

**ABNORMALITIES OF CARDIOVASCULAR STRUCTURE
AS MEDIATORS OF THE ASSOCIATION OF
HIGH BLOOD PRESSURE, INSULIN RESISTANCE
AND LOW BIRTHWEIGHT**

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Abstract : Abnormalities of Cardiovascular Structure As Mediators of the Association of High Blood Pressure, Insulin Resistance and Low Birthweight.

Many studies have demonstrated associations between low birthweight and higher subsequent blood pressure, insulin resistance and greater incidence of coronary heart disease. These findings suggest that the origins of vascular disease are to be found during intra-uterine life, and that the low birthweight individual may be "programmed" to develop vascular disease. This hypothesis suggests that the development of the individual is permanently altered by its response to the environmental conditions that are responsible for low birthweight. At present, evidence of altered functioning of potential mechanisms that might be susceptible to programming is beginning to emerge.

Increased vascular resistance is the hallmark of hypertension, and of the components of the vascular tree, microvessels contribute the greatest proportion to vascular resistance. Abnormalities of microvessels have been identified at early stages in the development of high blood pressure, and may contribute to variations in glucose homeostasis by limiting glucose uptake by metabolically active tissue. Thus if vascular structure is altered by the intrauterine environment it would have the potential to mediate associations with adult cardiovascular risk factors.

The introduction to this thesis reviews key papers describing associations between early life and adult cardiovascular risk factors. Factors that alter birthweight are considered in detail, especially the effect of gestational age, which has been

relatively neglected in previously published work. The chapter concludes with a review of the field of microvascular structure and hypertension.

The experimental chapters describe studies performed in three cohorts of healthy young people. The first cohort comprises 61 survivors of premature delivery or control individuals born at term. We demonstrated higher adult blood pressure, glucose and lipids in adult premature babies irrespective of whether they had been exposed to IUGR. Chapter 3 documents indices of cardiac and vascular structure in this cohort. We demonstrated that low birthweight in this cohort was associated with greater left ventricular mass, over and above the effect from the higher blood pressure in these individuals.

Chapter 4 describes the influence of birthweight, current size and parental size on blood pressure in a cohort of 107 boys attending a fee-paying school. Low birthweight is associated with higher blood pressure after adjustment for current size, but is not associated with parental influence on current size. We also measured dermal capillary density in these boys but did not demonstrate associations with blood pressure or birthweight. Chapter 5 investigates associations between structure and function of microvessels, and blood pressure, insulin resistance and fasting plasma glucose concentrations in 105 healthy young men. We did not find evidence that insulin resistance was associated with altered structure or function of microvessels, although both higher blood pressure and higher blood glucose were associated with capillary rarefaction. The thesis concludes with a brief overview of conclusions and speculations.

Dedication

To my family, and to absent friends.

Abbreviations

A Ch:	Acetyl-choline	L-NMMA:	NG-monomethyl -L-arginine
AGA:	Appropriate for gestational age	LVH:	Left Ventricular Hypertrophy
ANOVA:	Analysis of Variance	LVMI	Left Ventricular Mass Index
ASE	American Society of Echocardiography	mmHg:	Millimetres of Mercury
BMI:	Body Mass Index (weight in kg / (height in m) ²)	NIDDM:	Non Insulin Dependent Diabetes Mellitus
BMJ:	British Medical Journal	O.G.T.T.:	Oral Glucose Tolerance Testing
BP:	Blood pressure	O.R.:	Odds ratio
C.I.:	Confidence Intervals	SD:	Standard Deviation
et al:	et alia	SHR:	Spontaneously Hypertensive Rat
HDL:	High Density Lipoprotein	SNP:	Sodium Nitroprusside
IUGR:	Intra uterine growth retardation		
IQ:	Intelligence Quotient		
LDL:	Low Density Lipoprotein		

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Declaration

I declare that this thesis has been composed by me, that the experimental studies have been carried out by me, except where acknowledged in the text. This thesis has not been submitted in candidature for any other degree, postgraduate diploma or professional qualification.

Chapter 1: Introduction

Vascular disease awaits all of us. In modern times, we are likely to elude premature death from infectious or neoplastic causes, and will become susceptible to the ubiquitous morbidity and mortality that result from atheromatous degeneration of conduit arteries. The clinical manifestations of myocardial ischaemia and infarction, stroke, and renal failure are the culmination of many years of pathophysiological processes. The rate of progression to clinical manifestations in individuals relates to a collection of risk factors which have been acknowledged for many years – cigarette smoking, high blood pressure, and disordered metabolism of glucose and of lipids. Observations that events during intra-uterine, and early post-natal life might influence the development of conditions in the twilight years have been hailed as “a breath of fresh air to both perinatal and cardiovascular disease epidemiology” (KRAMER 1996). This is a relatively young field which has developed since 1986.

Many associations between early life and indices of vascular disease risk and mortality have been described. The most consistent observation is an association between low birthweight and higher blood pressure in childhood and adulthood, and this is also the main emphasis of this thesis. Low birthweight is also associated with increased prevalence of glucose intolerance, disordered lipid metabolism and higher cardiovascular mortality. Other indices of size at birth: length, thinness, circumference of head and abdomen, ratio of placental size to birthweight have also correlated with increased cardiovascular risk. (BARKER 1990, JOSEPH 1996, THAME 2000)

Many of the studies describing the adult associations have used data preserved by chance, which has been not collected with the intention of exploring potential confounding influences on associations with adult outcomes. Causation has been asserted for associations between birthweight and adult outcomes, but at present this is essentially speculative. Factors influencing birthweight may potentially programme the individual, and have a causative effect on adult outcomes. Alternatively low birthweight may be a marker for individuals who will develop adult outcomes that have been influenced through genetic or environmental influences acting during adult life, with no causative mechanisms operating in early life. Most of the studies published in this field are observational; there are many practical and ethical problems with an experimental approach to this question, at least in humans, the question of causality will not be answered for some time.

Professor Barker and colleagues who are based in Southampton have published many of the studies that will be cited in this thesis. The group has reproduced their initial observations in many populations throughout the world, and established a theoretical framework for elucidating underlying mechanisms. The rapid development of the field is due in no small measure to their energy.

In this thesis, an initial introduction will examine in detail key observations that are representative of many studies that describe these associations. I will expand definitions of programming and discuss alternative interpretations of the associations. Having set out this stall, I will consider what processes are represented

by measurements of birthweight and infant size. Early development could influence subsequent vascular physiology in a plethora of ways. The next section will consider potential mechanisms. The experimental studies that I performed pursued the hypothesis that vascular structure might be altered by early development; this chapter concludes with a detailed review of vascular structure in hypertension, and an outline of the theoretical basis to support investigation in this area.

Key observations

Ecologic evidence

One of the first studies to link cardiovascular mortality with conditions in early life examined disease prevalence rates in each of 212 local authorities in England and Wales. The authors noted a strong correlation ($r=0.73$) between infant mortality in 1921-25 (291 082 deaths) and ischaemic heart disease mortality rates between 1968 and 1978 (922 834 deaths). Correlations with other disease processes were also noted: Bronchitis ($r=0.82$), stomach cancer ($r=0.79$), stroke ($r=0.54$), and lung cancer ($r=0.46$). The associations were consistent across age ranges of adults, and between sexes. Infant mortality is divisible into neonatal (first month after delivery) and post-neonatal. Correlations with ischaemic heart disease mortality were similar for both neonatal ($r=0.69$), and post-neonatal ($r=0.68$), whereas for chronic bronchitis ($r=0.58$, vs. $r=0.83$ respectively), and lung cancer ($r=0.13$, vs. $r=0.55$) post-neonatal mortality was the stronger predictor. Post-neonatal death was most commonly due to respiratory illness suggesting a common link with social conditions and cigarette

smoking. Neonatal death was ascribed to congenital causes in 80% of cases. (BARKER 1986)

These results were interpreted to indicate that influences on intra-uterine life may predispose to coronary heart disease in later life, and suggests maternal and infant nutrition as a potentially causative factor.

Earlier claims to originality

An editorial in the BMJ (Early origin of coronary heart disease (the "Barker hypothesis")) (PANETH 1995) provoked a letter from Norwegian academics (GRAM 1995), noting that ecologic associations between poverty in childhood and later cardiovascular disease had been described long before the paper that is normally credited as the original description (BARKER 1986). This earlier paper (FORSDAHL 1973) was written in Norwegian which may explain its lack of prominence, although another paper by Forsdahl (FORSDAHL 1977) was cited in the Barker paper. This thesis will not adjudicate on ownership of eponymous status.

Low birthweight and higher blood pressure

The association between low birthweight and higher subsequent blood pressure has been comprehensively reviewed by Huxley *et al.* (HUXLEY 2000) These authors have built upon their earlier review (LAW 1996) which identified 34 studies published up to March 1996. 46 further studies were published up to March 2000. They excluded a further 18 studies from the original review, and 12 from 1996 to 2000. Studies were excluded if published only in abstract form, if the paper did not describe the magnitude of change in blood pressure associated with birthweight, or if all participants were drawn from a “pathological group”, such as prematurity.

These reviews record numbers, age and sex of participants, location of study, mean birth weight and blood pressure of participants and the strength of the association (as regression coefficients with confidence intervals). The strength of the association was typically 2-3 mmHg/kg birthweight, after adjustment for current size, in the 1996 review and 1-2mmHg/kg in the 2000 review. Thus the difference in blood pressure attributable to birthweight is approximately 6-8 mmHg. This is similar in magnitude to the blood pressure lowering effect of a single anti-hypertensive agent, which can be clinically significant for an individual. We should note that the association between birthweight and blood pressure pertains to the entire range of birthweight and not merely a tendency for very low birth weight babies to develop hypertension.

I do not propose to reproduce the information summarised in these reviews. I will describe in detail a few studies that made key observations in different patient groups, and general points about the extent to which these observations have been reproduced in other population samples.

Adulthood

Evidence relating intra-uterine factors to adult disease in individual patients, followed from the discovery of unusually detailed birth records for in-hospital births in two cohorts in England: East Hertfordshire and Preston. Individuals who had not moved away from the area were traced and invited to participate in studies of cardiovascular risk.

Of 1298 men and women, born in the labour ward at Sharoe Green Hospital, Preston, Lancashire between 1935 and 1943, 449 were traced and agreed to participate (BARKER 1990). In this cohort higher blood pressure was predicted by lower birthweight (11 mmHg (95% C.I. 3-19 mmHg) higher in the quintile with lowest birth weight than the greatest). This study showed that greater placental weight predicted higher blood pressure, independently from birthweight (15 mmHg (95% C.I. 8 – 23 mmHg) higher in the tertile with greatest placental weight). Information on social class at birth or at the time of the study did not seem to confound the relationship between placental weight and blood pressure; no statistical test is quoted. The authors acknowledge that hospital birth was less prevalent at that time although inspection of the records does not suggest that mothers were ill, as many of the beds had been booked early in pregnancy.

The Hertfordshire blood pressure data was published along with 3 other cohorts (LAW 1993). For individuals born in Hertfordshire, a 1kg decrease in birthweight predicted a 4.0 mmHg increase in blood pressure, although this was not significant in female subjects. With the other cohorts, it appeared that the inverse relationship with birthweight became stronger as the age of the subjects increased. This has not been supported by all further studies.

These observations have been repeated in 20 populations reporting multiple regression analyses (HUXLEY 2000). After adjusting for current size, in all but three, an inverse relationship between birthweight and blood pressure was shown (95% confidence intervals traversed zero in 9 of these cohorts).

Childhood

Blood pressure is known to increase, and to track throughout childhood. Individuals tend to maintain their rank within the population, at least until the onset of adolescence (LEVER 1992). Blood pressure in childhood is also predictive of blood pressure in adulthood.

In a long-term follow up study of 1739 individuals performed in Farnborough (LAW 1993), individuals with lower birthweight had a tendency to have higher blood pressure at annual measurements up until the age of 10 years.

Whincup *et al.* performed repeated measures of blood pressure in children attending primary schools in Guildford and Carlisle (WHINCUP 1989, WHINCUP 1995). At 5-7 years of age, low birthweight predicted higher systolic BP (1.83 mmHg/kg, 95% C.I. 1.31 – 2.35). This relationship was stronger at age 9-11 years for both systolic (2.80 mmHg/kg, 95% C.I. 3.84 to 1.76) and diastolic BP (1.42 mmHg/kg, 2.14 to 0.70) after adjustment for current size. Lower birthweight predicted greater increase in blood pressure between the two measurements (1.71 mmHg/kg decrease in birthweight, 95% C.I. 3.35 to 0.07).

In the recent review (HUXLEY 2000) 17 papers report multiple regression analyses of birthweight and systolic blood pressure. 15 showed an inverse association which achieved statistical significance in 8 of these.

Adolescence

Many studies of blood pressure in adolescence have not demonstrated significant associations between birth weight and blood pressure (HUXLEY 2000, MATTHES 1994). It has been suggested that tracking (maintenance of rank within the population) of blood pressure is disturbed by rapid somatic growth (LEVER 1992). This may weaken the association below the threshold at which it can be detected by small studies.

Two very large studies have matched data on blood pressure, collected at compulsory medical examinations on conscripts into armed services (LEON 2000, SEIDMAN 1991). Linkage with centrally held birth records was possible through the

unique national identification number of every participant. In the Israeli study of 32 580 recruits (SEIDMAN 1991), there was a weak, positive correlation between birthweight and blood pressure in conscripts, whether or not current size was adjusted for. The Swedish study (NILSSON 1997) of 149 378 recruits showed an inverse association between birthweight and blood pressure (0.78 mmHg (95% C.I. 0.64 – 0.92) per kg birthweight). Interestingly, a reanalysis of a more complete dataset (165 136 subjects) was published by a different group of authors (LEON 2000) which also showed an independent association with gestational age (0.136 mmHg (0.94 – 0.178) per week of prematurity. Huxley *et al.* (HUXLEY 2000) identified 20 studies published on measures of blood pressure in adolescence. An inverse relationship with birthweight is shown in 14, a positive relationship in 4 and contrasting results in male and female subjects in 2.

Birthweight and other risk factors

Glucose intolerance and diabetes mellitus

The first report of an association between low birthweight and glucose intolerance was in 468 men, average age 64 years, from the Hertfordshire cohort who had undergone oral glucose tolerance testing (OGTT). Impaired glucose tolerance was found in 30% of subjects \leq 5.5 pounds birthweight versus 14% in $>$ 9.5 pounds (HALES 1991). Similarly in young men (21 years) 30 minute plasma glucose levels after OGTT was inversely related to birthweight (ROBINSON 1992). Prevalence of diabetes in 60 year old Swedish men was inversely related to birthweight, after

adjustment for current BMI (LITHELL 1996). Pima Indians from Arizona have a remarkably high incidence of NIDDM. A study of offspring demonstrated a U shaped relationship between diabetes incidence and birthweight (MCCANCE 1994). The higher incidence in individuals with higher birthweight was largely explained by maternal gestational diabetes. The authors note that individuals with low birthweight accounted for 6% of the overall incidence of diabetes, and suggest that these individuals may have had a survival advantage from insulin resistance, as many low birthweight babies in this population die in infancy. This would explain the association without invoking programming.

Lipid disorders

Elevated triglyceride levels (1.6 mmol/l vs 1.2) and decreased HDL levels (1.32 mmol/l vs 1.57) were reported in women in the lowest and highest birthweight quintiles, respectively (FALL 1995).

Low Birthweight and Coronary Artery Disease

This association occupies a small portion of this thesis, as the experimental studies relate to mechanisms which might explain the association with blood pressure. Clearly, the relationship between premature mortality and low birthweight is of greater importance to public health.

Following up their initial observation (BARKER 1986), Barker and colleagues unearthed detailed birth records for infants born in Hertfordshire between 1911 and 1930. They matched birth records with mortality data for 5654 men. Death from cardiovascular causes was higher in men who were lighter at birth, and especially in men who were lighter at one year of age. (BARKER 1989 Lancet)

Low birthweight also predicted higher incidence of coronary artery disease (fatal and non-fatal) in a cohort of 3447 Finnish women. Length at birth was a much stronger predictor in this cohort. A large study (LEON 1998), with 14 611 participants and remarkably complete follow up showed that low birthweight predicted increased cardiovascular mortality in men.

A similar trend in coronary disease incidence was observed in a smaller cohort (517 people) born in Mysore, India (STEIN 1996), and in the American Nurses Study (RICH-EDWARDS 1997). This very large study of 70 297 women demonstrated relative risk of non-fatal first incidence of cardiovascular event decreasing from 1.49 in the lowest birthweight group to 0.68 in the highest.

Other infant measurements and later risk factors

Many other infant measurements have been shown to correlate with indices of vascular risk. It is difficult to draw consistent conclusions from these data. Many of the studies are contradictory (HUXLEY 2000, JOSEPH 1996), and doubt remains as to what precise processes are reflected in relative dimensions of cranial or abdominal circumference, or placental weight.

Potential Mechanisms: Programming

One potential explanation of these observations is that development of structure and function of organs and systems could be permanently influenced by conditions during early life. Initial development is a plastic process, and may be limited by environmental influences imposed upon the genetic blueprint. For example, cellular proliferation contributes to organ growth for a limited timespan. Subsequently, differentiated cells lose the ability to divide. If a planned expansion of cell numbers fails to occur at the correct time, this may impose functional limits upon that organ throughout the life of the organism. Adaptations that optimise function to the prevailing conditions in a growth retarded foetus, would become maladaptions in a nutritionally replete adult, manifested for example, as insulin resistance or higher blood pressure. Barker cites (BARKER 1998) development of sweat glands as an example of development in which conditions during a crucial window dictate subsequent sweat gland density. Babies are born with a greater density of sweat glands than adults have. Children exposed to a hot climate before 3 years of age retain a greater proportion of their sweat glands. Sexual differentiation has been recognised for many years to depend upon a surge of male hormones to change the phenotype that would otherwise be female. (SADLER 1985A)

Functional limitations in control mechanisms might be programmed by a similar process, establishing feedback loops that respond within different limits in the organism that was exposed to the programming stimulus.

Prenatal or postnatal influences?

Birthweight is the summary of many factors that influence intrauterine development. It is important to bear in mind that it may also predict subsequent postnatal development. This section considers interpretations of the existing studies in humans, that suggest events and processes occurring during postnatal development may be more important in determining adult outcomes.

A close relationship between the normal rise in blood pressure and childhood growth has been identified for many years (LEVER 1992). Lever and Harrap (LEVER 1992) proposed that the origins of essential hypertension may lie in mismatching of the rise in blood pressure and bodily growth in childhood, followed by self-perpetuating mechanisms in adulthood. The central role of the kidney in maintenance of blood pressure (GUYTON 1972), and the requirement for increased blood pressure to maintain renal homeostasis during somatic growth, has led to speculation (WEDER 1994) that disproportion between kidney volume and body size in childhood would be a potent initiator of hypertension.

It may be that environmental factors influence this mismatching, but interactions between genetic factors are in themselves extremely complex. Establishing the role that is played by programming of postnatal influences will require a great deal of research.

Postnatal growth

In many studies, associations with birthweight are not seen unless adult factors (in the studied individual, not their parents) are adjusted for. For some adult factors a direct association with birthweight is unlikely for example cigarette smoking, or alcohol intake. These factors could be proxy for socio-economic deprivation, which is a separate issue (see deprivation section below). Most studies adjust blood pressure for adult size, using height, weight or body mass index. The authors argue that obesity is positively associated with both birthweight and blood pressure and may thus obscure the inverse relationship between birthweight and blood pressure. Lucas *et al* have argued that to adjust birthweight for adult size in multiple regression analysis in effect describes the association between blood pressure and growth velocity between the two measurements of size (LUCAS 1999). It is known that many low birthweight babies grow at a faster rate during early childhood, although babies with severe IUGR tend not to undergo “catch-up growth”. Barker and colleagues have argued that the programming influences on the baby predispose it to gain weight as an infant. An alternative, equally valid interpretation is that low birthweight may be a marker for babies that will undergo rapid catch up growth, which is the mechanistic determinant. Associations between adult obesity and blood pressure are, in general, stronger than those between birthweight and blood pressure. From a public health perspective, adulthood obesity is therefore more important. (PANETH 1995)

Lucas *et al.* (LUCAS 1999) argue that intermediate sizes in large longitudinal studies will help resolve this issue. Weight at, for example, one year is included in a simultaneous multiple regression model with birthweight and adult weight, predicting systolic blood pressure. If the association with birthweight is weakened it implies that growth between one year and adulthood is the important determinant of blood pressure. If the relationship with birthweight is strengthened then the model suggests that earlier factors are likely to be important. To date few studies have had measurements recorded at multiple time points, and no papers have published analyses along these lines.

Postnatal growth and blood pressure

In the review by Huxley *et al.* (HUXLEY 2000) 3 studies report positive associations between skeletal growth, that is to say adult height relative to birthweight, and blood pressure. 13 studies report associations between blood pressure and weight gain, or non-skeletal growth, 9 of these report a positive outcome.

Postnatal growth and cardiovascular mortality

Studies of incidence of overt clinical vascular disease have demonstrated different associations in men and women. Risk in women relates to length at birth and linear growth (FORSEN 1999), and in men relates to thinness at birth and increase in weight (ERIKSSON 1999). Neither study examined the data specifically to test

whether postnatal growth explained the association better than indices of foetal growth.

In summary, it has not yet been established that low birthweight predicts higher adult blood pressure through programming, which could occur during intrauterine life or later. This issue will be addressed in the experimental chapters of the thesis.

Influences on Birthweight

Interpretation of the associations described above requires understanding of the processes that lead to differences in birthweight. This section considers these factors in detail, and the current state of knowledge regarding our ability to alter them.

Birthweight is the sum of many influences on the developing foetus, both environmental and genetic, and the relative strength of these factors will vary between individuals. Most research on prediction and outcome of low birthweight has been performed by Obstetricians or Neonatologists, and the main outcome measures are neonatal. Many studies are designed to identify individuals in pathological groups, identifying intrauterine growth restriction (IUGR) as occurring in babies below the tenth centile of weight for gestational age, and premature babies having gestational ages <37 completed weeks. These babies are at high risk of adverse neonatal outcomes. Low birthweight is a risk factor for perinatal morbidity but 50% of British babies below the 3rd centile of birthweight at term, are

proportionally small and have no obstetric or neonatal complications. (CHARD 1993)

In 1948 the World Health Assembly defined premature delivery (less than 37 weeks gestation) as having occurred in all babies born weighing less than 2500g. Over the following decades it became apparent that one third of these babies were not premature, but were in fact inappropriately small. Normal foetal growth, in Caucasian populations, was defined in 2 papers published in 1966 (GRUENWALD 1966, SCOTT 1966). With the development of ultrasound, standard tables for foetal growth have been developed. (DETER 1992)

Because of the complexity of the influences on birthweight, beyond a statement that “large mothers tend to have large babies” (CHARD 1993), it is difficult to precisely quantify the genetic potential of individual babies. Maternal size has a stronger association with birthweight, and indeed adult size, than paternal size (WALTON 1938). Estimates of the influence of genetic factors on birthweight vary from 70% in one study of the singleton offspring of female twins (MAGNUS 1984) to 14% in a study of two generations of women who delivered in the same Aberdeen hospital (CARR-HILL 1987). Resolution of these disparate estimates depends upon whether twin or generational studies are more successful at separating maternal and foetal genetic factors, and environmental factors.

Kramer *et al.* report a study of 8719 singleton offspring from which babies with major congenital abnormalities or infections had been excluded, and in who there

was good agreement between estimates of gestational age from last menstrual period and ultrasound dating at 16-18 weeks (KRAMER 1990). Known risk factors for growth restriction, or augmentation predicted only 17.2% of the variance in foetal growth ratio (weight divided by predicted weight for gestational age calculated for that population).

In summary, variation in birthweight is caused by some degree of genetic factors and environmental factors, known and unknown.

Racial factors

Normal values for birthweight vary between different races, babies of black ethnicity are larger early in gestation but smaller at term (ABRAMS 1991). Moreover, data from 1975-80 in South Carolina, showed white babies were born averaging 270g heavier and one week later than non-white babies (ALEXANDER 1985). For low birthweight babies, at a given birthweight or gestational age, white babies had higher perinatal mortality. The reverse was true at higher birthweights.

Gestational age at delivery

The mean bodyweight of the foetus increases from 500g at 24 weeks gestation (which is approximately the most premature delivery compatible with independent existence) to 3500g at term. This growth in overall body size is linear. (ROBERTON 1999). Premature delivery therefore strongly predicts low birthweight. 5-10% of deliveries worldwide are premature (<37 weeks gestation). (VILLAR 1998)

Premature delivery is associated with many factors, which are listed and, where possible, quantified below.

Genetic factors

In a Swedish twin study, the correlation between gestational age of the singleton offspring of monozygotic twins was 0.31, and 0.13 for dizygotic twin offspring. It is not known what genes mediate this observation, the authors speculate it might be inheritance of the risk of pre-eclampsia or cigarette smoking. (CLAUSSON 2000)

Sociodemographic factors

Low socio-economic status is associated with premature delivery. Specific risk factors include low maternal age (<16 years, Odds Ratio 2.91), use of illicit drugs (O.R. 3.90), black ethnicity (O.R.2.10), exposure to trauma or surgery (O.R. 3.47),

low rate of maternal weight gain (O.R. 1.59), short inter-pregnancy interval.(O.R. 1.77) (ABRAMS 1989).

Obstetric factors

Obstetric problems that result in premature delivery include: uterine abnormalities, such as incompetent cervix (O.R. 6.15) (ABRAMS 1989); overdistention of the uterus (polyhydramnios, multiple pregnancy); premature rupture of amniotic membranes, which most commonly relates to the presence of infection; ante-partum haemorrhage; and pregnancy induced hypertension. Previous premature labour increases the chances of recurrence in subsequent pregnancies to 30% in a second confinement and 60% in a third. (SYMONDS 1992)

Interventions to prevent premature delivery

Administration of β -agonist drugs can slow labour. This effect is not prolonged, but can allow administration of dexamethasone which accelerate development of the foetal lungs and improve the outlook if the baby is <36 weeks gestation. (SYMONDS 1992)

An overview (VILLAR 1998) of randomised controlled trials of interventions designed to prevent preterm birth reported efficacy of screening for and treatment of asymptomatic bacteriuria. An appropriate course of antibiotics, which may be limited to a single dose, reduces the incidence of preterm birth by 47 % (Relative risk 0.53,

95% confidence interval 0.33 – 0.86). Malaria prophylaxis has not been studied in trials with sufficient power to determine an effect. Treatment of vaginal bacteriosis is unproven.

Nutritional supplements have insufficient evidence to support their routine use. An old study performed in London in the 1940s suggested that fish oil supplementation could help (THE PEOPLE'S LEAGUE OF HEALTH 1942) but no data relevant to modern populations is available. Mineral supplements have been studied and show promise, but better quality studies are required.

Intra uterine growth retardation

Almost all forms of pathology of mother or foetus during pregnancy are associated with growth restriction of the offspring. Clustering of multiple risk factors in socio-economically deprived populations makes dissection and quantification of individual risk factors problematical.

Foetal abnormality

Many foetal abnormalities will result in low birthweight, e.g. chromosomal abnormality, congenital infection, or congenital malformation. These individuals will have many other problems specific to their condition and the incidence of these major foetal abnormalities in populations of surviving adults is low. (ROBERTON 1999)

Foetal exposure to toxins

Alcohol

Heavy drinking during pregnancy (3 or 4 drinks every day) is associated with the foetal alcohol syndrome, which includes marked impairment of brain development. Birthweight is reduced by 150g if 3 or more standard drinks are consumed. Women who drink occasionally were shown to deliver babies 14g lighter, which may not be a clinically significant effect.

Cigarettes

Birthweight is 100 - 250g less if mothers smoke. (CONTER 1995) There seems to be a dose-related effect.

Pharmacological agents

Drugs of abuse are associated with low birthweight although affluent mothers rarely take them. (ROBERTON 1999)

β -adreno receptor blockers are widely prescribed for pregnancy induced hypertension, and for cardiac arrhythmias in pregnancy. Growth retardation seems to be a problem (25% of babies) if used early in pregnancy, but not if the prescription is confined to the third trimester. (BRACKLEY 1999)

Foetal Infection

Intrauterine infection leads to growth restriction (ROBERTON 1999).

Maternal illness

Maternal Blood pressure in pregnancy

Disorders of blood pressure control occur in pregnancy and are a major cause of maternal and infant morbidity and mortality. Hypertension is arbitrarily defined at >140/90 mmHg and is divided into two categories, pre-existing hypertension (essential hypertension) and pregnancy induced hypertension. The latter is more ominous, encompassing a spectrum of illness from mildly raised BP in the latter stages of the third trimester, to proteinuria (pre-eclampsia) and, with the onset of seizures, eclampsia. Eclampsia is an obstetric emergency, complicating 1 in 2000 pregnancies in Europe. (THE ECLAMPSIA TRIAL COLLABORATIVE GROUP 1995) Birthweight is reduced because of IUGR and prematurity following expedited delivery, which is indicated when the balance of risks to the premature foetus is outweighed by the risk to the mother. IUGR is particularly associated with early onset pre-eclampsia (18.2% early, vs. 5.6% in late onset). This combination is associated with a very high perinatal mortality rate (28.7%). (LONG 1980) Another study of 17,000 consecutive births found shortened gestational age and lower birthweight in offspring whose gestation had been complicated by pre-eclampsia. (HIMMELMANN 1996)

Women with chronic hypertension are at increased risk of pre-eclampsia (for women with diastolic BP \geq 110 mmHg, Odds ratio 5.2, C.I. 1.5 to 17.2). In the absence of superimposed pre-eclampsia their risk of delivering a baby below the tenth centile is approximately double the general population 10.9% vs. 4.1%. (MCCOWAN 1996)

Systemic illness

Women with significant medical disorders e.g. renal disease, heart disease, tend to deliver lower birthweight babies. (ROBERTON 1999)

High altitude

Babies born in Colorado or Bolivia are smaller than those born at sea level. (ROBERTON 1999)

Maternal Nutrition

Impaired maternal nutrition undoubtedly lowers birthweight. Studies of birth records of babies exposed to famine *in utero* during World War II show a reduction of birthweight. These populations had been reasonably well nourished before the tides of war turned against them, and relief of famine could be precisely dated to liberation. In Amsterdam the reduction was 200-300g. (RAVELLI 1998) During the siege of Leningrad birthweight was reduced by 18% in boys exposed *in utero* and 16% in girls. (STANNER 1997)

It is less clear the extent to which less extreme reductions in nutrition alter birthweight, and the extent to which it is possible to alter the effect of generations of poor nutrition. Studies of nutritional supplementation suggest that at most 100g may be added to birthweight by energy supplementation for expectant mothers. (GULMEZOGLU 1997)

Socio-economic status

The effects of socio-economic status on birthweight are complex. Individuals in the 1958 birth cohort study who weighed less than 6lb were more likely to experience deprivation throughout childhood. (BARTLEY 1994) A more recent, prospective study of pregnant women showed that the effect of social factors on intrauterine growth were explained by maternal smoking, (BROOKE 1989) although premature delivery was predicted by indices of social deprivation. (PEACOCK 1995)

Multiple births

Multiple births are reliably both premature and growth restricted.

Symmetrical vs. Asymmetrical growth retardation

It has been suggested that intrauterine growth restriction may result in either proportional, limited growth of all body parts, to babies with sparing of head circumference and body length. Symmetrical IUGR was thought to imply more severe and prolonged insult from factors present throughout intrauterine life. Disproportionate restriction would result from impaired growth during the third trimester only. This theory forms the basis for the hypothesis that foetal undernutrition at specific crucial windows of development will result in programming of diseases specific to that window (BARKER 1995).

It has also been suggested that different patterns of growth restriction have different prognostic significance, although contradictory studies suggesting either pattern is the more dangerous have been published. (CHARD 1993)

In their study of 8719 singleton offspring, (KRAMER 1990) Kramer *et al* demonstrated that disproportionate growth, calculated by *z* scores for 5 indices of proportion, occurred as a direct function of the severity of growth restriction. Babies with severe growth restriction were disproportionate, but no maternal features predicted disproportionality, over and above their effects on growth. Moreover, disproportionate growth did not predict perinatal mortality and morbidity, other than as a proxy for growth restriction. (KRAMER 1990)

Interventions to prevent IUGR

“ You canny make a small baby big, and you canny make a big baby small.”

(THOMPSON 1998) Consultant Obstetrician, St. Johns Hospital. Livingston

An overview of 126 randomised control trials evaluating 36 interventions commented on disparity between the quality of epidemiological trials describing the problem of IUGR, and the trials describing treatment strategies. Many of the studies reviewed were too small to be rigorous evaluations of the intervention under study. Most of them did not have power to assess the key outcome of perinatal mortality. (GULMEZOGLU 1997)

Strategies to stop smoking have been shown to increase birthweight, with greatest success in compliant populations. Nutritional supplementation strategies have involved various dietary manipulations. Energy supplementation may increase birthweight by up to 100g, especially in developing countries. Supplementation of minerals or vitamins has not demonstrated consistent effects. Management of hypertensive disorders in pregnancy have routinely included admission and bedrest for which there is no evidence of benefit. Treatment with antihypertensive agents does not reduce the risk of IUGR, and β - blocker treatment seems to increase this risk. (BUTTERS 1990 , RUBIN 1983)

Abdominal decompression by intermittent negative pressure was popular in the 1960s but seems to have fallen from clinical practice. It was claimed that it would

improve the IQ of babies undergoing this treatment in-utero. No such effect was demonstrated, although children who had undergone the intervention were noted to be “undisciplined and aggressive”, perhaps as a result of their parents’ perception of their superior intelligence (HOFMEYR 1990). Two randomised trials were performed in pregnancies at high risk of IUGR, which demonstrated a large treatment effect (odds ratio for developing IUGR 0.21 (0.12 – 0.34) (HOFMEYR 1990). Antimalarial prophylaxis is likely to be beneficial although the optimal agent is not known.

Summary of influences on birthweight

Birthweight is the sum of many influences on intrauterine growth and gestational length. Reflecting the complexity of these influences, at present there are no reliable interventions that substantially increase size at birth and reduce perinatal, and perhaps adult cardiovascular, morbidity and mortality.

Influences on Growth: Childhood

Post-natal growth diminishes in velocity from birth during the first year of life. Weight is lost during the first days of post-natal life as final functional maturation of gut function is delayed. Fluid losses during this time can total 10% of body weight. Once feeding patterns have been established in the healthy child, weight gain is steady. Birthweight is doubled by 5 months and tripled at one year. Growth to adult size is then more linear, with increased velocity during maturation of adrenal

androgen production and, during puberty, gonadal function. Parental size correlates with final adult size attained.

Deviation from a normal growth pattern is the hallmark of significant childhood illness. Growth may be postponed, and convalescence may be characterised by "catch-up" growth, but some individuals remain stunted and never fulfil their potential, genetic height. Socio-economic deprivation and particularly malnutrition are powerful inhibitors of childhood growth.

Potential mediators of birthweight: blood pressure association

Having considered the numerous influences upon birthweight that exist, the question arises as to which may influence subsequent blood pressure. The causes of essential hypertension remain obscure, although hypotheses include activation of the sympathetic nervous system, or excessive dietary intake of sodium. It is inevitable that associations between measurements made at remote time points may be explained by an enormous number of potential mechanisms. This next section considers plausible candidates.

Confounding?

Maternal blood pressure

High blood pressure is a familial trait (WATT 1991), and offspring of pregnant women tend to have low birthweight as discussed above. There is also evidence to

suggest that mothers who have had higher blood pressure measured during, or remote from, pregnancy, have offspring with higher blood pressure.

The Hypertension in Pregnancy Offspring Study followed up a cohort of 36 individuals whose mothers had had blood pressure >140/90mmHg during pregnancy. In these children gestation was shorter than 17 controls (38.2 weeks vs. 40.2, $p<0.01$), birthweight lower (3235 g vs 3655, $p<0.05$), and systolic blood pressure recorded at the age of 7-12 years, was higher (120 mmHg vs 112, $p<0.01$). (HIMMELMANN 1994)

The influence of parental blood pressure was examined in a study of 452 young adults whose parents had been screened for inclusion in the MRC mild hypertension trial. Maternal (but not paternal) blood pressure measured approximately 10 years after their pregnancy was shown to confound the relationship between birthweight and blood pressure in their offspring. (WALKER 1998) In this study, 24% of the association was explained by inherited factors, which may be genetic, or shared familial environment.

Early studies did not find maternal BP during pregnancy confounded the associations with later offspring blood pressure. Martyn *et al.* demonstrated that higher maternal blood pressure measured during pregnancy was associated with higher adult blood pressure, independently of the effect of birthweight on adult BP. (MARTYN 1995)

A paper studying the influence of maternal factors on blood pressure in 4 year old children showed that a relationship persisted between birthweight and current blood pressure when adjusted for maternal blood pressure measured 4 years after pregnancy, although no significance tests are quoted. (LAW 1991) The paper also does not mention whether maternal BP measured either during pregnancy or later influenced birthweight.

A study of 3591 school children showed that a history of maternal hypertension predicted current BP but did not confound the association between birthweight and BP. The paper does not show whether maternal hypertension predicted birthweight. (WHINCUP 1989)

It is acknowledged that blood pressure recordings during pregnancy may be less accurate. (SHENNAN 1996) Churchill *et al.* (CHURCHILL 1997) recorded ambulatory blood pressure monitoring during pregnancy in 209 nulliparous women demonstrating associations between 24 hour mean diastolic BP at 28 weeks and weight at term. A 5 mmHg increase in BP was associated with a 107 g decrease in weight at term and independently, a 2.3 day decrease in gestational age at birth. Women with pre-existing hypertension and women who developed pre-eclampsia were excluded from this study.

In summary, higher maternal blood pressure predicts offspring with lower birthweight and subsequent higher blood pressure. Acknowledging that it is difficult

to obtain accurate data for two generations of adults, this is an important factor to quantify in further, large, high-quality epidemiological studies.

Socio-economic deprivation

A 1998 overview of studies examining the influence of socio-economic status on blood pressure suggested that a difference of 2-3mmHg of systolic blood pressure (COLHOUN 1998). The influence was strongest in women and related to obesity. Differences in treatment rates did not seem to account for this effect, although were not examined in most of the studies.

As discussed above, low socio-economic status is a strong risk factor for low birthweight as a result of both prematurity and growth retardation. While many studies have attempted to allow for this source of confounding, the multiple, pervasive influences of socio-economic deprivation are difficult to fully quantify, and to make valid statistical adjustments to the relationships in question (JOSEPH 1996).

Some studies have not had information on social class in the available data (BARKER 1989 BMJ, LAW 1993). Others have assessed current social class but not examined for confounding of the relationship between birthweight and blood pressure, (LAW 1991) or the outcome measured e.g. arterial compliance (MARTYN 1995). These adjustments may be insufficiently subtle as errors in attribution of social class may disproportionately alter the results, and differences in other indices

of deprivation may be greater within social classes, than between classes. (JOSEPH 1996)

Potential mediators of birthweight: blood pressure association

Intra-uterine Programming?

Nutrition: "The Barker Hypothesis"

Professor Barker from Southampton has been extremely active in producing epidemiological studies. He has proposed a detailed theory describing developmental windows in which specific disease processes are programmed by nutritional deprivation at crucial times in development. This arises, at least in part, to explain associations between adult outcomes and measurements of proportionality. This is acknowledged by him to be a work in progress (BARKER 1995). The contradictions in the results of studies that look at these relationships (HUXLEY 2000, JOSEPH 1996) makes this hard to sustain, and more recent work from Southampton has been emphasised the role of postnatal growth. Although considerable emphasis is given to maternal nutrition as a potential cause of low birthweight and subsequent raised offspring blood pressure, little consistent data support this assertion.

Researchers contacted offspring of women who had participated in a survey of diet during pregnancy in 1948 – 1954 (CAMPBELL 1996). Lower birthweight predicted higher adult blood pressure (4.8 mmHg / kg, $p=0.01$), but placental weight did not (9.2 mmHg per kg placental weight, $p=0.2$). No index of maternal dietary

constituents predicted birthweight. After division of the study into two groups according to animal protein intakes of greater than or less than 50g per day, statistically significant associations between carbohydrate intake and placental weights and between carbohydrate intake and adult blood pressure emerged. At low animal protein intakes higher carbohydrate intake predicted higher blood pressure and lower placental weight. The converse was true at high intakes of animal protein.

Other investigators contacted 77 children born to women who had participated in a study of haemoglobin concentrations and of weight gain and skinfold thickness in pregnancy (GODFREY 1994). In this study decreased maternal triceps skinfold thickness predicted higher offspring systolic blood pressure (10.7 mmHg per log mm decrease in skinfold thickness, $p=0.0001$). There was also a relationship between maternal haemoglobin and offspring blood pressure, although this was abolished by the relationship with triceps skinfold thickness in multiple regression. Low maternal weight gain between weeks 15 and 35 of gestation was also associated with higher blood pressure in offspring (-0.6 mmHg per kg gained, $p=0.02$).

Maternal haematological status and childhood blood pressure were studied by Whincup et al in 1311 children age 9-11 years (WHINCUP 1994). Low haemoglobin concentrations, and change in mean corpuscular volume did not predict blood pressure, although they did predict lower birthweight. The authors comment that changes in haematological indices in pregnancy is a complex area with an uncertain relationship with foetal and maternal nutrition.

Two studies have reported blood pressure in adults exposed to famine *in utero*. (ROSEBOOM 1999, STANNER 1997) (see nutrition and IUGR above). Neither showed a significant difference in blood pressure in exposed infants compared to non-exposed.

Postnatal diet

Alan Lucas and colleagues randomised 758 children with low birth weight (<1850g) to 4 different diets which contained very different compositions of nutrients, and recorded blood pressure at follow up at 7.5-8 years of age. No component of diet, nor pattern of postnatal growth predicted blood pressure (LUCAS 1994). Further follow up of the cohort showed lower blood pressure in the group randomised to pooled breast milk (SINGHAL 2001A) (Mean arterial pressure 81.9 mmHg vs. 86.1 mmHg, $p=0.006$). These researchers are exploring what aspect of early nutrition might mediate influences on blood pressure (SINGHAL 2001B). Although sodium intake has been implicated in an animal model of early feeding (GOULDSBOROUGH 1998), this may not be the case in humans.

Twins

One implication of the programming hypothesis is that higher blood pressure should be particularly prevalent in twins, as they are inevitably growth restricted during the third trimester, but this is not the case. In a prospectively studied birth cohort in Dunedin, twins had lower blood pressure than singleton children at the ages of 9 and 18. (WILLIAMS 1999)

Within twin pair birthweight differences showed correlations with differences in adult blood pressure in a study of 492 female twin pairs studied in London (POULTER 1999). The regression coefficient was 5.8 mmHg for every kg difference in birthweight. Similarly, a study of 8 year old twin pairs in Tasmania (DWYER 1999), within twin pair weight difference was inversely associated with BP (-5.3 mmHg per kg birthweight). In these studies similar trends were seen if only monozygotic twins were included in the analysis, although limited numbers meant that these analyses did not reach statistical significance. Izjerma *et al.* report significant in pair correlations for dizygotic but not monozygotic twins (IZJERMAN 2000). However, no between twin differences were seen in a much larger study of 8000 female Australian twin pairs. (NOWSON 2000)

Smoking

This factor has been controlled for in some studies (LEY 1997, WHINCUP 1992) and not demonstrated to influence the association between birthweight and blood

pressure, but information is not available for many studies (HIMMELMANN 1993, LAW 1991, LAW 1993, LEON 2000, VALDEZ 1994, WHINCUP 1989). A study of 795 members of a New Zealand cohort demonstrated that systolic blood pressure was 1.54 mmHg higher in individuals whose mothers had smoked during the pregnancy (WILLIAMS 1999). As maternal smoking is a potent and potentially avoidable, factor influencing it is an important unresolved issue to be considered in more detailed epidemiological studies.

Gestational age

The "Barker hypothesis" does not include a role for premature delivery in the mediation of the observed effect (BARKER 1998). This assessment is based on the lack of an association with gestational age in many of the previously published studies. The data chapters that follow will challenge this interpretation, so this point is considered in some detail.

The largest study of the association between birthweight and blood pressure examined the birth records and records of medical examinations performed for conscription into the Swedish armed forces (LEON 2000). Records for 165,135 eighteen year old men were complete, which comprised 78% of singleton male births (1973 – 1975). This study showed that systolic BP increased by 1.47 mmHg per kg birthweight (after adjustment for adult size) and an independent decrease of 0.25mmHg per completed week gestation. Babies born earlier than 35 weeks were excluded because of evidence of inaccurate gestational age assessments.

In a study of 430 Swedish men with mean age 49 years, there was no relationship between birthweight and blood pressure, but a weak relationship between gestational age and blood pressure was shown for the entire cohort ($r = -0.10$, $P = 0.04$; $n = 430$). A stronger association between systolic blood pressure and gestation was shown when premature (<38 weeks gestation) babies were considered alone ($r = -0.46$, $P = 0.001$). The relationship was particularly strong for low birthweight (<2.5kg) babies, ($r = -0.86$, $p < 0.001$; $n = 14$) although this is a very small subgroup of the population. (SIEWERT-DELLE 1998)

Several papers by Whincup and colleagues have shown similar results in children. In 3 year old children, ($n = 1860$) systolic blood pressure decreased by 0.37 mmHg per completed week of gestation (WHINCUP 1999), although this was not independent of birthweight (adjusted regression co-efficient 0.13 mmHg, $p = 0.34$). A similar result was demonstrated in 3360 children selected from towns with widely contrasting cardiovascular mortality rates. (WHINCUP 1992) Information on birth information was collected by questionnaire. This study included children with gestational ages from 28 to 44 weeks. After adjustment for current size lower birthweight predicted higher systolic blood pressure (regression coefficient 2.05 mmHg per kg), as did premature delivery (0.35 mmHg decrease in systolic blood pressure per completed week, However simultaneous adjustment for birthweight and blood pressure abolished the association with gestational age (adjusted 0.06, $p = 0.57$). The authors note that gestational age is less likely to be accurately recalled, and the stronger association with birthweight may reflect greater precision in measurement.

In a third study (TAYLOR 1997) of 8 –11 year old children similar associations were found between birthweight and blood pressure for preterm, (-1.16 mmHg per kg, $p=0.07$), and term infants (-1.39 mmHg / kg, $p=0.0005$).

Reasons for lack of observed effect of gestational age at birth

In the 80 studies reviewed by Huxley *et al.*, 58 did not have information on gestational age (HUXLEY 2000). 16 studies did not demonstrate a relationship between gestational age and birthweight. They are examined in the table. Gestational age, as has been noted above, tends to be inaccurately assessed, especially before ultrasound was available, and is frequently rounded to completed weeks of gestation, which introduces further inaccuracy. Many studies recruited only babies born around term, which may be in part due to the poor prognosis of premature babies born before modern neonatal care.

Table 1

First author	Number		Age in years		Gestational age		adjustment	result	notes
	mean	S.D.	mean	S.D.	mean	S.D.			
Hashimoto (HASHIMOTO 1996)	195	0.1	38.6	1.5	simple regression	no effect	gestation did not predict systolic (r=0.03, p=0.72) or diastolic (r=0.12, p=0.10) blood pressure.		
Woelk (WOELK 1998)	756	0.63	not stated (N.S.)	N.S.	tertiles of gestation	no effect	≤ 38 weeks 91,98 mmHg against 89.89mmHg for 38-39 weeks, 89.93 mmHg for 40 to 44 weeks. No statistical test is quoted		
Yiu (YIU 1999)	2958	0.2	39.8	2.7	multiple regression	no effect	adjusted B mmHg / week -0.08, p=0.5		
Barker (BARKER 1989)	9921	N.S.	N.S.	N.S.	Simple regression	No effect	Illustrated, no statistical test quoted		
Forrester (FORRESTER 1996)	1610	-	38.87 (range)	1.92	multiple regression	No effect	1409 were born at term, between 38 and 42 weeks' gestation		
Ley (LEY 1997)	68	0.7	37.9	2	multiple regression	not shown			

Himmelmann (HIMMELMANN 1994)	53	12.5	1.5	39.2	1.8	simple regression	No effect	$r = -0.12$, $p =$ not significant
Zureik (ZUREIK 1996)	210	13.2	3.7	not stated	N.S.	multiple regression	not shown	
Matthes (MATHES 1994)	330	15.7	N.S.	38 - 42	N.S.	multiple regression	$p = 0.66$	Case control study, low range of birthweight
Leger (LEGER 1997)	516	20.6	2.1	39.8	1.2	simple regression	not shown	Only >37 weeks, neither birthweight or gestation predicted BP
Campbell (CAMPBELL 1996)	253	~40		40.2	1.84	multiple regression	not shown	
Jie Mi (MI 2000)	627	45	1.4	39.5	2	multiple regression	not shown	
Barker (BARKER 1990)	449	46 - 54 (range)	-	N.S.	N.S.	multiple regression	no effect	
Martyn (MARTYN 1996)	337	50 - 53	-	40.4	2	multiple regression	not shown	



1995)

Roseboom (ROSEBOOM 1999)	739	50.4	0.9	40.9	1.7	multiple regression	not shown
Thame (THAME 2000)	428	1 - 3.5 (range)		39.4	1.86	Multiple regression	Not shown
Alves (ALVES 1999)	634	12 - 36 hours (range)	-	39.3	N.S.	Multiple regression	Not shown Excluded <37 weeks, APGAR scores <7

Table 1

This table shows the main features of the studies that do not show an influence of gestational age on subsequent blood pressure. Blood pressure was higher in premature individuals in the study of Woelk *et al.* although this was apparently not statistically significant. These studies tend to be smaller than the studies that have shown effects from gestation, but most seem to have explicitly considered the possibility and made appropriate adjustments, although the results of the tests are not always quoted in full.

Potential mediators of birthweight blood pressure association:

Endocrine systems

In this introduction, the evidence considered has consisted of epidemiological studies, demonstrating a consistent association between birthweight and blood pressure, and less consistent associations between aspects of life during pregnancy for mother and foetus, and subsequent blood pressure. This section will consider potential hormonal mediators which might link early life to cardiovascular disease. The next section will outline a theoretical basis supporting altered vascular structure as a programming mechanism.

Foetal Insulin theory

Insulin is an important growth factor during intrauterine life. Hattersley and Tooke (HATTERSLEY 1999) have suggested that lifelong insulin resistance could explain associations between birthweight and blood pressure. Insulin is an important mediator of foetal growth, therefore insulin resistance would impair intrauterine growth. The adult would remain insulin resistant and display the phenotype of glucose intolerance and elevated blood pressure. Mechanisms that may explain the link between insulin resistance and hypertension are explored in chapter 5. Their theory is supported by studies of babies with single gene disorders of insulin metabolism. Low birthweight is a feature of these illnesses. This theory explains the associations without invoking programming.

Placental steroid metabolism

A different hypothesis was also published in *The Lancet* (EDWARDS 1993). In rats, decreased activity of the placental enzyme that protects the foetus from exposure to maternal glucocorticoids (11- β hydroxysteroid dehydrogenase type 2) is associated with decreased birthweight. Moreover, administration of dexamethasone to pregnant rats results in reduced birthweight, and higher blood pressure in their offspring. The authors suggest that increased foetal exposure to glucocorticoids may permanently alter metabolic control, with resulting hypertension and insulin resistance.

Hypothalamic Pituitary Adrenal Axis abnormalities

Abnormalities of glucocorticoid metabolism have been demonstrated in numerous cohort studies. Early morning fasting plasma cortisol concentrations were increased in low birthweight members of cohorts from Adelaide, Preston and Hertfordshire (PHILLIPS 2000). A more detailed study of 370 men from the Hertfordshire cohort demonstrated associations between their fasting cortisol concentrations and features of insulin resistance (PHILLIPS 1998). In a further study of the Hertfordshire cohort, 205 men had overnight suppression of endogenous glucocorticoid production following oral dexamethasone (REYNOLDS 2001). Their response to intravenous ACTH was then measured, and was greatest in the low birthweight individuals.

Thus, there is evidence that it is possible to programme the Hypothalamic Pituitary Adrenal Axis, and evidence of abnormalities of the functioning of components of the

axis in adult males with low birthweight. It might be that different influences on birthweight have similar, long lasting effects upon the functioning of the Hypothalamic Pituitary Adrenal Axis, and subsequent higher blood pressure.

Potential mediators of birthweight: blood pressure association:

Vascular Structure

The vascular tree is a supremely efficient mechanical system. It takes a lifetime for the subtle increase in work required of the vasculature in hypertension to manifest itself in the catastrophic events of stroke or myocardial infarction. This section will discuss the changes that are present in vascular and cardiac structure in many patients with hypertension. These changes are important in the altered haemodynamics in hypertension, and have prognostic significance. This leads to the hypothesis that altered vascular structure could be programmed by early development, which would in turn, programme the development of increased blood pressure.

Increased peripheral resistance with a normal cardiac output is the hallmark of hypertension. Resistance varies throughout the vascular tree and vessels of less than 500 micrometers in diameter form the greatest component (BORDERS 1986, STRUIJKER BOUDIER 1992). Abnormalities of these vessels have been described in human skin (PRASAD 1995), muscle (HENRICH 1988, PEDRINELLI 1990), conjunctival (HARPER 1978), retinal (STANTON 1995) and gut vasculature (SHORT 1958) in hypertension and can be shown to increase resistance (FOLKOW

1995). However, in the presence of normal renal function, the effect on blood pressure from increased peripheral resistance would be cancelled out by pressure natriuresis. (GUYTON 1972) In other words, over a remarkably wide range of blood pressure, any rise in pressure is followed by increased sodium excretion which restores the original blood pressure. Transplantation experiments performed in immunologically compatible rats, between hypertensive and normotensive individuals show that the transplanted kidney dictates the blood pressure of the recipient (BIANCHI 1974, DAHL 1975). A series of patients with endstage renal failure as a result of severe hypertension who had therapeutic renal transplantation gained remission from hypertensive disease maintained over five years (CURTIS 1983). This study preceded routine cyclosporin use which confers hypertension upon most recipients. Therefore if increased peripheral vascular resistance is a primary change in hypertension, factors involved in the generation and maintenance of increased vascular resistance must be considered for both renal and non renal vasculature.

Structural changes in peripheral vessels

The vasculature of an individual with established hypertension has several distortions from normal. Vessel walls are thickened in the small arteries and resistance arterioles, and vessels are diminished in number or rarefied. These changes have been described for more than one hundred years.

Thickening of vessel walls with consequent increase in wall to lumen ratio is termed hypertrophy. The methods used to investigate this change in humans have been reviewed by Schiffrin (SCHIFFRIN 1997). The term implies growth and division of wall components but this may be misleading. Baumbach and Heistad (BAUMBACH 1989) were first to suggest that it is remodelling of existing vessel components that results in the thicker wall. They demonstrated this in cerebral arteries of Stroke prone Spontaneously Hypertensive Rats (SpSHR). Work reviewed by Heagerty *et al* (HEAGERTY 1993) has shown that in most studies this is the dominant effect in essential hypertensive patients and in Spontaneously Hypertensive Rats (SHR). Growth and cell division contribute much less.

Rarefaction refers to reduced vessel number per volume of tissue. This phenomenon has been demonstrated in multiple tissues in humans and rats. Short described arteriolar rarefaction in hypertensive cadavers in the 1950s (SHORT 1958, SHORT 1966); *in vivo* rarefaction has been demonstrated in conjunctivae (HARPER 1978), skeletal muscle (HENRICH 1988) and nail beds (GASSER 1992) in hypertension. This may be a response to local pressure in the studied network and a functional state may precede anatomical changes. SHR have diminished arteriolar numbers which can be overcome by high dose vasodilators in young animals but not in older animals (HASHIMOTO 1987). However, this interpretation is challenged by data from experiments inducing hypertension by coarctation (BOEGEHOLD 1991). The hindquarters of the animal are not exposed to the elevated systemic pressure but nevertheless rarefaction occurs. The cellular mechanism involved in development of rarefaction may well involve apoptosis. Electron micrographic studies demonstrated

cellular markers of apoptosis (GOBE 1997) in endothelial tissues in Goldblatt hypertensive rats (one kidney, one clip).

Left Ventricular Hypertrophy

Thickening of the left ventricle is an important feature of hypertension as it is associated with higher risk of death (LEVY 1990). Left ventricular hypertrophy (LVH) can be detected by electrocardiography, or more accurately by ultrasound or magnetic resonance imaging (KANNEL 1998). The prevalence of LVH increases with age, systolic blood pressure and obesity (LEVY 1988), and the prognostic implication remains when these, and other cardiovascular risk factors are controlled for in multiple regression analysis. Although population derived cut off points are often used in studies, there is a linear association with risk of death at all levels of LV Mass. A 50g increase in LV Mass predicts a 1.49 increase in relative risk of death (LEVY 1990). The processes involved in the development of LVH are discussed later.

Haemodynamic effect of structural vascular changes

The key experiments that first illustrated the functional significance of these changes were performed by Bjorn Folkow in Sweden in the 1950s. Prior to this, decreases in the minimum vascular resistance of tissues had been described in hypertensive patients with plethysmography (PICKERING 1950). Folkow showed for the first time (FOLKOW 1958) that the relationship between minimum vascular resistance

and pressor responses to noradrenaline was identical in hypertensives and normotensives. He inferred that for any given pressor tone, resistance from hypertensive vessels will be greater as a result of the increased luminal encroachment by the thickened wall. Thus, the structural change in the vessel wall is translated into the functional change of increased resistance without requiring alterations in smooth muscle activity or pharmacological sensitivity to vasoactive agents.

The "Structural Factor" Folkow described is a generally accepted principle in hypertension. The relative significance of rarefaction arouses more controversy. Hallback et al (HALLBACK 1976) simulated rarefaction in the hindquarters of a rat by injection of microspheres. These were 50 μm in diameter and blocked 50% of microvessels of that size. No enhancement of the effect of vasoconstrictors was observed and he concluded that rarefaction did not significantly increase resistance. However, a mathematical model study by Greene *et al* (GREENE 1989) demonstrated that rarefaction of 42% of 3rd or 4th order vessels would lead to 21% increased resistance. This model was based on the hamster cheek pouch which forms an idealised branching structure. This may have different characteristics to vascular beds which contribute more to peripheral resistance. Modelling techniques are not yet sufficiently advanced to describe interactions between hypertrophied vessels and rarefied networks. It has been suggested that vessel remodelling proximally and distal rarefaction will act in a synergistic manner (ZWEIFACH 1983) and the combination will lead to greater resistance than the simple product of each

component. Sophisticated modelling may be the path for future research to establish the relative importance of observed changes in different vessels.

Changes in the renal vessels

The cardinal histological change seen in hypertensive kidneys is narrowing of the afferent arteriole. The classic studies by Sommers (SOMMERS 1958) identified a spectrum of disorder throughout 1800 renal biopsies. This varied from focal spasm of arterioles with concentric overlapping of smooth muscle in the mildest cases to more severe changes with endothelial oedema leading to luminal encroachment, smooth muscle hypertrophy, degenerative changes with hyalinisation and irregular and focal luminal narrowing. Traditionally these changes were thought to develop after some years of established hypertension (CASTLEMAN 1948) A recent study examined renal tissue from young adults who died traumatically and demonstrated a correlation between arteriolar narrowing with increasing incidence of hypertension in their country of origin (TRACY 1992).

These changes are not uniformly distributed through the kidney and will lead to relative ischaemia in the more severely effected nephrons. Sealey (SEALEY 1988) *et al* have proposed that the renin secreted by the ischaemic nephrons will have a disproportionate effect on blood pressure. The majority of normal nephrons will ensure adequate renal function, and increases in blood pressure will tend to lead to correction through sodium natriuresis. However, this response will be incomplete due to the promotion of sodium retention though angiotensin II from the ischaemic

nephron population. This phenomenon may explain in the variation between an individual's responses to sodium loading, and the residual circulating renin in some hypertensive patients when renin should be suppressed by increased perfusion pressure.

The origins of vascular structural changes: Developmental Relics or Compensatory Plasticity?

So far this section has described structural changes which have been reported and commented on the haemodynamic alterations which result. We have shown that vascular structure can influence renal function, peripheral vascular resistance and hence blood pressure. Could they be a programming mechanism linking events in early development with higher blood pressure?

Hypertrophy and rarefaction are maladaptive changes in view of the demonstrable decrease in mechanical efficiency that they cause and the vascular catastrophes that they predate, but they result from the same developmental principles that create efficient structures with minimal mechanical work. Control of vessel growth and development is determined by nutritive demands from the dependant tissues (ADAIR 1990). Feedback from the resulting flow in the form of shear stress and wall stress alters growth and development of cellular wall components. These processes determine embryonic development of a vascular tree and subsequent remodelling to the changing demands of the growing organism. This plasticity of vascular structure is retained in the mature adult and can be demonstrated in animal models.

The interaction between the ability of structures to remodel towards the ideal for their function, and the limits imposed by the other components of the cardiovascular system can be illustrated by the following example. Wall remodelling has been shown mathematically to reduce tension in the vessel wall when it is exposed to increased pressure (ZWEIFACH 1983) and is thus adaptive to the circumstances of that vessel. However remodelling results in luminal encroachment and increased resistance which increases the pressure loading on the system, and is maladaptive. This also illustrates the central idea of programming; adaptive responses to circumstances early in life may become maladaptive for the adult organism.

We suggest that structural changes may be present at an early stage of development as a result of relative increases in flow and pressure during intra-uterine life in the low birthweight baby. These changes, or an increased capacity to develop these changes, may persist and manifest themselves in adult life as hypertension. To support our hypothesis, we will describe normal vascular development, animal experiments demonstrating retained adult plasticity, and human studies showing altered vascular structure early in the development of hypertension.

Vascular Embryology

Vascular cells derive from mesoderm, differentiating into blood cellular components and vessel components before flow is established. Growth of vessel walls is initially controlled by VEGF primarily leading to differentiation and division. As marshes of

blood vessel components stir into flowing channels, tissue demands mediated through oxygen and adenosine as paracrine factors will stimulate further growth (RISAU 1997). A combination of vessel wall factors in response to circumferential and shear stress will stimulate development of additional wall components and accumulate smooth muscle cells and extra-cellular matrix including elastic fibres that characterise arteries and arterioles. The cellular and molecular mechanisms governing this process are beginning to be identified and are comprehensively reviewed by Cowan and Langille (COWAN 1996). Network factors are less well studied in vivo than cellular mechanisms. The methods by which genetic blueprints for a vascular tree or organogenesis are translated into functioning structures are beginning to be understood. A family of homeobox genes has been identified and linked to tissue polarity in wing development in *Drosophila* (BLANKESTEIJN 1996) and myocardial repair in rats (DE ROBERTIS 1990).

Cardiac Embryology

Embryological development of the heart occurs during the early weeks of gestation. Formation of the initial tube occurs at day 21 (SADLER 1984). This coils around itself and initially divides into two chambers. Septa sprout from opposite poles of the ball like heart and grow towards each other establishing the twin pumping systems of pulmonary and systemic circulations. Ventricular growth will be stimulated by stretching of myocytes, as the functioning vasculature responds to the demands of the growing organism, and by sequences of gene activation, cascading in concert. Further demands will be made on the left ventricle at birth, when the output of the

right ventricle switches from the systemic circulation to the pulmonary circulation with closure of the ductus arteriosus. This will lead to further growth of left ventricle establishing adult proportions between the ventricles. Hyperplasia of myocytes continues throughout intrauterine development, although during the final trimester the ability of myocytes to divide is lost (RAKUSAN 1985) and further growth of the muscle occurs through growth of individual cells and laying down of extracellular matrix.

Plasticity of mature vasculature

There are limits to the further differentiation of the mature vascular tree. Clearly it is not possible to grow a second aorta. Animal models suggest that there is considerable plasticity of vessels supplying muscles. Pig right ventricles hypertrophy in response to a pulmonary artery banding (WHITE 1992). Haemodynamic studies of the right coronary arteries in these animals demonstrated no additional resistance compared to control arteries indicating that the increased demands of the muscle produce an ideal, mechanically minimised network. The characteristics of the new network are an increased ratio of arterioles to capillaries; the number of vessel divisions from coronary ostium to capillary was found to be increased from 10 to 11. The increased branching begins in proximal vessels indicating residual plasticity in well differentiated structures. Tissue demands will not be the sole stimulus to vessel growth in this model and it is possible that chronic vasodilatation and muscle pressures may also be transduced into vascular growth.

Another model that demonstrates adult vascular plasticity is the cremaster muscle of rats with unilateral orchidectomy (WANG 1991). The operated side has diminished vessel density in an identical milieu other than loss of load bearing function in the muscle.

Mathematical modelling

Mathematical models of vascular networks support the theory that shear stress and circumferential stress in combination as modifiers of growth can lead to idealised flow (KIANI 1991). Alternatively with different criteria in the model the network would remodel into a single vessel (HACKING 1996). These models are relatively crude, limited to describing possible mechanisms to create capillary meshworks to supply homogenous tissue metabolic demands.

Plasticity of Renal Vasculature

Irregular involvement of nephrons in pathological responses could be accentuated in the presence of limited absolute nephron number. This theory was first outlined by Brenner in 1988 (MACKENZIE 1995). Nephron number varies between individuals from 300,000 to 1,100,000 (NYENGAARD 1992) and is determined at birth (BRENNER 1985). Further renal growth is finite and limited to increasing the size of components of the nephron. Individuals with numerically limited functional reserve will be more susceptible to the pathological processes of high blood pressure

described above. The supporting evidence is accumulating but is circumstantial. Populations with different susceptibilities to salt sensitive hypertension have been shown to have smaller average size of kidneys (LUFT 1979). Experimental renal mass reductions increase the risk of hypertension (BRENNER 1985) as does surgical reduction in removal of renal tumours (NOVICK 1991) or unilateral nephrectomy for donation (HABERAL 1998).

Development of Left Ventricular Hypertrophy

Ventricular growth continues during childhood, once adult size has been determined LV Mass is dependent upon demands. Obesity is associated with increased LV mass (DE SIMONE 1992). Men have greater LV mass than women, but this is mostly accounted for by increased lean body mass (DE SIMONE 1995). There is probably no growth with advancing age in maturity, although it is very difficult to separate the effect of age and the increasing incidence of hypertension that occurs with age. Physically active people will have greater LV Mass than sedentary people, but when the demands decrease, regression of hypertrophy occurs rapidly. This is in contrast to the limited regression seen in treatment of hypertension associated LVH (SCHMIEDER 1998).

Hypertensive Cardiopathy?

The additional growth in left ventricular size that occurs in association with hypertension is qualitatively different from the normal growth and development

processes, or the hypertrophy associated with athletic training. These differences have pathophysiological implications.

Pathological hypertrophy is characterised by growth of individual myocytes, and increase in the collagen content of extra-cellular matrix, leading to fibrosis (WEBER 1998). Increasing tissue mass is not accompanied by matched vascular growth, and coronary reserve, the capacity of the vascular bed to accommodate increased flow, is decreased (SCHWARTZKOPFF 1998). These changes lead to increased ventricular stiffness and therefore increased myocardial oxygen demands. Tissue ischaemia promotes further fibrosis, cardiac arrhythmias, and eventual heart failure.

LVH and prediction of subsequent blood pressure

Increased left ventricular mass predicts individuals who will go on to develop the greatest rise in blood pressure in follow up studies. (DEVEREUX 1991) This has been found in the Framingham study (POST 1994), in a population of employed adults of average age 47 (DE SIMONE 1991), a population of middle aged Japanese men (ISO 1994) and in children aged 6 – 15 years (MAHONEY 1988). This is in contrast to other haemodynamic variables which do not predict risk of hypertension independent of stature (POST 1994).

It seems plausible that the development of higher blood pressure, and left ventricular hypertrophy develop in parallel. This is predicted by the theory proposed by Lever and Harrap (LEVER 1992) that the central abnormality in hypertension is of

mismatch of blood pressure rise and somatic growth in childhood. It may be that common hormonal and growth factors are involved in both processes.

Vascular Structural Changes in early Hypertension

Evidence supporting programming of vascular structure as an important process in the development of hypertension comes from studies showing structural changes early in the process, especially in people with a familial tendency to develop hypertension. Japanese subjects with a strong family history of hypertension have diminished reactive hyperaemic forearm blood flow (TAKESHITA 1982). Subjects with early essential hypertension characterised by higher cardiac output had capillary rarefaction demonstrated in conjunctival micrographs (SULLIVAN 1983).

Familial determinants of blood pressure have been studied through a novel epidemiological method. The population attending a single medical centre at Ladywell in Edinburgh had their blood pressure screened. By studying groups of offspring defined by a function of their own and their parents' blood pressure, factors associating with their familial tendency to develop high blood pressure can be separated from factors consequent upon their blood pressure. Non invasive studies of microvascular blood flow demonstrated abnormalities in the offspring who shared above average blood pressure with their parents (NOON 1997). They had 50% lower maximal blood flow in heated skin and diminished capillary number following venous occlusion. These abnormalities are of the magnitude observed in established hypertension but the differences in blood pressure between the subject groups were

small (6 mmHg). Thus abnormalities in the microvasculature are present prior to a rise in pressure, and not present in everyone with a higher pressure suggesting a possible causative role in the inherited rise in blood pressure.

These changes will tend to increase blood flow in the fewer vessels, which we know from the animal studies will provide the stimulus to develop more vessels to correct the mismatch between growth and flow. Yet the abnormalities persist. This might be due to decreased sensitivity to the stimulus of increased flow or to the growth message transduced by that flow. Alternatively the peripheral microvessel structures may be an appropriate response to the pressure set by the kidney which may have fewer nephrons or populations of ischaemic nephrons influencing the pressure natriuresis relationship.

Whatever the precise mechanism we can appreciate that these subtle maladaptions can develop, persist and feedback to increase peripheral resistance. Adaptive structural alterations from the ideal may be beneficial during times of rapid growth and development, or protective during nutritional insufficiency but burden the organism with the structural basis for subsequent high blood pressure and increased risk of premature death.

Microvascular structure and function, and Insulin resistance

In addition to the effects on peripheral vascular resistance that have been discussed, it has been postulated that altered microvascular structure could also contribute to

insulin resistance, by limiting the rate of glucose delivery and therefore insulin-mediated glucose uptake (LILLIOJA 1987). Normotensive individuals with fasting hyperglycaemia have a decreased skin hyperaemic response which is associated with a higher fasting insulin concentration (JAAP 1997) Moreover, glucose uptake may be enhanced by insulin-mediated vasodilatation, which is largely dependent upon nitric oxide synthesis (BARON 1995). Insulin resistance is associated with high blood pressure in many patients with hypertension (FERRANNINI 1987) and also those at risk of hypertension (HULTHEN 1995), although the mechanism is unknown.

This review has focussed on vascular structural abnormalities present in hypertension and in individuals at risk of hypertension. Blood vessel function is also altered in hypertension (PANZA 1990) and in individuals at risk (TADDEI 1992), demonstrated by impaired vascular responses to the endothelium dependant vasodilator acetylcholine.

Endothelial dysfunction may therefore contribute both to insulin resistance and hypertension. These observations raise the possibility that diversity of vascular function or structure contributes to the variance of both insulin sensitivity and blood pressure in health. It also follows that programming of vascular structure or function could mediate insulin resistance in addition to higher blood pressure.

Summary of introduction, and statement of hypotheses

To summarise the introductory chapter, low birthweight predicts a range of cardiovascular risk factors in later life. This may or may not represent a causative relationship. If it is causative, and "programming" has occurred this may have been during intra-uterine life, or during postnatal development. Furthermore, it has not been established what systems are susceptible to programming in humans, and we have outlined a rationale for programming of vascular structure.

Many of the studies describing this area are retrospective analyses of fortuitously preserved records. The relationship between low birthweight and high blood pressure is robust, but there are several issues which have not been clarified:

- What is the effect of length of gestation?
- What is the role of confounding from maternal factors and from socio-economic deprivation?
- What is the role of catch up growth?
- What is the role of infant nutrition?

These questions are considered in the study described in the next chapter, which investigates associations between adult cardiovascular risk factors and observations made in a prospectively recruited cohort of low and normal birthweight individuals born in Edinburgh.

If a system has been programmed by events in intrauterine life then abnormalities of that system should be detectable early in the development of abnormal functioning of that system. Evidence for programming of vascular structure is considered in the premature delivery cohort, and described in chapter 3, and in a separate cohort of schoolboys in chapter 4. Chapter 4 also examines parental influences on the relationship between blood pressure and birthweight, and considers the role of socio-economic factors in more detail.

Interrelationships between blood pressure, insulin resistance and vascular structure have been described in the final section of the introduction. Chapter 5 reports an analysis of assessments of microvascular structure and function in healthy young men drawn from a cohort of differing familial predisposition to hypertension. This work explores functional implications of alterations in microvessel physiology which are relevant to the hypothesis that vascular structure may be programmed by events that influence birthweight.

Chapter 2: The influence of premature delivery on adult

cardiovascular risk factors.

Premature delivery exposes an infant to greatly increased perinatal morbidity and mortality. It also accounts for an important proportion of population birthweight, as 5-10% of individuals are born prematurely, and a large part of attained birthweight occurs during third trimester growth. It is perhaps curious that this profound event did not seem to influence relationships between birthweight and subsequent blood pressure in the early studies. Closer examination of the literature shows that an influence of birthweight can be demonstrated, and that it has been a minority of studies that have had information on gestation. This study examines the hypothesis that premature delivery programmes subsequent cardiovascular risk.

In the Simpson Memorial Maternity Pavilion in Edinburgh between November, 1973, and February, 1975, mothers of 72 low-birthweight (<2000 g) babies agreed to participate in the study. As controls, mothers of 54 normal birthweight (>2000 g) babies born on the same day and matched for sex, birth order, and father's social status, were also recruited. This was conceived as a study of outcome of low birthweight, with particular reference to nutritional factors. Gestational age was estimated from the mother's last menstrual period and by developmental assessment by a paediatrician. Low-birthweight babies were classified as either appropriate weight for gestational age (n=30) or having IUGR (n=42) by means of a cut-off of the 10th centile on Gairdner-Pearson growth charts (GAIRDNER 1971). Detailed records were compiled for parents and offspring. Parental data included adult height

and weight and birthweight. Categorized details were recorded of the neonatal period, unfortunately not including maternal BP. The infants were closely followed up with detailed anthropometric measurements recorded at least every 4 weeks until 1 year of age, and less frequently until 7 years of age. The infants diet was based on powdered milk which allowed for remarkably detailed calculations of dietary components. Mothers weighed every item of food 3 days per week for one year. Their childhood growth, nutrition and development has been reported previously (BELTON 1986).

In 1998-99 we contacted 81 of the original 126 babies. Of these, 61 consented to a further study approved by the local ethics committee. Parental and offspring measurements in infancy did not differ between participants and non-participants. (table 2.1)

Table 2.1

	Non-responders (mean)	Responders (mean)	p-value
Mothers age (years)	26.0	24.6	0.10
Social class	3.2	2.9	0.12
Placental Weight (g)	587.0	559.1	0.50
Apgar 1 minute	6.6	6.8	0.63
Apgar 5 minutes	8.9	9.0	0.49
Maternal Height (cm)	158.5	158.9	0.73
Maternal weight (kg)	55.4	55.3	0.97
Paternal Height (cm)	173.6	174.6	0.49
Paternal weight (kg)	73.4	71.0	0.12
Days of prematurity	22.9	25.4	0.59
Birthweight (g)	2358	2321	0.80

Participants attended the hospital at 0900 h having fasted from midnight. Females attended during the follicular phase of their menstrual cycles. Height, weight, and abdominal and hip circumferences were recorded before participants sat and acclimatised to a temperature-controlled environment (23-25°C) for 10 min. Blood pressure was recorded in the right arm by means of an automated sphygmomanometer (Omron HEM 705CP Hutchings Healthcare, Henfield, UK). The mean of two recordings was used for analysis, unless they differed by >5 mm Hg in which case a third recording was made and the mean of two closest matches

was used. Venous blood was obtained to measure glucose by an enzymatic technique (Capas Mira Plus, Roche Diagnostics, Lewes, UK), insulin by enzyme immunoassay (Eurogenetics Tasah, Hampton, UK), and lipids by dry slide principle (Vitras 950, Ortho Clinical Diagnostics Amersham, UK). The clinical investigator (myself) was masked to the birthweight of participants until data collecting was complete.

Statistics

Data are presented for the three groups, low birthweight babies who were appropriate for gestational age (AGA), low birthweight babies that had intra-uterine growth retardation, (IUGR) and controls. Group comparisons were tested by unpaired student *t* test. Regression analyses were made using the combined groups.

Results

Low-birthweight premature babies had higher adult blood pressure and fasting plasma glucose than normal birthweight controls born at term (table 2.2). Among low-birthweight babies there was no difference in blood pressure or plasma glucose between those whose low birthweight was appropriate for gestational age and those who had IUGR. Other cardiovascular risk factors were not significantly different between groups, but there were trends for an adverse metabolic profile (higher plasma insulin, triglyceride, and total cholesterol, and lower high-density lipoprotein cholesterol) in the group with appropriate weight for gestational age rather than the IUGR group.

Table 2.2 Data are mean \pm SD. Comparisons were made between controls and all low birthweight subjects, and between AGA and IUGR, by unpaired Student's t test:

*p<0.02; **p<0.001.	Controls	All Low Birthweight	Low Birthweight Appropriate for Gestational Age (AGA)	Low Birthweight Intra-Uterine Growth Retardation (IUGR)
Number of Males/Females/ Females on oral contraceptive	11 / 16 / 4	13 / 21 / 4	9 / 10 / 2	4 / 11 / 2
Birthweight (kg)	3.13 \pm 0.45	1.68 \pm 0.22 **	1.66 \pm 0.22	1.70 \pm 0.22
Gestational age at birth (wk)	39.3 \pm 1.9	33.4 \pm 2.3 **	31.9 \pm 1.5	35.2 \pm 1.7 **
Weight at term (kg)	3.08 \pm 0.41	2.82 \pm 0.33	3.05 \pm 0.24	2.60 \pm 0.24 **
Age when studied (y)	24.0 \pm 0.7	24.3 \pm 0.5	24.4 \pm 0.4	24.3 \pm 0.6
Adult height (m)	1.68 \pm 0.10	1.67 \pm 0.09	1.70 \pm 0.08	1.63 \pm 0.09 *
Adult weight (kg)	64.9 \pm 11.1	66.9 \pm 12.7	71.7 \pm 13.4	60.8 \pm 8.7 **
Adult systolic blood pressure (mmHg)	115 \pm 9	122 \pm 12 *	123 \pm 9	120 \pm 14
Adult diastolic blood pressure (mmHg)	73 \pm 7	78 \pm 7 *	80 \pm 7	77 \pm 6
Fasting plasma glucose (mmol/l)	4.2 \pm 0.4	4.5 \pm 0.4 *	4.4 \pm 0.4	4.6 \pm 0.3
Fasting plasma insulin (mU/l)	4.9 \pm 2.8	5.1 \pm 2.0	5.6 \pm 2.1	4.3 \pm 1.6
Fasting plasma triglyceride (mmol/l)	0.8 \pm 0.3	0.9 \pm 0.3	0.9 \pm 0.4	0.8 \pm 0.3
Total plasma cholesterol (mmol/l)	4.0 \pm 0.9	4.3 \pm 0.9	4.5 \pm 1.0	4.0 \pm 0.6
plasma HDL cholesterol (mmol/l)	1.4 \pm 0.3	1.4 \pm 0.3	1.3 \pm 0.3	1.5 \pm 0.3

Eight twin pairs participated in the study; after exclusion of twins from the analysis, the differences between groups described in table 2 remained significant except for fasting plasma glucose (4.2 [0.3] mmol/L in controls; 4.4 [0.3] in low-birthweight babies, $p=0.06$).

Confounding by parental factors?

These observations were confirmed in linear regression analyses; both lower birthweight and shorter gestation were associated with higher blood pressure (systolic $r=-0.26$; $p<0.05$ for birthweight and $r=-0.32$; $p<0.02$ for gestation), fasting plasma glucose ($r=-0.32$, $p<0.02$ and $r=-0.27$, $p<0.05$, respectively), and total plasma cholesterol ($r=-0.31$, $p<0.03$ and $r=-0.32$, $p<0.02$, respectively). However, the effects of birthweight were not independent of the effects of gestation (for birthweight adjusted for gestation $r=0.10$, $p=0.70$ for systolic blood pressure; $r=-0.34$, $p=0.20$ for plasma glucose; and $r=-0.15$, $p=0.85$ for cholesterol). That is to say that growth retardation is not *sine qua non* for the association with adult phenotype. These associations with birthweight were independent of other potential confounders, including sex, placental weight, indices of adult obesity, smoking, current medication, and social class in fathers of offspring. Very few mothers had high blood pressure during pregnancy, unfortunately actual blood pressure values were not recorded, but only 5 had high blood pressure. There was no detectable influence from maternal blood pressure on birthweight or adult blood pressure.

Table 2.3

	Beta	St. Err. of Beta	B	St. Err. of B	p-level
Birthweight	-0.28	0.12	-3.81	1.70	0.03
Male sex	0.38	0.12	8.52	2.74	<0.01
BMI (kg/m ²)	0.05	0.13	0.17	0.45	0.70
Current smoker	0.03	0.12	0.07	0.30	0.82
Current medication	-0.11	0.13	-2.11	2.37	0.38
Maternal smoking	-0.02	0.13	-0.24	1.29	0.86
Social class	-0.01	0.12	-0.09	1.21	0.94
Placental weight	-0.07	0.12	0.00	0.01	0.54

Table 2.3

Multiple regression model predicting systolic blood pressure.(R= 0.51, R²= 0.26). Sex, smoking and regular prescription medication were scored as dummy variables, Social class was scored from 1 to 5 according to paternal occupation. In simultaneous multiple regression, male sex and lower birthweight remain significant predictors of higher blood pressure. For birthweight the effect is 3.81 mmHg per kg birthweight (95% CI 0.41 – 7.21).

Influence of catch-up growth

By the time all babies had reached term, the appropriate for gestational age-group were similar in weight to controls, while IUGR babies remained smaller. Even by this early stage of infancy, the inverse links between bodyweight and adult blood pressure ($r=0.21$), plasma glucose ($r=-0.05$), and total cholesterol ($r=-0.02$) were no longer apparent; nor did weights measured in any later 4-week period up to the age of 1 year predict adult blood pressure or biochemistry (data not shown).

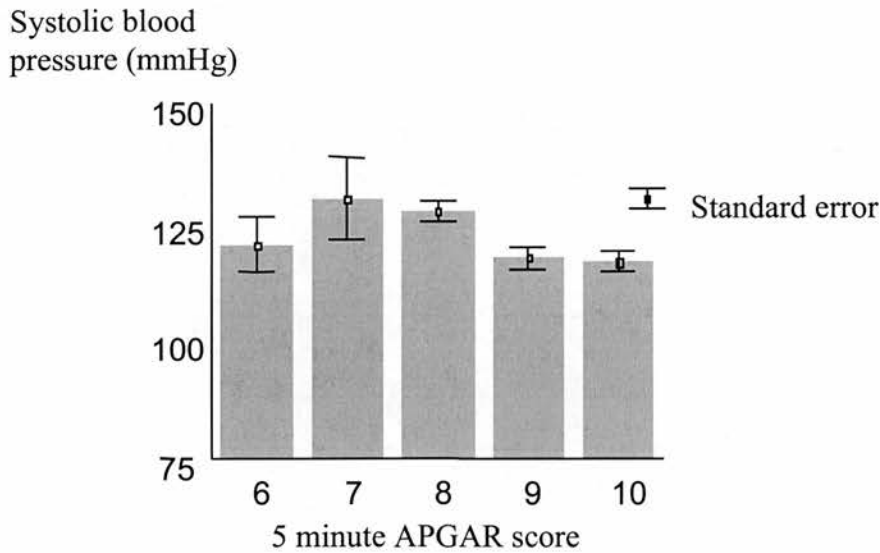
We calculated growth rates in absolute terms, and in growth per kg weight, from birth to term and from term to 44 weeks after last maternal menstrual period, but no measure of growth rate predicted adult blood pressure, (data not shown). In a multiple regression model using sex, birthweight and current weight as predictors, birthweight was a strong independent predictor of blood pressure but current weight was not a significant predictor. The same result was obtained for each of glucose, cholesterol and LDL cholesterol.

Potential programming influences of early neonatal life

Premature delivery exposes the neonate to a very different environment to the baby growing safely attached to a placenta. Information was collected on numerous obstetric and neonatal factors, but they did not predict adult factors independently of gestational

age. This study is too small to answer questions as to which aspect of premature delivery might have effects on adult factors.

Figure 2.1



This graph shows adult blood pressure according to 5 minute Apgar score. This is a clinical score on 5 criteria scored from 0 to 2 on criteria of heart rate, respiration, muscle tone, response to pharyngeal catheter and colour of trunk. A low score indicates that parturition has exposed the infant to hypoxia. In addition a low score predicted higher blood pressure as an adult ($r=-0.27$, $p=0.03$). A similar association was seen with Apgar score at 1 minute ($r=-0.25$, $p=0.05$). However, these associations were confounded by premature delivery in multiple regression analysis, (not shown).

Table 2.4 Infant characteristics of participants

	Males	Females	p-value for sex differences
n	24	37	
Birthweight (kg)	2.37 ± 0.92	2.29 ± 0.73	0.68
Gestational age at birth (weeks)	35.8 ± 0.74	36.7 ± 0.66	0.89
Body weight at term (kg)	3.06 ± 0.33	2.86 ± 0.40	<0.05
Body weight at 1 year after estimated term date (kg)	9.05 ± 1.1	8.39 ± 0.9	0.01
Average nutrition from term to 8 weeks of age			
Fat (g/day)	21.65 ± 4.2	19.06 ± 3.4	0.02
Carbohydrate (g/day)	54.33 ± 11.5	53.93 ± 12.1	0.91
Protein (g/day)	19.83 ± 4.4	18.52 ± 3.4	0.24
Energy (kcal/day)	482.1 ± 82	451.6 ± 74	0.18
Sodium (mg/day)	364.0 ± 97	345.4 ± 70	0.40

Data are mean ± SD.

Infant nutrition was measured for major dietary components (see table 2.4). Daily intake was averaged for time period until 1 year of age (measured from term). There were no associations between intake of macro-nutrients or minerals during infancy and adult characteristics for any time period (e.g. average daily sodium intake during the first eight weeks after birth versus adult systolic blood pressure $r=-0.18$, $p=0.17$).

Discussion

By contrast to the hypothesis that growth retardation in utero mediates programming of adverse cardiovascular risk in babies born at term, we found that among premature babies, those with IUGR were not measurably more disadvantaged than those with birthweights appropriate for gestational age. This is a novel finding that challenges the hypothesis that adult outcomes are the result of decreased intrauterine growth. Careful attention to measurement of, and adjustment for, gestational age should be part of every study of the influence of birthweight. This study, and the other studies showing a relationship between prematurity and higher blood pressure, suggest that there may not be a single mechanistic explanation for the associations between adult cardiovascular risk factors and low birthweight due to premature delivery, and low birthweight due to IUGR, as prematurity and IUGR result from many and disparate influences (described in detail in the introduction).

This is a small study with limited power to determine the relative effects on adult outcomes from IUGR with or without premature delivery. Much larger follow up studies have been performed and demonstrated independent influence of premature delivery, and of birthweight (LEON 2000) on blood pressure in adolescence, although babies born more than 35 weeks gestation were excluded. Similarly, although we had detailed, prospectively collected data on obstetric and neonatal problems, we have insufficient power to determine what specific features of the experience of the premature infant predict higher blood pressure, glucose and lipid concentrations in adulthood. Larger

cohorts of survivors of extremely premature deliveries have been studied in regard to their educational attainment. It would be interesting to measure cardiovascular risk factors in further studies of these cohorts (HALL 1995, LEFEBVRE 1988, WARIYAR 1989).

In this study adult size had little predictive effect on adult risk factors, and growth rates in childhood did not predict adult outcomes. Indeed, repeated measurements of weight during infancy confirmed that participants in the in all groups grew at similar rates. Individuals who had IUGR did not catch up with the AGA group, who had caught up with the controls at 40 weeks gestation. According to Lucas' analysis (LUCAS 1999) this implies that pre-natal factors are more important than post-natal growth. In this study, the dominant pre-natal influence on birthweight is premature delivery, but this is not a single parameter, resulting as it does from many influences. The dominant statistical influence of prematurity may still be a surrogate for the extra-uterine environment during what otherwise would have been the third trimester.

Lower Apgar score was associated with higher blood pressure but no control individuals had very low Apgar scores, so we have no power to test whether hypoxia at birth predicts higher pressure independently of prematurity. This could be tested in analyses of other cohorts described above.

We examined early nutrition using nutritional values obtained by weighing the formula intake of the infants, but did not demonstrate an influence on adult factors. This is compatible with the results reported by Lucas *et al.* demonstrated that blood pressure

was lower in grown-up premature babies who were randomised to pooled breast milk, and not measurably different between groups randomised to different formula preparations (SINGHAL 2001).

Prematurity is frequently associated with pre-eclampsia, and though we could not demonstrate an association with maternal hypertension in our data, this reflects lack of power. Maternal pre-eclampsia could influence blood pressure through shared genetic influences on blood pressure in addition to mechanisms that relate to premature delivery.

In conclusion, this study, though small, raises interesting questions about many aspects of neonatal life that might act as programming influences on later cardiovascular risk. Larger studies of survivors of extreme prematurity, matched to appropriate controls, may provide more definitive answers to these questions.

Chapter 3: Higher adult blood pressure in premature low birthweight babies, evidence for programming of vascular structure.

The introduction to this thesis described vascular structural abnormalities that are present in high blood pressure, and an outline of how the developmental principles that govern these changes could lead to programming of high blood pressure by early events. This possibility has been investigated in other studies.

Intrauterine manipulations which retard fetal growth in animals can permanently alter fetal cardiac and peripheral vascular structure (MUROTSUKI 1997). There is evidence that adult men born at term with low body weight have altered structure of conduit vessels, indicated by decreased compliance assessed by ultrasound (MARTYN 1995), and altered structure of smaller vessels in the retina, indicated by altered arteriolar branching that suggests a rarefied microvasculature (CHAPMAN 1997). In studies of young adults, changes in microvascular structure have been associated with predisposition to hypertension (NOON 1997) and diabetes mellitus (JAAP 1997), so these may be an important link between events in early life and subsequent cardiovascular risk factors.

It has proved more difficult to relate cardiac structure, measured by transthoracic echocardiography, with parameters at birth, although two studies suggested a link between body weight between 9 months and 2 years of age with left ventricular mass in adulthood (VIJAYAKUMAR 1995, ZUREIK 1996). There is a large body of evidence suggesting that ventricular hypertrophy occurs early in the development of hypertension

(DEVEREUX 1991). Moreover, left ventricular hypertrophy is an important cardiovascular risk factor, independently of its association with hypertension (LEVY 1990), so programming of cardiac development could contribute to the high prevalence of adult cardiovascular disease in low birthweight babies.

Many epidemiological studies in this area have examined babies born at term, but in the modern era of neonatal care, most surviving low birthweight babies are born prematurely. The second chapter described cardiovascular risk factors in a cohort of individuals including premature, low birthweight babies. In this chapter we explore the possibility that vascular structure has been programmed in these individuals.

Methods

The characteristics of participants have been described in chapter 2.

Protocol

In addition to the measurements described in chapter 2, further detailed assessments of vascular structure were made. Subjects completed a nutritional and exercise questionnaire (exercise scored as 0 or 1, according to whether subjects took aerobic exercise at least three times each week). The following measurements were made in sequence by a single observer (myself) who was blind to the birthweight group of the participants. Further details of several of these measurements and their validation have been published previously (NOON 1997).

2. Pulse wave analysis was performed in the right radial artery (Sphygmocor, PWV Medical, Australia). Augmentation index (that is the ratio between calculated central pressure, and measured peripheral pressure) was derived using the validated transformation factor. This measure has been validated in our laboratory (WILKINSON 1998).

3. Maximum vasodilatation of skin microvessels was measured by laser Doppler fluximetry in response to local heating to 42°C. A 1cm brass heating element (Moor instruments Ltd. Devon) was applied to the volar aspect of the left forearm. The skin temperature was monitored with a thermocouple which passed through a hole in the heating element. After 30 minutes the thermocouple was removed and a laser Doppler probe was placed through the hole. The heater was rotated through 8 positions and the signal recorded in each area for 30 seconds. To record biological zero flux, a sphygmomanometer cuff was applied to the upper arm and inflated to supra systolic pressure for one minute. Minimum dermal vascular resistance was calculated as $(\text{mean arterial pressure}) / [(\text{mean of 8 maximum flux recordings}) - (\text{biological zero})]$ (NOON 1997).

4. Dermal capillary density was recorded on the dorsum of the middle phalanx of the right index finger by intravital videomicroscopy. The skin was prepared with a coating of clear nail varnish. Dermal capillaries were visualised using a microscope (Leitz; Leica UK, Milton Keynes, UK) under illumination with a mercury filament lamp (Leitz). Six adjacent fields of 0.25 mm² were recorded via a television camera (Phillips LDH0703; KRP Power Source, Newbury, UK) onto videotape for one

minute at baseline and again after 10 minutes of venous occlusion, achieved by inflating a cuff (Peni-cuff; Hokanson, Bellevue, WA) to 40mmHg around the base of the finger. Calibration was checked periodically with a graticule (Graticules Ltd., Tonbridge, UK). Counting of capillaries was performed at a later date. Thus the investigator was not aware of the blood pressure of participants during analysis.

5. Ultrasound images of the heart were recorded using an Accuson xl750 (Mountain View, CA). Using 2D echo in the parasternal long axis view, the M mode cursor was positioned through the tips of the mitral valve leaflets. M-mode images of sufficient clarity to analyse were obtained in 59 individuals, but in 12 the orientation of the probe was oblique and these images were not included in the final analysis. Characteristics of subjects in whom echocardiography was technically unsuccessful were not different from other participants (data not shown). Analysis was performed at a later date. Left ventricle (LV) dimensions were calculated according to the ASE convention. The mean dimensions from 3 cardiac cycles were used to calculate LV mass according to the formula: $LV\ mass\ (g) = 0.832[(IVS + LVEDD + PWT)^3 - (LVEDD)^3] + 0.6$, where IVS is intra-ventricular septum width, LVEDD is left ventricular end diastolic diameter, and PWT is posterior wall thickness. LV mass index (LVMI) was calculated by dividing LV mass by body surface area, calculated as $0.20247 \times height(m)^{0.725} \times weight(kg)^{0.425}$, as previously validated (DEVEREUX 1986). Aortic root area was also quantified using 2D echo via the parasternal long axis. From the apical view, continuous wave Doppler flows through the mitral and aortic valve were recorded during 6 cardiac cycles and the mean calculated to

overcome effects of respiration. Cardiac output was calculated as (aortic velocity time integral) x (aortic root area) x (heart rate). Cardiac index was calculated as cardiac output/body surface area. Diastolic function was assessed using E/A ratio (SCHIRMER 2000).

6. Common carotid artery intima-media thickness was measured using an Accuson 7mHz linear phase array probe. The posterior wall of the artery was imaged 1 cm proximal to the carotid bifurcation. Measurements were averaged from left and right. (HOWARD 1993)

7. Forearm blood flow was measured by venous occlusion plethysmography, at baseline and after ischaemia in the left arm. The arm was supported above the level of the heart. Cuffs were applied to the upper arm and wrist and a mercury-in-silastic strain gauge applied to the forearm, with internal calibration (Hokansen, Bellevue WA). During measurements, the wrist cuff was inflated to 220 mmHg to exclude the hand circulation and the upper arm cuff was inflated to 40 mmHg for 10 sec in every 15 sec to occlude venous return and measure forearm blood flow. Baseline flows were recorded for three minutes and calculated from the average of the last five flows. Then, an upper arm cuff was inflated to supra-systolic pressure for 12 minutes. Flows were recorded immediately after release of the cuff and the average of the first five flows used for analysis of maximum forearm blood flow. Forearm vascular resistance was calculated as (mean arterial pressure)/(forearm blood flow) (NOON 1997).

Statistics

Data were tested for normal distribution using a Shapiro Wilks *W* test. Birthweight was not normally distributed and relationships with adult parameters were tested using Spearman rank correlation. All indices of vascular structure were normally distributed except maximum forearm blood flow, which was normalised by logarithmic transformation, and LVMI, which was normalised using a negative inverse root transformation (ie $LVMI^{-0.5}$). No simpler transformation (natural or base ten logarithms) conferred normality. Multiple linear regression analyses were used to examine the influence of birthweight after adjustment for potential confounders in early and adult life. Data are presented in the text as mean \pm SD.

Results

Characteristics and confounders

Characteristics of participants, and differences between adult measurements in male and female participants are shown in Table 2.4. No differences between these variables were observed in participants who were twins or triplets ($n=17$) compared with singletons, or in those taking regular medication ($n=18$: including 6 taking oral contraceptives). Current smokers ($n=11$) differed from non-smokers only in having a higher E/A ratio (2.1 ± 0.37 vs 1.8 ± 0.36 , $p=0.03$). Subjects taking exercise at least 3 times per week ($n=15$ males, $n=10$ females) tended to have higher LVMI (LVMI amongst men $107.8 \text{ g/m}^2 \pm 25.5$ with exercise vs 91.8 ± 18.4 without, $p=0.06$; amongst women 82.2 ± 21.4 with exercise vs 69.8 ± 10.1 without, $p=0.12$), than subjects not taking exercise.

Relatively few haemodynamic variables correlated with each other. Higher blood pressure was associated with higher LVMI (for mean arterial pressure $r=0.47$, $p<0.001$). Lower heart rate was associated with higher LVMI ($r=-0.52$, $p<0.001$) and higher E/A ratio ($r=-0.36$, $p<0.01$).

Relationships between measurements in early and adult life

Relationships between measurements in infancy and adult haemodynamic variables are shown in Table 3.2 after adjustment for potential confounders identified above. Low birthweight or premature delivery were associated with higher adult blood pressure and LVMI, independently of measured confounders including gender, blood pressure (for LVMI only), and exercise. (See table 3.1) The effects of gestational age at delivery and birthweight were not independent of each other. Measurements in infancy did not predict any other measured haemodynamic variables.

Table 3.1

Multiple regression model predicting (transformed) LVMI

	BETA	St. Err. of BETA	B	St. Err. of B	p-level
Exercise	-0.300	0.105	-0.008	0.003	0.007
Male sex	-0.231	0.124	-0.006	0.003	0.071
Systolic BP	-0.321	0.117	0.000	0.000	0.009
Heart rate	0.221	0.116	0.000	0.000	0.063
Birthweight	0.213	0.103	0.004	0.002	0.046

R=0.79, R²= 0.63 Adjusted R²= 0.58, F(5,41)=13.671 p<0.001

This multiple regression model describes independent predictors of adult (transformed) LVMI. The transformation reverses the polarity of the association. Thus greater birthweight is associated with greater (transformed) LVMI. Reversal of the transformation following multiple regression analysis is not mathematically possible.

Table 3.2 Adult characteristics of participants and relationships with infant characteristics.

	Males n=24	Females n=37	Male vs Female p	Correlation coefficients ^a		
				Birthweight (g)	Gestational age (days)	Weight at 1 year (g)
Heart rate (min ⁻¹)	59 ± 9	71 ± 13	<0.001	-0.16	-0.07	-0.32 *
LVMl (g/m ²)	101 ± 24	73 ± 14	<0.001	-0.21 *	0.20	0.08
Cardiac index (l/min/m ²)	3.8 ± 0.8	4.0 ± 1.1	0.45	-0.17	-0.11	-0.13
E/A ratio	2.0 ± 0.3	1.8 ± 0.4	0.20	0.19	-0.06	-0.07
Common Carotid intima-media thickness (mm)	0.54 ± 0.04	0.53 ± 0.05	0.34	-0.04	-0.14	0.10
Radial artery augmentation index (%)	-5 ± 13	+10 ± 14	<0.001	0.14	0.13	-0.12
Basal forearm vascular resistance (mmHg.ml ⁻¹ .100ml ⁻¹ .min ⁻¹)	33.5 ± 12.5	33.7 ± 21.4	0.96	-0.14	-0.10	-0.24
Minimum forearm vascular	3.01 ± 1.64	3.42 ± 1.32	0.30	-0.05	-0.16	0.01

resistance ($\text{mmHg}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$)									
Minimum resistance (unit^{-100})	18 \pm 4	23 \pm 7	<0.01	-0.13	-0.11	-0.06	-0.04		
Resting dermal vascular flux ($\text{mmHg}\cdot\text{arbitrary}$ unit^{-100})									
Resting dermal capillary density (0.25 mm^{-2})	25 \pm 4	29 \pm 4	<0.005	-0.08	-0.02	-0.07	0.14		
Maximum capillary density (0.25 mm^{-2})	27 \pm 4	31 \pm 5	<0.005	-0.10	0.02	-0.15	0.16		

Data are mean \pm SD. Comparisons between men and women are by unpaired t tests.

^aCorrelation coefficients are from multiple regression analysis after adjustment for potential confounding effects of sex, exercise, heart rate, and systolic blood pressure where appropriate.

*denotes $p < 0.05$

Figure 3.1

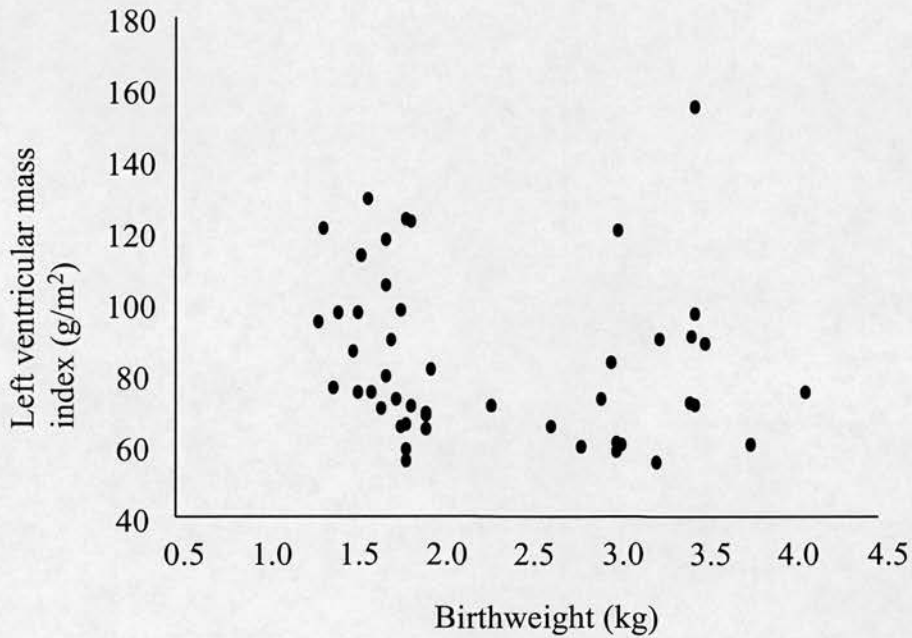


Figure 3.1 Relationship between low birthweight and higher adult left ventricular mass index

This graph shows the effect of birthweight on (unadjusted) left ventricular mass index (LVMI). Spearman rank correlation coefficient $R=-0.29$, $p<0.05$. This relationship persisted in multiple regression analysis (following normalisation) adjusting for potential confounding effects of sex, exercise, heart rate, and systolic blood pressure (adjusted $r = -0.20$, $p<0.05$).

By contrast with the inverse relationship between birthweight and adult LVMI and blood pressure, body weight measured at term (ie 40 weeks after the mother's last menstrual period) and at intervals up to 1 year of age did not predict adult haemodynamic variables (Table 1). This suggests that premature babies destined to higher adult LVMI had completed their 'catch up' growth by their term date. However, calculated growth rates between birth and term did not predict adult variables (eg growth rate between birth and term versus adult systolic blood pressure $r=-0.04$, $p=0.79$; and LVMI $r=0.04$, $p=0.81$).

Remarkably detailed records were kept of infant nutrition during this period of 'catch-up' growth. An apparent correlation between increased intake of fat and higher LVMI, (eg daily fat intake weeks 1 to 8 vs LVMI, ($r=0.47$, $p=0.004$) was confounded by sex in multiple regression.

Discussion

These data show that adults in whom low birthweight was associated with premature delivery, have increased left ventricular mass over and above that which is predicted from their elevated blood pressure. Moreover, the increased adult left ventricular mass occurs in small babies who had normal body weight by one month after their estimated term date (IRVING 2000). Since premature babies who have 'caught up' to normal size by term are unlikely to have suffered intra-uterine growth retardation this suggests that increased LVMI may be the consequence of premature delivery rather than impaired

intrauterine growth. Alternatively, catch up growth in premature babies may predict LV Mass, but we did not confirm this.

This interpretation is consistent in part with larger studies of babies born at term (ie excluding those delivered prematurely) which showed no relationship between birthweight and adult LVMI, albeit that in these studies there were inverse relationships between adult LVMI and body weight between the ages of 9 months and 2 years (VIJAYAKUMAR 1995, ZUREIK 1996). Offspring of mothers who had hypertension (>140/90 mmHg) during pregnancy have higher blood pressure than controls born to normotensive mothers, but LVMI was not different between the groups (HIMMELMANN 1993). In 435 Indian men and women, there was no relationship between birthweight and LVMI (KUMARAN 2000). Length of gestation is not recorded in these papers.

The "Barker Hypothesis" infers from detailed anthropometric measurements at birth, that there are specific temporal 'windows' during development when retarded growth can programme subsequent disease (BARKER 1993) Specifically, late intrauterine growth retardation, indicated by disproportionate linear growth and weight (low ponderal index), is thought to predict insulin resistance while early intrauterine growth retardation, indicated by proportionate growth retardation, predicts hypertension (BARKER 1993). Extending this model, the current data suggest that altered early post-natal growth and development may predict left ventricular hypertrophy. In animals, a temporal window of cardiac myocyte development is recognised, since there is a switch from a hyperplastic to a hypertrophic response to stimulation near term eg in rats

(RAKUSAN 1985, RAKUSAN 1998). In humans, left ventricular mass index increases by around 20% in the first month of life to accommodate the demands of sustaining the systemic circulation without the assistance of the right ventricle provided *in utero* (ICHIHASHI 1999). In premature babies, the increasing mechanical demands on the left ventricle are imposed earlier, perhaps at a time when there is still the capacity for myocyte hyperplasia rather than hypertrophy.

We did not find any evidence to support the hypothesis that peripheral vascular structure is 'programmed' by early life events. However, these results must be interpreted with caution. The number of participants in this study was relatively small. Moreover, the low birthweight babies in this study were all delivered prematurely, and the study had insufficient power to address factors associated with variation in birthweight amongst babies born at term. Previous studies have demonstrated altered vascular structure in adults with low birthweight. Martyn *et al* found decreased iliac artery compliance in middle aged men who had been low birthweight babies (MARTYN 1995). It is possible that this finding represents a consequence of their increased blood pressure as birthweight was not shown to have an influence on arterial compliance in 951 men and women aged 25 years (MONTGOMERY 2000). Chapman *et al* showed decreased retinal vascular density in low birthweight members of a study of 100 middle aged men (CHAPMAN 1997). Gestational age was not recorded in this cohort.

Animal studies have suggested that early nutrition may have a long term influence on adult outcomes (GOULDSBOROUGH 1998, LANGLEY-EVANS 1996), but a previous study of human neonates who were randomised to different diets showed no effect on

blood pressure at the age of 8 years (LUCAS 1994). Our study had detailed, prospectively collected nutritional data for the first year of life and cardiovascular data in adulthood but we did not find associations between different compositions of early nutrition and adult cardiovascular risk factors.

In summary, in addition to higher blood pressure and other biochemical risk factors for cardiovascular disease, premature babies have greater left ventricular mass in early adulthood. This may represent both a marker for greater subsequent rises in blood pressure in these individuals, and a marker for their early vascular death.

Chapter 4

Higher childhood blood pressure in boys attending a fee paying school, evidence for programming of vascular structure

The study described in the previous chapters has described cardiovascular risk factors in a group of adults in whom low birthweight was the result of premature delivery. This chapter discusses the results of a different study that examines further questions that arise from the descriptions of associations between early growth and adult blood pressure.

It has been a persistent criticism of the programming hypothesis that potential confounding has not been eliminated (PANETH 1995, JOSEPH 1996, KRAMER 2000). Social deprivation is associated with both low birth weight and higher blood pressure in later life (COLHOUN 1998), and assessments of social class in cross-sectional studies may be insufficiently subtle to eliminate confounding (JOSEPH 1996). Deprivation may vary to a greater extent within social class than between classes, or may change between birth and measurement of the risk factor in question.

A further uncertainty is whether, if programming of high blood pressure occurs, it results from events occurring before or after birth. Several studies have examined relationships between birthweight and measurements in childhood. (FORRESTER 1996, HASHIMOTO 1996, TAYLOR 1997, WHINCUP 1995, WHINCUP 1996, WHINCUP 1996, WOELK 1998) As in some studies of adults, lower birthweight predicts higher

blood pressure only when adjusted for indices of current size. One interpretation of these associations is that faster childhood growth determines a more rapid rise of blood pressure, and there is no programming effect from foetal growth. (LUCAS 1999)

Against this background, this study examined the hypothesis that low birthweight is associated with higher blood pressure and diminished capillary density in childhood. By studying boys attending a fee paying school we aimed to minimise confounding from socio-economic deprivation.

Subjects and methods

We studied boys attending George Watson's College, a private, fee paying school in the south of Edinburgh. Participants were chosen at random from each year group from the ages of 6 to 16 years. Parents of 110 boys were sent a questionnaire requesting details of parental size, offspring birthweight, and their informed consent. Only 3 declined to allow their sons to participate. Maternal recall of birthweight has been previously shown to be accurate in adults (WALKER 1998, TROY 1996, LUMEY 1994). Ethical approval was obtained from the local research ethics committee.

The study protocol lasted for half an hour. The studies were performed during the school day in a quiet room. Height, weight and waist hip ratio was recorded before the subject reclined on a couch. After one minute blood pressure was recorded once in the left arm using an automated sphygmomanometer (Omron HEM 705CP).

A similar technique for assessment of capillary density was used in both studies in this thesis. To maximise the number of perfused capillaries, venous occlusion was achieved by inflating a cuff (Peni-cuff; Hokanson, Bellevue, WA) to 40mmHg around the base of the finger. Capillary density following venous occlusion represents the maximum capillary number (ANTONIOS 1999 B). After 10 minutes six adjacent fields of 0.25 mm² were recorded via a television camera (Phillips LDH0703; KRP Power Source, Newbury, UK) onto videotape for 30 seconds. Calibration was checked periodically with a graticule (Graticules Ltd., Tonbridge, UK).

Capillary counting was performed between 6 and 12 months after the study. Although I had collected the data on birthweight and blood pressure, the passage of time ensured I was effectively blinded to these data. Statistical tests were performed using Statistica software (Statsoft Inc.). Regression slopes (B coefficients) were calculated and are expressed with 95% confidence intervals.

Results

Characteristics of participants are recorded in Table 4.1. Blood pressure increased with greater age (slope=2.02 mmHg per year, $r=0.50$, $p<0.0001$), height (slope=0.32 mmHg per cm, $r=0.53$, $p<0.0001$) and weight (slope=0.47 mmHg per kg, $r=0.66$, $p<0.0001$). Birthweights were normally distributed in the population (Mean 3.41 kg, range 1.35 – 5.06 kg, Shapiro Wilks test not significant).

Predictions from Birthweight:

Lower birthweight predicted lower childhood height, after adjustment for age (2.90cm per kg birthweight (95% confidence intervals 0.59 - 5.21, $p=0.01$)), but did not predict age adjusted weight (1.37kg per kg birthweight (-1.62 - 4.35, $p=0.36$)), or BMI(-0.10kg/m² per kg birthweight (-1.03 - 0.82, $p=0.82$)). In simple regression, birthweight did not predict systolic BP ($r=-0.14$, $p=0.15$), or diastolic BP ($r=0.05$, $p=0.61$). In multiple regression, after adjusting for the effects of age, height and weight on blood pressure, lower birthweight was shown to predict higher systolic blood pressure (Table 2). Diastolic BP was not significantly predicted by birthweight in similar analyses (data not shown).

Table 4.1 Characteristics of participants

Age (complete years)	n	Height (cm)	Mean	Std.Dev.	Weight (kg)	Mean	Std.Dev.	Systolic BP (mmHg)	Mean	Std.Dev.	Diastolic BP (mmHg)	Mean	Std.Dev.	Capillary density (per 0.25mm ²)	Mean	Std.Dev.
5	7	116.1	6.5	21.0	3.0	101.7	9.4	63.6	13.2	27.2	5.2					
6	10	119.4	4.5	22.9	2.6	103.5	8.4	65.0	10.4	22.9	3.8					
7	10	124.7	6.1	26.1	4.0	104.4	8.4	64.6	7.4	22.9	2.5					
8	11	130.7	8.2	29.8	5.5	102.9	7.4	63.9	4.5	25.1	2.6					
9	12	136.1	4.9	31.4	4.0	98.6	4.9	66.1	6.2	24.9	1.9					
10	13	142.8	6.2	37.1	6.3	107.7	6.5	64.5	7.2	22.9	3.8					
11	14	149.5	7.7	41.9	7.4	108.3	12.1	69.8	8.9	23.3	3.0					
12	5	158.5	17.1	53.2	13.1	114.8	7.8	70.8	8.9	24.1	2.8					
13	12	162.8	6.8	53.2	8.1	117.4	13.1	66.5	8.3	25.2	4.2					
14	5	170.2	8.0	58.4	9.9	116.4	9.2	63.4	5.9	22.2	2.4					
15	7	171.0	11.2	66.7	28.2	116.4	15.7	69.9	10.7	22.8	3.9					
16	1	178.0	0.0	66.6	0.0	144.0	0.0	80.0	0.0	19.3	0.0					
Correlation with age		r	p	r	p	r	p	r	p	r	p	r	p	r	p	
		0.92	<0.001	0.83	<0.001	0.50	<0.001	0.19	0.05	-0.12	0.24					

Predictors from parents:

Parental size predicted birthweight and size of the offspring in childhood. The associations were stronger for mothers than fathers, and for parental height rather than weight or obesity. Thus, lower birthweight was associated with shorter mothers (37g per cm maternal height (19 – 56, $p < 0.0005$)), and shorter fathers (17g per cm paternal height (-10 – 360, $p = 0.06$)). Taller children were born to taller parents (0.47cm per cm maternal height (0.24 - 0.70, $p = 0.0001$) and 0.34 cm per cm paternal height (0.11 - 0.56, $p = 0.003$)). However, parental size did not predict offspring blood pressure. There were no significant correlations between systolic blood pressure and maternal height ($r = -0.05$, $p = 0.62$), maternal weight ($r = -0.07$, $p = 0.48$), paternal height ($r = -0.03$, $p = 0.75$) or paternal weight ($r = -0.001$, $p = 0.99$).

Capillary Density:

Capillary density was not measurable in 9 subjects. Data were rejected from a further 4 subjects in whom fewer than 5 fields were countable. Technical failures occurred because of poor skin preparation or excessive movement. Younger age correlated with fewer countable fields ($r = 0.30$, $p = 0.002$).

In simple regression, capillary density was not significantly associated with birthweight ($r = -0.01$, $p = 0.91$, graph 4.1), systolic ($r = -0.03$, $p = 0.77$) or diastolic blood pressure

($r=0.01$, $p=0.96$) In multiple regression, capillary density did not confound associations between birthweight, current height or weight and systolic blood pressure (Table 4.2).

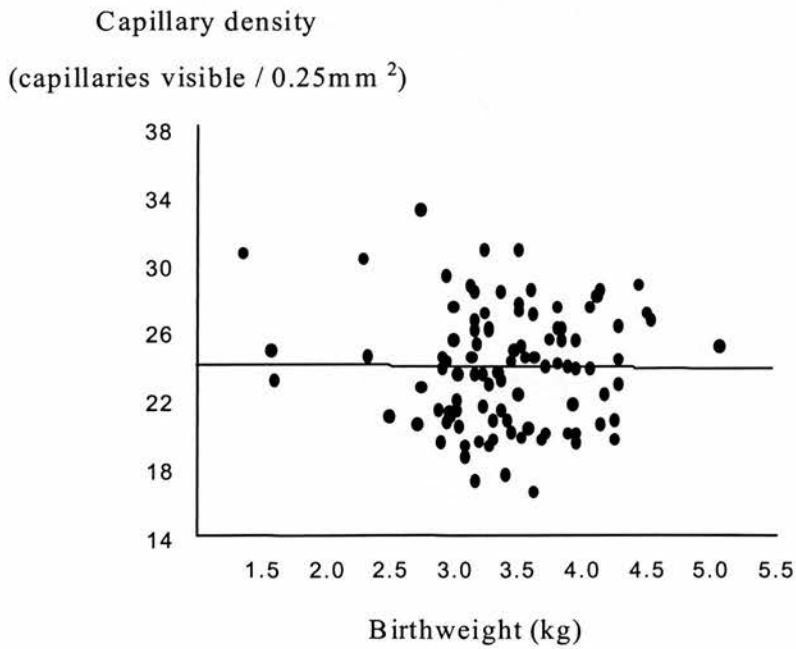


Fig. 4.1

This graph illustrates the lack of relationship between capillary density and birthweight.

($r=-0.01$, $p=0.91$)

Table 4.2

	Regression Coefficient (B coefficient) with systolic BP	95% confidence limits		p
		Lower	Upper	
<i>Model 1:</i>				
birthweight	-3.57	-6.75	-0.38	0.03
age	-0.40	-2.23	1.42	0.66
height	0.39	0.11	0.67	0.01
<i>Model 2:</i>				
birthweight	-3.22	-5.95	-0.50	0.02
age	-0.74	-1.76	0.28	0.15
weight	0.58	0.40	0.76	<0.01
<i>Model 3:</i>				
birthweight	-3.36	-6.27	-0.46	0.02
age	-0.67	-1.76	0.43	0.23
weight	0.58	0.39	0.77	0.00
capillary density	0.23	-0.29	0.76	0.38

Table 4.2

Multiple regression models showing predictors of systolic blood pressure. Omitting age from the analyses did not alter the association between birthweight and blood pressure.

Discussion

This study examined relationships between birthweight and blood pressure, dermal capillary density, height, and weight during childhood. In 107 private school pupils low birthweight was associated with higher systolic blood pressure, after adjustment for current size. As in the other study cohort described in chapter 3, we did not find evidence of changes in capillary density that might mediate the association between birthweight and blood pressure.

Low socioeconomic status predicts low birthweight through several mechanisms. Features of low socio-economic status during pregnancy such as lower maternal age, exposure to illicit drugs, low maternal weight gain and shorter inter-pregnancy interval are independent predictors of premature delivery. (ABRAMS 1989) An association between low socio-economic status and intrauterine growth retardation is explained by maternal smoking. (BROOKE 1989) Participants in our study were drawn from a narrow and privileged social spectrum, and though we cannot exclude the possibility of some confounding as we collected limited information on parental factors and did not request information on maternal smoking, it is unlikely that the subjects in our study had been exposed to the pervasive influences of poverty. This supports the conclusion of previous cross-sectional studies that the observed association between low birthweight and higher blood pressure is mediated by influences other than socioeconomic deprivation.

As previously reported (FORRESTER 1996, HASHIMOTO 1996, TAYLOR 1997, WHINCUP 1995, WHINCUP 1996, WHINCUP 1996, WOELK 1998) the association between birthweight and blood pressure was only seen after adjustment for the effect of current size. Current size is the product of both genetic and environmental influences, and potentially subject to the influence of deprivation. In this population parental height was a strong predictor of birthweight and also a predictor of current size. However, parental size did not predict blood pressure, suggesting that the factors that mediate the inheritance of birthweight and of current size are not those that mediate the associations between low birthweight and higher blood pressure in an advantaged population.

We did not find a role for programming of dermal capillary density in boys aged 6-16 years, in keeping with similar observations in infants. (GOH 2001) Neither of these studies exclude the possibility that capillary density in other vascular beds may be influenced by birthweight. An association between birthweight and post ischaemic capillary recruitment in nail fold capillaries has been reported by Serne *et al.* (SERNE 1999) in a small study (n=30) which did not report any association with absolute capillary number. A detailed discussion of the differences between the methods used by Serne and our lab appears in the next chapter. The current study had similar power to detect capillary rarefaction as a previous study of young adults with contrasting inherited predisposition to high blood pressure. (NOON 1997) It seems that capillary rarefaction may occur later in the pathogenesis of hypertension than childhood. This is consistent with Lever's hypothesis (LEVER 1992) that childhood growth is associated

with rising blood pressure, but subsequent vascular structural change is responsible for maintenance of increased pressure. Indeed, a previous study of middle-aged men did show a relationship between low birthweight and altered microvascular structure, albeit in the retinal circulation. (CHAPMAN 1997)

In conclusion, we have demonstrated that the effects of low birthweight can be seen in the blood pressures of relatively privileged schoolchildren. This study also adds to the evidence from the previous chapter that dermal capillary density is not programmed by early life events. In the next chapter, we shall review evidence for a link between insulin resistance and hypertension that is mediated by capillary density.

Chapter 5

Relationships between blood pressure, fasting glucose concentrations and microvascular structure and function in healthy young men.

The studies described in the preceding chapters tested the hypothesis that altered structure or function of microvessels mediated the association between low birthweight and higher blood pressure, and did not find evidence to support this hypothesis. It may be that capillary rarefaction develops later in the development of hypertension than childhood or young adulthood. Structural or functional abnormalities of the microcirculation have been postulated to influence insulin resistance as well as vascular resistance by limiting glucose delivery to tissues (see section in introduction). Tooke and Hattersley postulate (HATTERSLEY 1999) that diminished capillary network development might occur as a consequence of foetal resistance to vascular growth promotion by insulin. This would be an initiator of higher blood pressure because of increased peripheral resistance, and would contribute to the development of metabolic consequences of insulin resistance. This chapter describes an analysis of experimental work performed by Dr. Joe Noon in our laboratory, relating microvascular structure and function to indices of blood pressure and glucose metabolism.

Serne *et al.* addressed this hypothesis in a small group of healthy normotensive subjects aged 20-64 years (SERNE 1999) and demonstrated an association between insulin

sensitivity and blood pressure and a strong relationship between both parameters and microvascular function assessed by vasodilator responses to iontophoresis of acetylcholine, and capillary recruitment following arterial occlusion. In order to eliminate the possible confounding factors of age and sex in these findings, the present study examined a large group of 105 healthy men aged 23-33 years.

Methods

Participants

These studies were approved by our local Research Ethics Committee and written informed consent was obtained. Subjects were selected from a cohort which has been described elsewhere (WATT 1991, WATT 1992). In brief, blood pressure was measured in 603 married couples in 1979 and in 864 of their offspring, then aged 16-24 years, in 1986. Age-adjusted Z-scores were used to define tertiles for both offspring and mean parental blood pressures. Offspring for whom both their own blood pressure and the mean blood pressure of their parents were outwith the middle tertile were identified as belonging to one of 'four corners'. Sub-groups of offspring randomly selected from these corners have participated in further investigations (WATT 1992, NOON 1997) which have identified correlates of the inherited predisposition to high blood pressure. We studied 105 Caucasian male offspring drawn at random from the four corners.

Protocol

After an overnight fast from 2200 h, and abstention from proprietary drugs including aspirin for 10 days, subjects attended the Clinical Research Centre at 0900 h. Height and

weight were recorded before subjects rested supine and acclimatised to a controlled environmental temperature of 23-24°C. After 20 minutes, a 30 ml venous blood sample was obtained and recordings of blood pressure were made 4 times at 5 minute intervals using a validated (WIINBERG 1988) semi-automated machine (Takeda UA 751 sphygmomanometer; Takeda Medical Inc., Tokyo, Japan). The means of the last three recordings were used in subsequent analyses. After 40 minutes, the following observations were made in sequence: (i) in the right arm, maximum vasodilatation of skin microvessels was measured by laser Doppler fluximetry in response to local heating to 42°C; (ii) in the left arm, nailfold capillary blood velocity and red cell column width, and capillary numbers on the dorsum of the ring finger were measured by intravital videomicroscopy; (iii) in the right arm, forearm blood flow was measured before and after 12 minutes of ischemia applied using a sphygmomanometer at supra-systolic pressure, using strain gauge plethysmography; (iv) in the left arm, dermal vasodilatation was measured by laser Doppler fluximetry in response to transdermal delivery of acetylcholine by iontophoresis.

Further details of measurements in these subjects have been published previously (NOON 1997), except for iontophoresis and assessment of insulin sensitivity. Iontophoresis was performed as previously validated in the same lab (NOON 1998). Briefly, acetylcholine (Sigma Chemicals Ltd., Poole, Dorset, UK) was prepared in 2% methylcellulose gel at a final concentration of 2 g/100 ml. Acetylcholine or vehicle (0.5 ml) were injected in random order into an iontophoresis chamber (Moor Instruments Ltd., England) positioned on the volar aspect of the left forearm and flux was measured

continuously by laser Doppler. Currents of increasing duration and intensity (100 μ A for 10 s; 200 μ A for 10 s; 200 μ A for 20 s; 200 μ A for 40 s; and 200 μ A for 80 s) were applied to deliver charges of 1, 2, 4, 8 and 16 mC. Response periods were allowed after each charge (60 s for 1 and 2 mC, 90s for 4 mC, 120 s for 8 and 16 mC) which were sufficient for the response to plateau consistently. The iontophoresis chamber was then removed and cleaned before the procedure was repeated on a neighbouring site with the next solution (drug or vehicle). Mean flux, measured at the plateau of the response for each charge, was expressed in arbitrary flux units. We calculated % increase in flux and area under the curve, and used both as summary statistics. However, there was no difference between these measures for any relationship with iontophoretic response, and % change in flux is quoted for all results. In preliminary experiments, laser Doppler 'biological zero' values were recorded during ischaemia, but flux values were negligible in relation to the vasodilatation to iontophored drugs so we did not adjust for biological zeros in this study.

Laboratory methods

Plasma was stored at -80°C until analysis. Insulin was measured by radio-immune assay (WALKER 1995). Glucose was measured by autoanalyser. Insulin resistance was estimated using the Homeostasis Model Assessment - HOMA. (MATTHEWS 1985)

Statistics

Comparison between subjects from the four corners was performed by ANOVA. Fasting plasma insulin, HOMA resistance index and maximal forearm blood flow were log

transformed to normalise their distribution. Correlations in the whole sample were identified by linear regression. Multiple regression analysis was used to identify confounding influences between interrelating variables.

Results

Summary of results and comparison between four corners

Characteristics of subjects were: age (28.7, ± 2.5 years, 23-33; mean, standard deviation, range); body mass index (BMI) (24.3, ± 3.02 , 18.6-36.8 kg/m²); systolic BP (119, ± 9.7 , 93-154 mmHg); diastolic BP (69.2, ± 7.4 , 51-98 mmHg); fasting plasma glucose (4.9, ± 0.5 , 3.0-6.5 mmol/l); fasting plasma insulin (5.3, ± 4.5 , 1.1-29.6 mUnits/l); and HOMA Resistance index (14×10^{-6} , 12×10^{-6} , $2-78 \times 10^{-6}$). Comparison of measurements of microvascular structure in these members of the four corners has been published previously (NOON 1997). Fasting plasma glucose was not different between corners (ANOVA $p=0.24$), but there were trends for higher HOMA insulin resistance index amongst subjects with higher blood pressure (ANOVA $p=0.09$), as previously described in a different sample of men and women (WATT 1992).

Iontophoresis of acetylcholine caused dose dependent vasodilatation while there was no significant dilatation with vehicle (Figure 5.1). There was no difference in response to iontophoresis between the four corners (ANOVA $p= 0.4$)

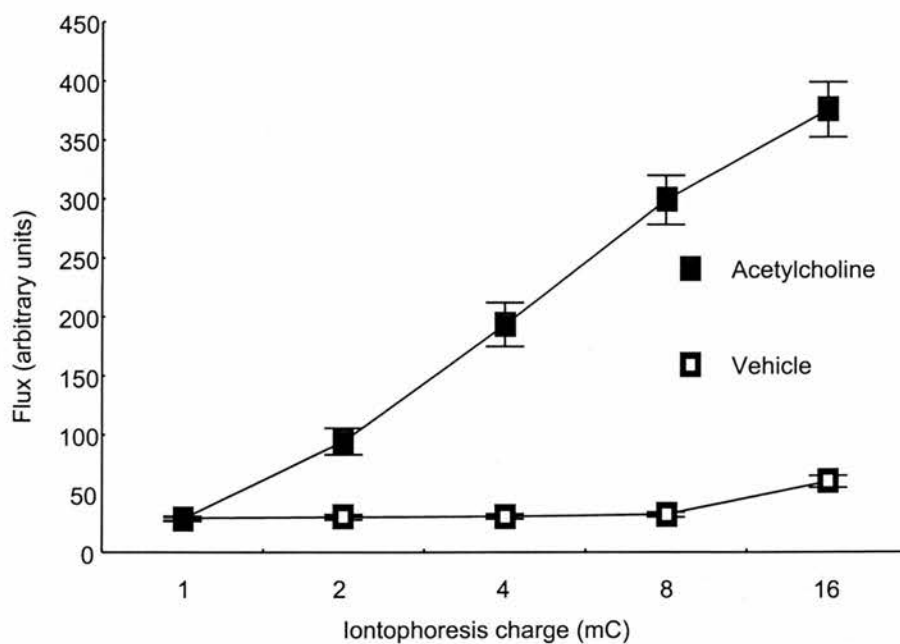


Fig 5.1 Graph of iontophoresis responses to acetylcholine.

This graph shows the dose dependant vasodilatation resulting from iontophoresis of drug or vehicle. Measurements were made by laser Doppler fluximetry and expressed as mean \pm standard error. (Repeated measures ANOVA $p < 0.001$)

Relationships with blood pressure

Higher systolic blood pressure was associated with higher fasting plasma glucose ($r=0.21, p<0.05$), and higher indices of insulin resistance (log fasting plasma insulin $r=0.32, p=0.001$; log HOMA Resistance index $r=0.31, p<0.005$), and with higher body mass index ($r=0.25, p<0.01$). Higher blood pressure was also associated with fewer dermal capillaries at baseline and during venous occlusion, and reduced maximal flow in dermal vessels. However, blood pressure did not correlate with maximal forearm blood flow following ischaemia or dermal vascular vasodilatation to acetylcholine. Diastolic Blood pressure similarly correlated with insulin resistance (log HOMA $r=0.21, p<0.05$; log plasma insulin $r=0.21, p<0.05$) and with skin hyperemia, but not other measurements of microvascular structure or function. (See Table 5.1)

Relationships with insulin sensitivity

(See Table 5.1). As expected, greater insulin resistance was associated with higher BMI ($r=0.53, p<0.001$). There were no relationships between insulin resistance and indices of dermal microvascular structure or response to acetylcholine. However, higher fasting plasma glucose was associated with fewer dermal capillaries at baseline and during venous occlusion, and with increased capillary blood velocity. These effects were independent of systolic blood pressure in multiple regression analyses ($r=-0.24, p<0.05$ and $r=-0.21, p<0.05$ for plasma glucose versus capillary density at baseline and after venous occlusion, respectively, $r=0.29, p<0.01$ for plasma glucose versus capillary blood velocity after adjustment for effect of systolic blood pressure).

Table 5.1

Microvascular variable	Mean (S.D.)	Correlation with							
		Systolic BP	Diastolic BP	BMI	Plasma Glucose	log Plasma Insulin	logHOMA		
log Maximal forearm blood flow (ml/min)	5.28 (0.41)	-0.11	-0.07	-0.15	0.04	-0.13	-0.12		
Maximal skin flow (arbitrary flux units)	525 (211)	-0.27†	-0.31†	-0.08	0.03	-0.09	0.02		
Capillary blood velocity (mm/sec)	1.22 (0.65)	0.07	0.06	0.16	0.30*	0.11	0.15		
Capillary density before venous occlusion (/0.25 mm ²)	23.6 (4.0)	-0.21*	-0.11	-0.13	-0.27†	-0.04	-0.11		
Capillary density after venous occlusion (/0.25 mm ²)	25.3 (4.2)	-0.25*	-0.15	-0.15	-0.25*	-0.05	-0.07		
Capillary recruitment (%)	7.7 (6.2)	-0.11	-0.13	-0.03	0.08	-0.03	0.13		
Iontophoresis of acetyl choline (% flux increase)	596 (410)	0.09	0.11	-0.04	0.06	-0.05	0.02		

Table 5.1 Correlations between microvascular structure and function, and blood pressure and insulin sensitivity. (* denotes p<0.05, † denotes p<0.01)

Discussion

It is intellectually attractive to link the common, associated conditions of high blood pressure and insulin resistance through shared abnormalities of microvascular structure and function, causing on the one hand, increased peripheral vascular resistance and high blood pressure, and on the other, impaired tissue delivery of glucose and insulin resistance. Few would deny that insulin can stimulate skeletal muscle blood flow in some circumstances, though controversy exists over its importance in the physiological control of skeletal muscle glucose uptake. For example, an elegant study (LAINE 1998) recently demonstrated that reversing the abnormality in insulin mediated vasodilatation failed to improve glucose uptake in the legs of insulin resistant young men, and studies have shown that other vasodilators do not improve insulin resistance.

Much of this controversy is likely to be explained by the lack of techniques sensitive enough to measure muscle capillary flow in human subjects. Animal studies clearly demonstrate, even in the absence of a change in *total* muscle flow, that insulin increases nutritive capillary recruitment and improves glucose uptake. Conversely, increases in total blood flow which bypass the nutritive capillary bed do not improve glucose uptake. In humans, insulin increases skin capillary blood flow independent of any glucose effects at least in subjects with type 1 diabetes (TOOKE 1985). If insulin mediated vasodilatation is indeed important in the physiological regulation of glucose uptake, the impaired insulin mediated vasodilatory responses described in hypertension and other insulin resistant states such as obesity, ageing, and type 2 diabetes could contribute to

insulin resistance. We have confirmed that insulin resistance and higher blood pressure are correlated in our cohort of 105 healthy young men but we found no evidence that microvascular structure or function influenced this relationship.

The relationships in our data between dermal microvascular structure and blood pressure were consistent with many previously published studies (ANTONIOS 1999A, GASSER 1992, PRASAD 1995) supporting the theory first proposed by Folkow in 1958 (FOLKOW 1958) that structural distortions in microvascular networks maintain increased peripheral vascular resistance.

We did not demonstrate relationships between blood pressure and microvascular function assessed by responses to iontophoresis of acetylcholine. Previous descriptions of endothelial dysfunction in hypertension have examined large arteries and the forearm muscle bed where nitric oxide is the major mediator of acetylcholine related vasodilatation (PANZA 1990). It is unlikely that iontophoresis of acetylcholine to dermal vessels assesses purely nitric oxide dependent vasodilatation. We have previously shown that blood flow assessed by laser Doppler in areas of skin that are supplied by essentially nutritive microcirculation (ie dorsum of hand or ventral surface of forearm) does not change during intra-arterial infusion of L-NMMA (NOON 1996). Moreover, vasodilator responses to iontophoresis of acetylcholine in these areas are not inhibited by L-NMMA (NOON 1992) although they may be inhibited by intravenous (NOON 1998) but not oral aspirin (MORRIS 1996). Conversely, laser Doppler fluximetry of flow in the pulp of the thumb, which has numerous thermoregulatory

arterio-venous anastomoses, was decreased by 33% during L-NMMA infusion (NOON 1996).

In contrast with the strong associations with blood pressure there were no relationships between markers of insulin resistance and dermal microvascular structure, (capillary number pre or post venous occlusion, and hyperaemia following local skin heating) or function (vasodilatation to acetylcholine). We have previously shown a correlation between fasting insulin levels and skin hyperaemia in a group of normotensive men and women with fasting hyperglycaemia, and a correlation between BMI and skin hyperaemia in healthy normotensive men and women. (unpublished observations). The influence of age and gender are the most likely explanations for the differences between these studies since both factors may influence both vascular function and insulin sensitivity. The lack of relationships with the skin microcirculation response to acetylcholine agree with those of Petrie and colleagues (PETRIE 1996) who failed to show a relationship between the forearm blood flow response to acetyl choline and measures of insulin resistance in healthy male subjects.

These results contradict those of Serne *et al.* (SERNE 1999) who showed associations between dermal microvascular function, blood pressure and insulin resistance. Several factors may contribute to this disparity. The populations studied were different; we studied 105 young men age 23- 35 while Serne studied just 18 men and women, with a wide age range. Both age and gender are known to have marked effects on vascular reactivity. Serne and colleagues quantified insulin sensitivity by euglycaemic

hyperinsulinaemic clamp which was not logistically possible in our study. We used the HOMA score which correlates closely with the result obtained by the clamp method (MATTHEWS 1985). Since M values from a clamp are influenced by the initial glucose concentration (BERGMAN 1985) our finding of a relationship between baseline capillary numbers and fasting plasma glucose may be a relevant confounder of the clamp results. Our study also differed from Serne *et al.* in the site where vessels were studied; they used the nail fold to assess capillary density whereas we used the dorsum of the finger. Nail fold capillaries are unusual in that they follow a prolonged course parallel to the skin surface. The visible length varies between individuals as a function of the degree of trauma to which their cuticles and nail folds are exposed. Capillary density in the dorsal finger skin is greater ($94/\text{mm}^2$ vs. $38.3/\text{mm}^2$) and not subjected to this additional source of bias.

Serne *et al* describe relationships with % recruitment of capillaries during reactive hyperaemia implying that this measurement relates to vascular function. Baseline capillary perfusion chiefly depends upon skin temperature, which tends to be lower in women despite acclimatisation to a temperature controlled environment. (unpublished observation) Recruitment of capillaries following venous occlusion in dorsal finger skin was much less than that observed in the nail fold following arterial occlusion (7.7% vs. 40.5%). Some of this discrepancy may result from the predominantly female group studied by Serne, as their lower resting capillary number will have greater scope for recruitment. However, Antonios *et al* (ANTONIOS 1999 B) found that capillary density *decreased* during reactive hyperaemia following 5 minutes of arterial occlusion

(111/mm² to 104/mm²) and that venous occlusion was the most effective way of demonstrating recruitment (ANTONIOS 1999 B). It is therefore unclear what this index actually represents.

Comparison of the strength of the relationship between insulin sensitivity and response to iontophoresis of acetylcholine in our subjects with those of Serne can be done by examining confidence intervals for the gradient of the regression. In the Dutch study, a 100% change in insulin sensitivity was associated with a 256% change in iontophoresis response. Conversely in our study 95% confidence limits for a 100% change in insulin sensitivity would be associated with 4% to 12% change in iontophoresis response.

Interestingly in the present study fasting plasma glucose, which is more a reflection of impaired pancreatic β -cell function than insulin resistance was independently associated with lower dermal capillary density both before and after venous occlusion. This reduction in capillary number was accompanied by an elevation in capillary blood velocity with no change in red cell column width (a marker of capillary diameter) suggesting that individual capillary blood flow is increased. Such an increase in flow and associated increases in shear stress on the endothelial cell may have profound effects on capillary function.

In summary, we have demonstrated strong links between microvascular structure and blood pressure in healthy young men that may play an important role in the familial

inheritance of high blood pressure. By contrast we found no relationship linking microvascular structure or function to insulin resistance.

Chapter 6: Conclusions and Speculations

This thesis has considered the role of structural changes in the microvasculature as a programmed mediator of the association between low birthweight and high blood pressure. We have found no evidence to support this hypothesis in two separate studies of capillary density, but have shown evidence that individuals born prematurely have greater left ventricular mass, over and above that predicted by their elevated blood pressure. We have also shown evidence of elevated blood lipids and fasting plasma glucose in premature low birthweight babies but no evidence of insulin resistance. Furthermore, we have demonstrated that low birthweight predicts higher blood pressure in a cohort drawn from a narrow and relatively privileged social spectrum. Finally we have considered evidence considering whether insulin resistance might be mediated through vascular responses in the dermal microvessels. This final chapter will briefly consider some of the broader issues.

Public Health Importance

The “foetal origins” hypothesis as an explanation for the burden of cardiovascular disease gains little support from ecologic trends. Cardiovascular disease has followed an epidemic dissemination through industrialised countries during the 20th Century. It has followed trends of altered diet, exercise and, perhaps most importantly cigarette smoking. No trends of diminished birthweight have been demonstrated preceding the

rise in incidence of clinical manifestations of vascular pathology. The decline in disease incidence during the latter decades was not preceded by a major rise in birthweight. Birthweights tend to be high in the Northern European countries that have had the greatest incidence of cardiovascular disease.

The small influence of birthweight on adult outcomes should reassure individuals with low birthweight that their expectation of future good health remains intact, and efforts to encourage and maintain healthy habits are equally relevant to all sectors of the population.

Gestational Age, IUGR and Catch-up growth

That disparate processes occurring during foetal or infant life can be shown to associate with higher adult blood pressure challenges reductionism. The relationship between size at birth and adult blood pressure depends upon the whole range of birthweight, and it seems logical that the explanation of the association might encompass more than one of the influences on birthweight. We have demonstrated, in a small study, that premature delivery can be associated with higher blood pressure. Very large studies have also demonstrated this, however several have not shown an influence from gestation. Similarly, in our study of premature babies there was no influence from growth between birth and the adult measurements, although this is important in many studies.

Reconciling differences in these studies requires recognition that the effect size is small, and large studies will be best able to precisely define the true effect of these variables. Opportunistic use of routinely collected data linked through unique national identification numbers has provided the largest study published to date. Accessing childhood growth records could add the data required to definitively calculate the contributions that these separate biological processes add to the association in question. Truly prospective studies with the scale to settle these questions would require resources exceeding the scientific merits of their purpose. The influence of birthweight on lifetime risk of cardiovascular morbidity and mortality is small and unlikely to be amenable to simple, safe interventions. The value of studies in this area is the insights gained from study of interactions between gene and environment in normal and abnormal development.

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