp. 180-184.

HARSHFIELD, G.A. and TREIBER, F.A., 1999. Racial differences in ambulatory blood pressure monitoring-derived 24 h patterns of blood pressure in adolescents. *Blood pressure monitoring,* **4**(3-4), pp. 107-110.

HATTERSLEY, A.T. and TOOKE, J.E., 1999. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet,* **353**(9166), pp. 1789-1792.

HAWORTH, J.C., ELLESTAD-SAYED, J.J., KING, J. and DILLING, L.A., 1980. Fetal growth retardation in cigarette-smoking mothers is not due to decreased maternal food intake. *American Journal of Obstetrics and Gynecology,* **137**(6), pp. 719-723.

HEDIGER, M.L., OVERPECK, M.D., MAURER, K.R., KUCZMARSKI, R.J., MCGLYNN, A. and DAVIS, W.W., 1998. Growth of infants and young children born small or large for gestational age: findings from the Third National Health and Nutrition Examination Survey. *Archives of Pediatrics & Adolescent Medicine,* **152**(12), pp. 1225-1231.

HUXLEY, R., NEIL, A. and COLLINS, R., 2002. Unravelling the fetal origins hypothesis: Is there really an inverse association between birthweight and subsequent blood pressure? *Lancet,* **360**(9334), pp. 659-665.

JACKSON, J.A., WAILOO, M.P., PETERSEN, S.A., THOMPSON, J.R. and DAVIES, T., 2001. Changes in body temperature and urinary cortisol after routine immunization in babies with intrauterine growth retardation. *Acta Paediatrica (Oslo, Norway : 1992),* **90**(10), pp. 1186-1189.

JACKSON, J.A., WAILOO, M.P., THOMPSON, J.R. and PETERSEN, S.A., 2004. Early physiological development of infants with intrauterine growth retardation. *Archives of Disease in Childhood. Fetal and Neonatal Edition,* **89**(1), pp. F46-50.

JACOBY, A.C., FIXLER, D.E. and TORRES, E.J., 1993. Limitations of an oscillometric ambulatory blood pressure monitor in physically active children. *The Journal of pediatrics,* **122**(2), pp. 231-236.

JOSEPH, K.S. and KRAMER, M.S., 1996. Review of the evidence on fetal and early childhood antecedents of adult chronic disease. *Epidemiologic Reviews,* **18**(2), pp. 158-174.

KAZUMA, N., OTSUKA, K., WAKAMATSU, K., SHIRASE, E. and MATSUOKA, I., 2002. Heart rate variability in normotensive healthy children with aging. *Clinical and experimental hypertension,* **24**(1-2), pp. 83-89.

KINGDOM, J., HUPPERTZ, B., SEAWARD, G. and KAUFMANN, P., 2000. Development of the placental villous tree and its consequences for fetal growth. *European journal of obstetrics, gynecology, and reproductive biology,* **92**(1), pp. 35-43.

KORPELAINEN, J.T., SOTANIEMI, K.A., HUIKURI, H.V. and MYLLYLA, V.V., 1997. Circadian rhythm of heart rate variability is reversibly abolished in ischemic stroke. *Stroke; a journal of cerebral circulation,* **28**(11), pp. 2150-2154.

KRAMER, M.S., 2000. Invited commentary: Association between restricted fetal growth and adult chronic disease: Is it causal? Is it important? *American Journal of Epidemiology,* **152**(7), pp. 605-608.

LEE, P.A., CHERNAUSEK, S.D., HOKKEN-KOELEGA, A.C., CZERNICHOW, P. and INTERNATIONAL SMALL FOR GESTATIONAL AGE ADVISORY BOARD., 2003. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics,* **111**(6 Pt 1), pp. 1253-1261.

LEGER, J., LIMONI, C. and CZERNICHOW, P., 1997. Prediction of the outcome of growth at 2 years of age in neonates with intra-uterine growth retardation. *Early human development,* **48**(3), pp. 211-223.

LEVINE BS, 2000. Nursing management: hypertension. In: H.M. LEWIS SM DIRKSEN SR, ed, *Medical-Surgical nursing: Assessment and management of Clinical Problems.* 5th edn. St. Louis,Mo: mosby, pp. 817-840.

LI, H., BARNHART, H.X., STEIN, A.D. and MARTORELL, R., 2003. Effects of early childhood supplementation on the educational achievement of women. *Pediatrics,* **112**(5), pp. 1156-1162.

LI, H., STEIN, A.D., BARNHART, H.X., RAMAKRISHNAN, U. and MARTORELL, R., 2003. Associations between prenatal and postnatal growth and adult body size and composition. *The American Journal of Clinical Nutrition,* **77**(6), pp. 1498-1505.

LIN, C.C. and SANTOLAYA-FORGAS, J., 1998. Current concepts of fetal growth restriction: part I. Causes, classification, and pathophysiology. *Obstetrics and gynecology,* **92**(6), pp. 1044-1055.

LODEMORE, M., PETERSEN, S.A. and WAILOO, M.P., 1991. Development of night time temperature rhythms over the first six months of life. *Archives of Disease in Childhood,* **66**(4), pp. 521-524.

LOMBARDI, F., 2000. Chaos theory, heart rate variability, and arrhythmic mortality. *Circulation,* **101**(1), pp. 8-10.

LUCAS, A., FEWTRELL, M.S. and COLE, T.J., 1999. Fetal origins of adult disease - The hypothesis revisited. *British Medical Journal,* **319**(7204), pp. 245-249.

LURBE, E., CREMADES, B., RODRIGUEZ, C., TORRO, M.I., ALVAREZ, V. and REDON, J., 1999. Factors related to quality of ambulatory blood pressure monitoring in a pediatric population. *American Journal of Hypertension : Journal of the American Society of Hypertension,* **12**(9 Pt 1), pp. 929-933.

LURBE, E. and REDON, J., 2000. Ambulatory blood pressure monitoring in children and adolescents: the future. *Journal of hypertension,* **18**(10), pp. 1351-1354.

LURBE, E., TORRO, I., RODRIGUEZ, C., ALVAREZ, V. and REDON, J., 2001. Birth weight influences blood pressure values and variability in children and adolescents. *Hypertension,* **38**(3), pp. 389-393.

MALIK, M., 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation,* **93**(5), pp. 1043-1065.

MALLIANI, A., LOMBARDI, F. and PAGANI, M., 1994. Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. *British heart journal,* **71**(1), pp. 1-2.

MARTIN, C.R., VAN MARTER, L.J., ALLRED, E.N. and LEVITON, A., 2001. Growth-restricted premature infants are at increased risk for low thyroxine. *Early human development,* **64**(2), pp. 119-128.

MARTINELLI, P., GRANDONE, E., COLAIZZO, D., PALADINI, D., SCIANNAME, N., MARGAGLIONE, M. and DI MINNO, G., 2001. Familial thrombophilia and the occurrence of fetal growth restriction. *Haematologica,* **86**(4), pp. 428-431.

MARTORELL, R., KETTEL KHAN, L., HUGHES, M.L. and GRUMMER-STRAWN, L.M., 2000. Overweight and obesity in preschool children from developing countries. *International journal of obesity and related metabolic*

disorders : journal of the International Association for the Study of Obesity, **24**(8), pp. 959-967.

MARTORELL, R., KHAN, L.K. and SCHROEDER, D.G., 1994. Reversibility of stunting: epidemiological findings in children from developing countries. *European journal of clinical nutrition,* **48 Suppl 1**, pp. S45-57.

MARTORELL, R., RAMAKRISHNAN, U., SCHROEDER, D.G., MELGAR, P. and NEUFELD, L., 1998. Intrauterine growth retardation, body size, body composition and physical performance in adolescence. *European journal of clinical nutrition,* **52 Suppl 1**, pp. S43-52; discussion S52-3.

MARTORELL, R., STEIN, A.D. and SCHROEDER, D.G., 2001. Early nutrition and later adiposity. *The Journal of nutrition,* **131**(3), pp. 874S-880S.

MASSIN, M. and VON BERNUTH, G., 1997. Normal ranges of heart rate variability during infancy and childhood. *Pediatric cardiology,* **18**(4), pp. 297-302.

MASSIN, M.M., MAEYNS, K., WITHOFS, N., RAVET, F. and GERARD, P., 2000. Circadian rhythm of heart rate and heart rate variability. *Archives of Disease in Childhood,* **83**(2), pp. 179-182.

MCCORMICK, M.C., 1985. The contribution of low birth weight to infant mortality and childhood morbidity. *The New England journal of medicine,* **312**(2), pp. 82-90.

METGES, C.C., 2001. Does dietary protein in early life affect the development of adiposity in mammals? *The Journal of nutrition,* **131**(7), pp. 2062-2066.

MING, X., JULU, P.O., BRIMACOMBE, M., CONNOR, S. and DANIELS, M.L., 2005. Reduced cardiac parasympathetic activity in children with autism. *Brain & development,* **27**(7), pp. 509-516.

MULLER, J.E., TOFLER, G.H. and STONE, P.H., 1989. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation,* **79**(4), pp. 733-743.

NATIONAL HIGH BLOOD PRESSURE EDUCATION PROGRAM WORKING GROUP ON HYPERTENSION CONTROL IN CHILDREN AND ADOLESCENTS, 1996. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. *Pediatrics,* **98**(4), pp. 649-658.

NICHOLSON, W.R., MATTHEWS, J.N., O'SULLIVAN, J.J. and WREN, C., 1993. Ambulatory blood pressure monitoring. *Archives of Disease in Childhood,* **69**(6), pp. 681-684.

O'BRIEN E, PICKERING T, ASMAR R, ET AL, 2002. Working Group on Blood Pressure Monitoring of the European Society of HypertensionInternational Protocol for validation of bloodpressure measuring devices in adults. *Blood Pressure monitoring,* **7**, pp. 3-17.

O'BRIEN E, ASMAR R, BEILIN L, IMAI Y, MALLION JM, MANCIA G, MENGDEN T, MYERS M, PADFIELD P, PALATINI P, PARATI G, PICKERING T, REDON J, STAESSEN J, STERGIOU G, VERDECCHIA P and EUROPEAN SOCIETY OF HYPERTENSION WORKING GROUP ON BLOOD PRESSURE MONITORING., May 2003. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *Journal of Hypertension 2003,* **21**(5), pp. 821-848.

O'BRIEN, E., WAEBER, B., PARATI, G., STAESSEN, J. and MYERS, M.G., 2001. Blood pressure measuring devices: recommendations of the European Society of Hypertension. *BMJ (Clinical research ed.),* **322**(7285), pp. 531-536.

OGDEN, C.L., FLEGAL, K.M., CARROLL, M.D. and JOHNSON, C.L., 2002. Prevalence and trends in overweight among US children and adolescents, 1999-2000. *JAMA : the journal of the American Medical Association,* **288**(14), pp. 1728-1732.

O'KEEFFE, M.J., O'CALLAGHAN, M., WILLIAMS, G.M., NAJMAN, J.M. and BOR, W., 2003. Learning, cognitive, and attentional problems in adolescents born small for gestational age. *Pediatrics,* **112**(2), pp. 301-307.

PANETH, N. and SUSSER, M., 1995. Early origin of coronary heart disease (the 'Barker hypothesis'). Hypotheses, no matter how intriguing, need rigorous attempts at refutation. *British Medical Journal,* **310**(6977), pp. 411-412.

PAZ, I., LAOR, A., GALE, R., HARLAP, S., STEVENSON, D.K. and SEIDMAN, D.S., 2001. Term infants with fetal growth restriction are not at increased risk for low intelligence scores at age 17 years. *The Journal of pediatrics,* **138**(1), pp. 87-91.

PEARCE, M.S. and O'SULLIVAN, J.J., 2003. Relationship between birth weight and blood pressure variability in children. *Journal of human hypertension,* **17**(10), pp. 677-680.

PELEG, D., KENNEDY, C.M. and HUNTER, S.K., 1998. Intrauterine growth restriction: identification and management. *American Family Physician,* **58**(2), pp. 453-60, 466-7.

PETERSEN, S.A., PRATT, C. and WAILOO, M.P., 2001. Relations between the development of patterns of sleeping heart rate and body temperature in infants. *Archives of disease in childhood.Fetal and neonatal edition,* **85**(2), pp. F133-6.

PIVIK, R.T., BUSBY, K.A., GILL, E., HUNTER, P. and NEVINS, R., 1996. Heart rate variations during sleep in preadolescents. *Sleep,* **19**(2), pp. 117-135.

POLLACK, R.N. and DIVON, M.Y., 1992. Intrauterine growth retardation: definition, classification, and etiology. *Clinical obstetrics and gynecology,* **35**(1), pp. 99-107.

PORTMAN, R.J. and YETMAN, R.J., 1994. Clinical uses of ambulatory blood pressure monitoring. *Pediatric nephrology (Berlin, Germany),* **8**(3), pp. 367-376.

PRYOR, J., SILVA, P.A. and BROOKE, M., 1995. Growth, development and behaviour in adolescents born small-for-gestational-age. *Journal of paediatrics and child health,* **31**(5), pp. 403-407.

RAHIALA, E., TENHOLA, S., VANNINEN, E., HERRGARD, E., TIKANOJA, T. and MARTIKAINEN, A., 2002a. Ambulatory blood pressure in 12-year-old children born small for gestational age. *Hypertension,* **39**(4), pp. 909-913.

READ, M.S., CATZ, C., GRAVE, G., MCNELLIS, D. and WARSHAW, J.B., 1984. Introduction: Intrauterine growth retardation--identification of research needs and goals. *Seminars in perinatology,* **8**(1), pp. 2-4.

REKAWEK, J., MISZCZAK-KNECHT, M., KAWALEC, W. and MIELNICZUK, J., 2003. Heart rate variability in healthy children. *Folia Cardiologica,* **10**, pp. 203-211.

RESNIK, R., 2002. Intrauterine growth restriction. *Obstetrics and gynecology,* **99**(3), pp. 490-496.

ROSEBOOM, T.J., VAN DER MEULEN, J.H., RAVELLI, A.C., OSMOND, C., BARKER, D.J. and BLEKER, O.P., 2001. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Molecular and cellular endocrinology,* **185**(1-2), pp. 93-98.

SCHERJON, S.A., OOSTING, H., SMOLDERS-DEHAAS, H., ZONDERVAN, H.A. and KOK, J.H., 1998. Neurodevelopmental outcome at three years of age after fetal 'brain-sparing'. *Early human development,* **52**(1), pp. 67-79.

SCHMIDT, G., MALIK, M., BARTHEL, P., SCHNEIDER, R., ULM, K., ROLNITZKY, L., CAMM, A.J., BIGGER, J.T.,JR and SCHOMIG, A., 1999. Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. *Lancet,* **353**(9162), pp. 1390-1396.

SHANNON, D.C., CARLEY, D.W. and BENSON, H., 1987. Aging of modulation of heart rate. *The American Journal of Physiology,* **253**(4 Pt 2), pp. H874-7.

SILVETTI, M.S., DRAGO, F. and RAGONESE, P., 2001a. Heart rate variability in healthy children and adolescents is partially related to age and gender. *International journal of cardiology,* **81**(2-3), pp. 169-174.

SILVETTI, M.S., DRAGO, F. and RAGONESE, P., 2001b. Heart rate variability in healthy children and adolescents is partially related to age and gender. *International journal of cardiology,* **81**(2-3), pp. 169-174.

SOERGEL, M., KIRSCHSTEIN, M., BUSCH, C., DANNE, T., GELLERMANN, J., HOLL, R., KRULL, F., REICHERT, H., REUSZ, G.S. and RASCHER, W., 1997. Oscillometric twenty-four-hour ambulatory blood pressure values in healthy children and adolescents: a multicenter trial including 1141 subjects. *The Journal of pediatrics,* **130**(2), pp. 178-184.

SOMMERFELT, K., ANDERSSON, H.W., SONNANDER, K., AHLSTEN, G., ELLERTSEN, B., MARKESTAD, T., JACOBSEN, G., HOFFMAN, H.J. and BAKKETEIG, L., 2000. Cognitive development of term small for gestational age children at five years of age. *Archives of Disease in Childhood,* **83**(1), pp. 25-30.

STEIN, P.K., BOSNER, M.S., KLEIGER, R.E. and CONGER, B.M., 1994. Heart rate variability: a measure of cardiac autonomic tone. *American Heart Journal,* **127**(5), pp. 1376-1381.

STEVEN G. GABBE,JENNIFER R. NIEBYL,JOE LEIGH SIMPSON,MIKKI SENKARIK, ed, 1996. ***Obstetrics**: **Normal** & **Problem Pregnancies***. 3rd edn. New York: Churchill Livingstone.

STRAUSS, R.S. and DIETZ, W.H., 1998. Growth and development of term children born with low birth weight: effects of genetic and environmental factors. *The Journal of pediatrics,* **133**(1), pp. 67-72.

STRAUSS, R.S. and DIETZ, W.H., 1997. Effects of intrauterine growth retardation in premature infants on early childhood growth. *The Journal of pediatrics,* **130**(1), pp. 95-102.

SZTAJZEL, J., 2004. Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss medical weekly : official journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine, the Swiss Society of Pneumology,* **134**(35-36), pp. 514-522.

TAPPIN, D.M., FORD, R.P., NELSON, K.P., PRICE, B., MACEY, P.M., DOVE, R., LARKIN, J. and SLADE, B., 1996. Breathing, sleep state, and rectal temperature oscillations. *Archives of Disease in Childhood,* **74**(5), pp. 427-431.

TOPP, M., LANGHOFF-ROOS, J., ULDALL, P. and KRISTENSEN, J., 1996. Intrauterine growth and gestational age in preterm infants with cerebral palsy. *Early human development,* **44**(1), pp. 27-36.

TORDJMAN, S., ANDERSON, G.M., PICHARD, N., CHARBUY, H. and TOUITOU, Y., 2005. Nocturnal excretion of 6-sulphatoxymelatonin in children and adolescents with autistic disorder. *Biological psychiatry,* **57**(2), pp. 134-138.

UVEBRANT, P. and HAGBERG, G., 1992. Intrauterine growth in children with cerebral palsy. *Acta Paediatrica (Oslo, Norway : 1992),* **81**(5), pp. 407-412.

VAN MONTFOORT, N., FINKEN, M.J., LE CESSIE, S., DEKKER, F.W. and WIT, J.M., 2005. Could cortisol explain the association between birth weight and cardiovascular disease in later life? A meta-analysis. *European journal of endocrinology / European Federation of Endocrine Societies,* **153**(6), pp. 811-817.

VAN RAVENSWAAIJ-ARTS, C.M., KOLLEE, L.A., HOPMAN, J.C., STOELINGA, G.B. and VAN GEIJN, H.P., 1993. Heart rate variability. *Annals of Internal Medicine,* **118**(6), pp. 436-447.

VANDENBOSCHE, R.C. and KIRCHNER, J.T., 1998. Intrauterine growth retardation. *American Family Physician,* **58**(6), pp. 1384-90, 1393-4.

VIK, T., VATTEN, L., MARKESTAD, T., AHLSTEN, G., JACOBSEN, G. and BAKKETEIG, L.S., 1996. Morbidity during the first year of life in small for gestational age infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition,* **75**(1), pp. F33-7.

VILLAR, J., SMERIGLIO, V., MARTORELL, R., BROWN, C.H. and KLEIN, R.E., 1984. Heterogeneous growth and mental development of intrauterine growth-retarded infants during the first 3 years of life. *Pediatrics,* **74**(5), pp. 783-791.

WALTHER, F.J., 1988. Growth and development of term disproportionate small-for-gestational age infants at the age of 7 years. *Early human development,* **18**(1), pp. 1-11.

WANG, H.S., LIM, J., ENGLISH, J., IRVINE, L. and CHARD, T., 1991. The concentration of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in human umbilical cord serum at delivery: relation to fetal weight. *The Journal of endocrinology,* **129**(3), pp. 459-464.

WANNAMETHEE, S.G., SHAPER, A.G., WHINCUP, P.H. and WALKER, M., 2002. Migration within Great Britain and cardiovascular disease: early life and adult environmental factors. *International journal of epidemiology,* **31**(5), pp. 1054-1060.

WHINCUP, P.H., COOK, D.G., ADSHEAD, F., TAYLOR, S.J., WALKER, M., PAPACOSTA, O. and ALBERTI, K.G., 1997. Childhood size is more strongly related than size at birth to glucose and insulin levels in 10-11-year-old children. *Diabetologia,* **40**(3), pp. 319-326.

WILKIN, T.J., METCALF, B.S., MURPHY, M.J., KIRKBY, J., JEFFERY, A.N. and VOSS, L.D., 2002. The relative contributions of birth weight, weight change, and current weight to insulin resistance in contemporary 5-year-olds: the EarlyBird Study. *Diabetes,* **51**(12), pp. 3468-3472.

WILLIAMS, S. and POULTON, R., 2002. Birth size, growth, and blood pressure between the ages of 7 and 26 years: failure to support the fetal origins hypothesis. *American Journal of Epidemiology,* **155**(9), pp. 849-852.

WOODS, K.A., CAMACHO-HUBNER, C., SAVAGE, M.O. and CLARK, A.J., 1996. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *The New England journal of medicine,* **335**(18), pp. 1363-1367.

WORLD HEALTH ORGANISATON, 1995. Maternal anthropometry and pregnancy outcomes. A WHO collaborative study. *Bull World Health Organ,* **73**, pp. 1-98.

YAMASAKI, Y., KODAMA, M., MATSUHISA, M., KISHIMOTO, M., OZAKI, H., TANI, A., UEDA, N., ISHIDA, Y. and KAMADA, T., 1996. Diurnal heart rate

variability in healthy subjects: effects of aging and sex difference. *The American Journal of Physiology,* **271**(1 Pt 2), pp. H303-10.

YETMAN, R.J. and PORTMAN, R.J., 1999. Technical aspects of blood pressure measurement in pediatric patients. *Blood pressure monitoring,* **4**(3-4), pp. 155-159.

ZAW, W., GAGNON, R. and DA SILVA, O., 2003. The risks of adverse neonatal outcome among preterm small for gestational age infants according to neonatal versus fetal growth standards. *Pediatrics,* **111**(6 Pt 1), pp. 1273-1277.

Publication and presentation from Thesis:

Foetal growth restriction: relation to growth and obesity at the age of 9 years. S. Chakraborty et al. Archive of Diseases in Childhood. Foetal and Neonatal Edition.2007; 92: F479-F483

Intrauterine growth restriction, compensatory growth and blood pressure: Annual Scientific meeting of British association of Community Child Health, Reading September 2007

Cardiovascular status in 10 year olds: Developmental Physiology conference. 21st June 2007 Leicester

Later growth of IUGR infants: Developmental Physiology conference. 15th June 2006 Leicester

IUGRs at 10 Years - cardiovascular status: Developmental Physiology conference. 16th June 2005 Leicester

Appendix A

Analysis of HRV profiles of four children with neurodevelopmental disorders

These four children belonged to the IUGR group. All of them showed a different pattern of the circadian rhythm of Heart Rate variability when measured in the form of HF:LF power spectral analysis, compared to the rest of the participants.

The first child suffered from unexplained developmental delay with moderate learning difficulty. Although she had a large parasympathetic dominance at the onset of sleep but that was quickly lost to be replaced by mostly sympathetic dominance throughout the night. She also had periods of marked parasympathetic dominance during morning.

The second child suffered from developmental delay and was being investigated for Autistic Spectrum Disorder. He had minimal parasympathetic dominance at the onset of sleep and mostly sympathetic dominance throughout the night. He also showed marked parasympathetic dominance during late afternoon.

The third child was diagnosed with Autistic Spectrum disorder and had mild learning difficulty. Throughout the whole twenty-four hour period his autonomic nervous system was mostly dominated by sympathetic nervous system and his sleep was reported to be very erratic.

The fourth child was diagnosed with Autistic spectrum disorder and moderate learning difficulty. His profile was totally opposite of the third child and showed persistent parasympathetic dominance throughout twenty four hours with very big fluctuations. The degree of parasympathetic dominance was different from what has been seen for the rest of the children.

These findings suggest that the functioning of ANS is different in children with Autism and other neurodevelopmental disorders and this may be linked to some of the clinical presentations.

Appendix B:

1. **Initial invitation letter to the parents**

2. **Participants' information leaflet**

3. **Participants' information leaflet (Child) with line drawings**

4. **Consent form**

5. **Letter to the participant's general practitioner**

6. **Line drawings for children**

7. **Data collection proforma**

Date: 29th July 2004

To: The parents of John Smith
39 AB ROAD
LEICESTER
LE1 2BB

Dear Parents,

Re: Invitation to take part in a research project.

We are doctors working for the University of Leicester, based at Leicester Royal Infirmary, and we are interested in learning more about how heart disease in adults may start during childhood. We are planning to make some simple measurements in children aged seven or eight years, including height, weight and blood pressure and to complete a simple questionnaire that tells us about the general health and activity of the children we see.

You may remember that when ……was a baby he/she helped us with a study of temperature changes that happen overnight. As a result of this we have some early information on your son/daughter and we are therefore keen to invite you and ……to take part.

We are writing to you now to ask if you and…….would be prepared to talk to a member of our team about the study, so that you could decide whether or not you are willing to take part. If you are happy to discuss this with us we will telephone you to make an appointment where we could give you all the information and answer any questions you may have. You would be under no obligation for..…….. to take part and you would have plenty of time to talk to other people before making up your mind. We would be very grateful if you could return the reply slip in the stamped and addressed envelope provided if you are interested. If you have any questions in the meantime, please do not hesitate to telephone me on 0116 252 3264.

Thank you for reading this letter.

Yours sincerely,

Dr. Mike Wailoo
Senior Lecturer in Child Health
University of Leicester
Robert Kilpatrick Building
Leicester Royal Infirmary

PARENT/GUARDIAN INFORMATION SHEET

Study Title: Study on Heart rate and Blood Pressure changes in seven-year old healthy children including some who were born with a low birth weight

Version 2 Date : MAY 2004

Principal Investigator: Dr Mike Wailoo
Senior Lecturer in Child Health
Leicester Royal Infirmary

Telephone Number: (0116) 2523264

We would like to invite your child to take part in a research study. Before you decide about your child's participation it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish for your child to take part.

What is the purpose of the study?

Heart disease and high blood pressure are two of the commonest causes of death in adults. It would be of great benefit to everyone if these diseases can be prevented. There is a view that the origins of these diseases lie in very early years of life. It has been suggested that children born with low birth weight are more prone to develop heart disease in adult life. With your help we aim to investigate this theory more in detail. We have observed in the past that babies with low birth weight tend to have a faster heart rate when compared to babies with normal birth weight. It is important to know whether this difference continues throughout the childhood.

Because heart rate and blood pressure are not constant they both change over short period of time. We plan to measure heart rate and blood pressure continuously over twenty-four hour period in a group of seven year old children with low birth weight and compare that to similar measurements in another group of seven year old children with normal birth weight. The techniques needed for these measurements are commonly used in hospital and are known to be painless and harmless. There will be no restriction on your child's lifestyle. We feel any significant differences between these groups may provide some clues about prevention of heart disease in adults.

In order to compare values between two groups we need to recruit fifty seven year old healthy children with normal birth weight and fifty seven year old healthy children with low birth weight

Why has my child been chosen?

You may recall that seven years ago your newborn child took part in a study on babies with low birth weight, conducted by Dr. Wailoo. Because your child had a low birth weight we would like to recruit him/her in this study.

What will happen to my child if she/he takes part?
It is up to you to decide whether or not for your child to take part. If you decide for your child to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide for your child to take part you are still free to withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives. If you consent to your child taking part in the study then the following steps will take place:

STEP 1. Your child will need to come to Leicester Royal Infirmary for a medical examination by one of the researchers. This will include medical history, examination and a few simple measurements like weight, height and head circumference. Only when the examiner is satisfied that your child is medically fit then we would proceed to the next step.

STEP 2. Your child will then undergo a standard Electrocardiogram (ECG) i.e. heart tracing.

STEP 3. If the ECG appears to be normal then two small pads (similar to the ones used for ECG) will be attached to the chest of your child and these will be connected to an ECG recording machine. This machine is not bigger than an average mobile phone and can be attached to the clothing or carried in the pockets. The time of beginning of the recording will be noted and your child will be allowed home to follow his/her normal activities without any restriction. This recording will be continued for twenty-four hours and after that we will visit you at home to take your child off the machine. We would request you to keep an approximate diary of his/her activities including the time spent in sleep during that period which will be handed over to the researcher.

STEP 4. We will provide you with two plastic pots to collect two urine samples from your child during the twenty-four hour period when he/she would be fitted with the ECG monitoring machine. One sample to be collected just before he/she is due to go to bed and the other one to be collected first thing in the morning before the breakfast. These will be collected from your home by the researchers.

STEP 5. After an interval of at least two days you need to bring your child once more to the hospital. A blood pressure cuff will be attached to the arm of your child and the cuff will be attached to a twenty-four hour blood pressure recording machine. This is a small lightweight portable machine and can be attached to the clothing. The machine will be programmed to record the blood pressure every twenty minutes from 0600hours to 2200 hours and every thirty minutes between 2200 hours and 0600 hours. During the recording of the blood pressure there will be a soft noise and your child will experience a sense of slight pressure on the arm. The time of beginning of recording will be noted and your child will be allowed home to follow his/her daily routine without any restriction. This recording will be continued for twenty-four hours and after that we will visit you at home to take your child off the machine. We would request you to keep an approximate diary of his/her activities including the time spent in sleep during that period which can be handed over to the researcher.

Please note that the researchers will visit you at home twice and you need to bring your child to the hospital twice to complete the research project.

What if something goes wrong?

We do not believe your child will be harmed by taking part in this research project, however if your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way your child has been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

What are the possible benefits for my child taking part?
There is no direct benefit to you or your child from taking part in this study. The information we get out of this study may help us to understand the origin of heart disease better in future.

Will my child taking part in this study be kept confidential?
All information, which is collected, about your child during the course of the research will be kept strictly confidential. Any information about your child will have his/her name and address removed so that your child cannot be recognised from it. Your own GP will be notified of your child's participation in the project as well as any relevant result or information.

We plan to finish the project within three years of starting. The result will be published in a medical journal. Your child will not be identified in any report or publication. We will provide you with a summary of the findings after the completion of the study.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the Committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information:

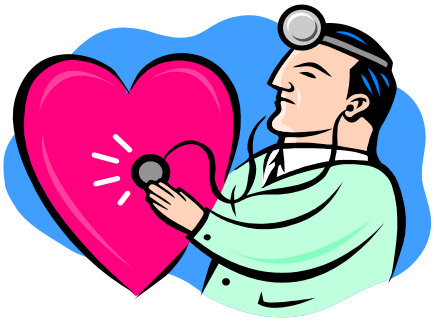
Dr. Mike Wailoo
Senior Lecturer in Child Health
Robert Kilpatrick Clinical
Sciences Building
University of Leicester.
PO Box 65
Leicester. LE2 7LX
Tel:01162523264
Fax:01162523282
E-mail: mw33@le.ac.uk

Dr. S. Chakraborty
Lecturer in Child Health
Robert Kilpatrick Clinical
Sciences Building
University of Leicester
PO Box 65
Leicester. LE2 7LX
Tel:01162525806 / 07714173050
Fax:01162523282
E-mail: sc154@le.ac.uk

If you decide to take part then you will be given a copy of the information sheet and a copy of the signed consent form to keep.

THANK YOU VERY MUCH FOR READING THE INFORMATION SHEET.

Information sheet for children



Would you help us to learn more about your heart?

We would like to ask you to help us with collecting some information about the way the heart works in children.

If you agree, we need to see you in the hospital one morning where a doctor will do a quick check up with your mum or dad present.

Then we will do a test called “heart tracing” which is simple, quick and painless. You will have a few sticky pads attached to your chest for this test.

After that we would attach a small machine to two sticky pads and clip that to your belt. This machine will count your heartbeats.

We would ask you to wear it through the whole day and the night and then we will come to your home the next morning to take that off.

We will also ask you to pass urine in two small pots, once before going to bed and then again before breakfast.

We need you once more in the hospital when another small machine will be attached to your belt with a shoulder strap. This machine will measure your blood pressure.

Just like the first machine this also needs to be worn for a whole day and night and we will come to your home to take that off.

These machines are the same size as a mobile phone and do not hurt you at all. A lot of children have used them in the past. You can do all the things you normally do while wearing the machines (but no swimming please!!).

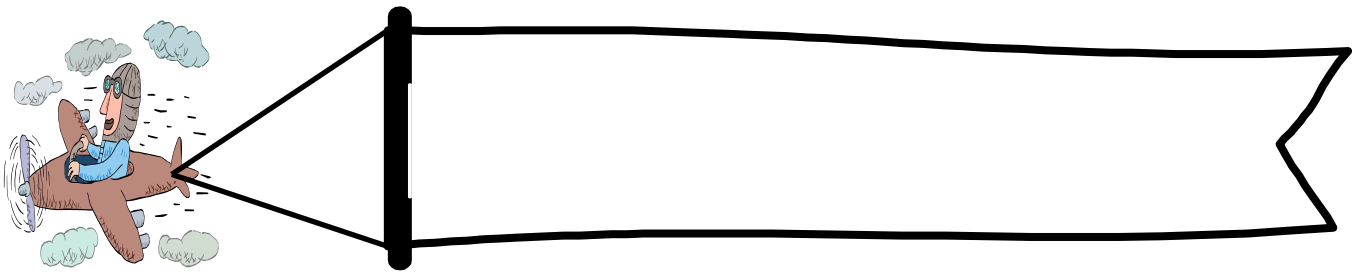
There are some pictures for you to look at.

If at any time you do not want to wear the machines any more then your mum or dad can take them off straight away.

We will not mind at all if you do not want to get involved, but if you would like to join in then please write your name below, inside the banner.

Thank you very much.

Mike.



PARTICIPANT CONSENT FORM FOR THE RESEARCH STUDY

Version No: 2 Date: MAY 2004

Participant identification number for this project:

Title of project:

Heart rate and Blood Pressure variability in seven-year old healthy children including some who were born with a low birth weight.

Name of principal researcher: Dr. Mike Wailoo, Consultant Paediatrician

This form should be read in conjunction with the Parent/Guardian information sheet, Version 2, May 2004

Please initial box

1. I confirm that I have read and understood the information sheet dated MAY 2004 (Version:2) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, and without affecting my child's medical care or legal rights. ☐
3. I understand that sections of any of my child's medical notes may be looked at by members of the research team or from regulatory authorities where it is relevant. I give permission for these individuals to have access to my child's records. ☐
4. I understand that all information collected in the research study will be held in confidence and that, if it is presented or published, all the personal details will be removed. ☐
5. I understand that medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs. ☐
6. I agree to allow my child to take part in the above study. ☐

_____ _____

Name of the Parent or Guardian | Signature | Date

I confirm that I have explained the nature of the research project, as detailed in the Parent/Guardian information sheet, in terms, which in my judgement are suited to the understanding of the Parent/Guardian and the child.

_____ _____

Name of Person taking consent (If different from researcher)	Signature	Date
_____	_____	
Researcher	Signature	Date

1 for parents; 1 for researcher; 1 to be kept with hospital notes if applicable

PARTICIPANT CONSENT FORM FOR THE RESEARCH STUDY
Version No: 2 Date: MAY 2004

Participant identification number for this project:

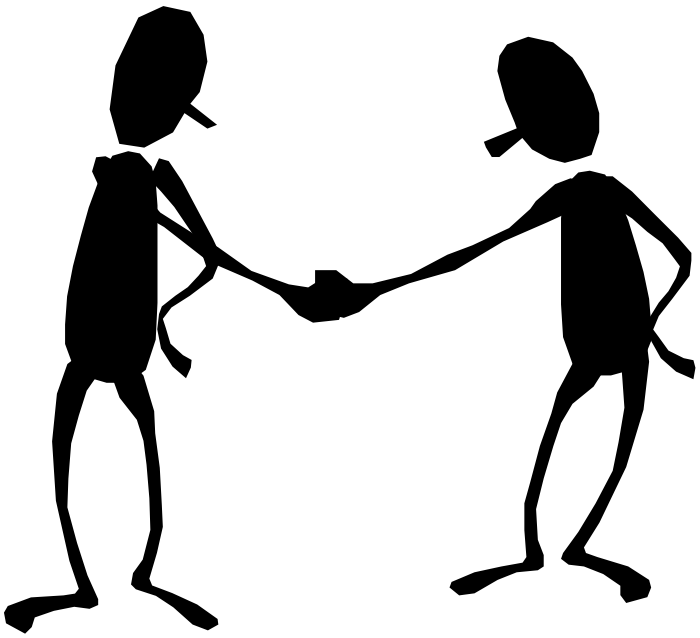
Title of project:

Heart rate and Blood Pressure variability in seven-year old healthy children including some who were born with a low birth weight.

Name of principal researcher: Dr. Mike Wailoo, Consultant Paediatrician

Consent form for the child

My name is ……..
The doctor has spoken to me about the research and I am happy to help him in his research.



12/08/05
Dr.
Health Centre

Re: A study on heart rate and blood pressure variability in seven year olds including some who were Intra Uterine Growth Retarded (IUGR) children.

Participant's name:
Date of birth:
Address:

Dear Dr,

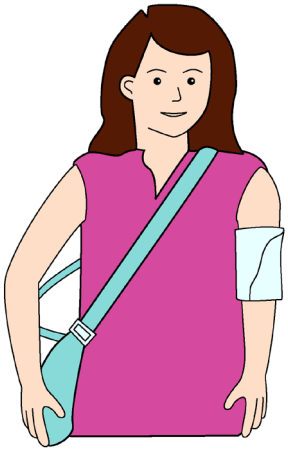
This is to inform you that the parents of your patient have agreed to his/her taking part in a research project conducted by the Division of Child Health, University of Leicester. This project will investigate heart rate and blood pressure variability and urinary cortisol excretion in healthy children. It will not involve blood sampling or use of any medication. There will be no restriction on the lifestyle of the participant. If during the course of the study we discover anything relevant to the health of the participant that was not known previously, then we will take appropriate steps and you will be informed. The parents have agreed to this arrangement.

If you would like to discuss this study, or if you think there is anything about this child you think we should be aware of, please telephone Dr. M. Wailoo on 01162523264.

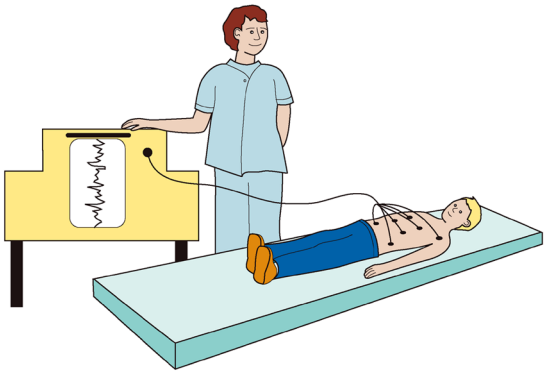
Thanking you,

Yours sincerely,

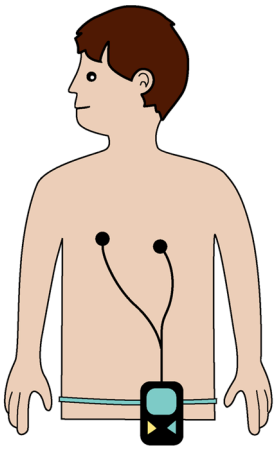
Dr. Mike Wailoo
Senior Lecturer in Community Child Health, Honorary Consultant
Division of Child Health
Robert Kilpatrick Clinical Sciences Building
University of Leicester
Tel: 0116 252 3264
Fax: 0116 252 3282



Girl with Blood Pressure Monitor



Boy having an ECG



Boy with a Heart Rate Monitor

Line drawings for Children

DATA COLLECTION SHEET FOR IUGR STUDY

DEMOGRAPHIC DETAILS

Unique ID Number
Name
Sex
Date of birth
Address

GP details

Invitation letter sent on
Parents happy to be approached
Information leaflet given on
Verbal consent obtained on
Signed and dated consent form obtained on
GP letter sent on
Parents happy to be contacted again

Date of first visit:

From history and previous medical records:

Age in months
Ethnic origin
Mother's name
Place of birth
Father's occupation
Mother's occupation
Social class (NS-SEC classification):
Townsend's score:
Number of cigarettes currently smoked per day by father
Number of cigarettes currently smoked per day by mother
Gestation at birth in weeks
Maternal smoking during pregnancy per day
Birth weight in grams
Head circumference at birth in centimetres
Length at birth in centimetres
Placental weight in grams
Comment on the placental state
Breast feeding
Red book obtained

Any significant medical condition present

Receiving any regular/as needed medication

Academic performance:
Attending mainstream school Yes No
Needing extra help: Yes No
Development: Normal Delayed

Physical examination:

Resting HR and BP

Any significant abnormality detected on examination Yes No

Current weight
Current height
Current head circumference
BMI
Ponderal Index
ECG : normal or abnormal

HEART RATE VARIABILITY DATA:

Date and time of starting the ECG recording
Date and time of finishing the ECG recording
Quality of data acceptable as per protocol
Total amount of hours of recording available
Diary card completed by parents
Any problem with the machine

Mean heart rate daytime
Mean heart rate nighttime
Mean heart rate over 24-hour period
Mean RR interval (msec)
SDNN (SD of all normal sinus RR intervals over 24 hour period in msec)
SDNN Index (mean of the standard deviation of all normal sinus RR intervals for all 5 minute segments in msec)
SDANN (SD of averaged normal sinus RR intervals for all 5 minute segments in msec)
RMSSD (root mean square of successive normal sinus RR interval difference in msec)
PNN 50 (percentage of successive normal sinus RR intervals longer than 50 msec in %)
High frequency power (msec)
Low frequency power (msec)
Very low frequency power (msec)
Total power (msec)
Ratio of High and low frequency power
Data within normal range

URINARY CORTISOL CREATININE DATA:

Quality of urine sample acceptable
Date and time of First urine sample collected
Date and time of Second urine sample collected
Cortisol: Creatinine for first sample
Cortisol: Creatinine for second sample

Date and time of second visit:

BLOOD PRESSURE DATA:

Blood pressure readings validated as per protocol

Blood pressure at rest
Right arm Left arm

Date and time of starting the BP recording
Date and time of finishing the BP recording
Diary card filled up by parents
Number of readings
Number of errors
Quality of data acceptable as per protocol
Any problem with the machine

24 hour mean Systolic BP
24 hour mean Diastolic BP

Daytime mean SBP
Nighttime mean SBP
Daytime mean DBP
Nighttime mean DBP
Day night ratio of SBP
Day night ratio of DBP
Percentage of nighttime dip of SBP
Percentage of nighttime dip of DBP
∗SD of SBP daytime
∗SD of SBP nighttime
∗SD of DBP daytime
∗SD of DBP nighttime
SD of SBP over 24 hours
SD of DBP over 24 hours
Data within normal range