Prenatal Development and Later Neuroendocrine Control of Cardiovascular Function

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Whilst midwife and friends assist a woman labouring, two birth-gazers attempt to predict the long-term outcome of the birth from prevailing environmental characteristics (by charting the position of the stars). Although no validation for astrology, there is now evidence that season of birth may be predictive of size at birth and later cardiovascular health.1,2

Jost Amman (Swiss, 1539-91), from Kunstbüchlin ('Art booklet'), Sigmund Feyerabund (printer & publisher), 1580, woodcut, Frankfurt.
Small size at birth, a marker of fetal growth, is associated with hypertension and cardiovascular disease in adulthood. Explanatory mechanisms for this association are not well characterized but may involve alterations of stress response systems such as the hypothalamic-pituitary-adrenal axis (HPAA) and autonomic nervous system (ANS). Animal studies show that adverse prenatal environments lead to sex-specific, lifelong alterations in the activity of these systems both at rest and during stress. The extent to which such prenatal adaptations occur in humans is unknown but may be of clinical importance, given emerging evidence that stress responsivity is a risk factor for cardiovascular disease. The sparse published human data comes from older populations and may be confounded by existing cardiovascular disease. Therefore, I have studied younger populations – young adults from Adelaide, Australia and pre-pubertal children from Southampton, UK. In these studies, healthy individuals, born at term, underwent psychological stress testing whilst measures of ANS, cardiovascular and HPAA function (salivary cortisol) were recorded. In adults, continuous finger arterial pressure was used to derive indices of autonomic and baroreflex function whilst in children, electrocardiography, impedance cardiography and blood pressure tonometry were used to assess cardiovascular function. Women, but not men, who were small at birth had increased sympathetic activity at rest \( (r = .28, P < .05) \) and during stress \( (r = .42, P < .001) \), reduced parasympathetic activity \( (r = .22, P < .05) \) and reduced baroreflex sensitivity \( (r = .34, P < .01) \). In boys, birth weight was inversely related to salivary cortisol responses to stress \( (r = -.56, P < .001) \) but not to morning cortisol levels, whilst in girls, morning peak cortisol was inversely related to birth weight \( (r = -.36, P < .05) \).

In boys, lower birth weight was also associated with higher arterial pressure and systemic vascular resistance, particularly following psychosocial stress \( (r = -.62, P < .01, \text{ and } r = -.47, P < .05, \text{ respectively}) \). In girls, lower birth weight was associated with greater cardiac sympathetic activation, indicated by shorter pre-ejection period \( (r = .53, P < .01) \) and corrected QT interval \( (r = .45, P < .01) \). These associations were independent of gestational age and potential confounding factors such as obesity, social class and educational achievement. These results suggest that, as in animals, intrauterine influences can have lasting, but sex-specific, effects on endocrine, autonomic, and cardiovascular function which may promote the development of cardiovascular disease in both sexes.
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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>11 β-HSD</td>
<td>11 β-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>α</td>
<td>Baroreflex sensitivity</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>Ang II</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>AR</td>
<td>Autoregressive</td>
</tr>
<tr>
<td>ARMA</td>
<td>Autoregressive moving-average</td>
</tr>
<tr>
<td>BHR</td>
<td>Borderline hypertensive rat</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BNST</td>
<td>Bed nucleus of the stria terminalis</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CARDIA</td>
<td>Coronary Artery Risk Development in Young Adults</td>
</tr>
<tr>
<td>CBG</td>
<td>Corticosteroid binding globulin</td>
</tr>
<tr>
<td>CES</td>
<td>Centre for Epidemiologic Studies</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>cQT</td>
<td>Corrected QT interval</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotrophin releasing hormone</td>
</tr>
<tr>
<td>CSS</td>
<td>Carotid sinus stimulation</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
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<tr>
<td>DELFIA</td>
<td>Dissociation-Enhanced Lanthanide Fluorescent Immunoassay</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier transform</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptors</td>
</tr>
<tr>
<td>GREs</td>
<td>Glucocorticoid Response Elements</td>
</tr>
<tr>
<td>HF</td>
<td>High-frequency</td>
</tr>
<tr>
<td>HP</td>
<td>Heart period (the interval between adjacent heart beats)</td>
</tr>
<tr>
<td>HPAA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>HP_{hf}</td>
<td>High-frequency heart period variability</td>
</tr>
<tr>
<td>HP_{lf}</td>
<td>Low-frequency heart period variability</td>
</tr>
<tr>
<td>HP_{ratio}</td>
<td>Ratio of low to high frequency heart period variability</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HR_{inc}</td>
<td>Increment of heart rate from rest to stress</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>IRSD</td>
<td>Index of Relative Socioeconomic Disadvantage</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LF</td>
<td>Low-frequency</td>
</tr>
<tr>
<td>LREC</td>
<td>Local Research Ethics Committee</td>
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<tr>
<td>LVET</td>
<td>Left ventricular ejection time</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MR</td>
<td>Mineralocorticoid receptors</td>
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<td>Abbreviation</td>
<td>Full Name</td>
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<td>--------------</td>
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</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MSNA</td>
<td>Muscle bed sympathetic nerve activity</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PEP</td>
<td>Pre-ejection period</td>
</tr>
<tr>
<td>PNS</td>
<td>Parasympathetic nervous system</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
</tr>
<tr>
<td>RSNA</td>
<td>Renal sympathetic nerve activity</td>
</tr>
<tr>
<td>SA</td>
<td>Sinoatrial</td>
</tr>
<tr>
<td>SAP</td>
<td>Systolic arterial pressure</td>
</tr>
<tr>
<td>( SAP_{inc} )</td>
<td>Increment of systolic arterial pressure from rest to stress</td>
</tr>
<tr>
<td>( SAP_{lf} )</td>
<td>Low-frequency systolic arterial pressure variability</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>STR</td>
<td>Systolic time ratio (ratio of PEP to LVET)</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic vascular resistance</td>
</tr>
<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index (SVR normalised by BSA)</td>
</tr>
<tr>
<td>TSST-C</td>
<td>Trier Social Stress Test for children</td>
</tr>
<tr>
<td>VLF</td>
<td>Very low-frequency</td>
</tr>
<tr>
<td>VVC</td>
<td>Ventral vagal complex</td>
</tr>
<tr>
<td>WPT</td>
<td>Wavelet packet transform</td>
</tr>
<tr>
<td>WT</td>
<td>Wavelet transform</td>
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</table>
Chapter 1. INTRODUCTION

The major clinical drive behind the work presented in this thesis is a desire to better understand cardiovascular disease and its related conditions such as hypertension. Despite the fact that diseases of the heart and vasculature are amongst the most prevalent and serious of all human health disorders, and despite the vast body of research on these conditions, a comprehensive understanding of their development has still not emerged. This introduction deals with a number of questions which arise from relatively recent research which departs from the traditional viewpoint that the significant pathogenesis of cardiovascular disease occurs in mid to late adulthood when cardiovascular diseases usually appear. Although it is now clear that lifestyle choices, such as the decision to smoke, lead a sedentary existence or eat a poor diet, are significant risk factors for cardiovascular disease, is it possible that these apparent choices are not simply a matter of free will or sociocultural pressures but have potent underlying biological imperatives too? Do the processes leading to cardiovascular disease begin in early life, even before birth? If so, are they determined entirely by the influence of genes or are there mechanisms by which environmental factors may influence them also? Although stress is known to influence the development of cardiovascular disease – what is stress? How is it measured and how does a psychological factor, a process of the mind, connect with cardiovascular disease, a condition of the body? Finally, is it possible that the structure and function of physiological pathways that are activated by stress (the wiring between mind and body) are altered in response to early demands on an individual with a detrimental impact later in life?
Addressing these questions, this introduction proceeds as follows: Initially, I have summarised the evidence that individual differences in adult physiology and, therefore, in risk of adult disease, do not simply come from differences in genotype but may be influenced by a variety of environmental agents during vulnerable periods of development. As this thesis is focused on cardiovascular disease, I go on to address the burden of cardiovascular disease, the processes (or risk factors) involved in cardiovascular disease, and the evidence that cardiovascular disease is related to prenatal development. Focus is then given to stress and psychosocial processes as specific cases of factors which influence risk of cardiovascular disease. The issue of the definition of stress is dealt with and an argument is forwarded that stress, through its effects on neuroendocrine physiology and behaviour, may affect a whole range of the risk factors known to determine cardiovascular disease, such as hypertension, dyslipidaemia, glucose intolerance, and perhaps even lifestyle behaviours. The physiology of the neuroendocrine pathways involved is then detailed and the hypotheses of this thesis are stated.

1.1 Developmental Origins of Health and Disease

The search for determinants of health and disease is at the core of modern medical science. Understanding of non-communicable diseases has come largely from a focus on lifestyle factors in adult life and from genetics. Recently, however, attention has shifted to prenatal and childhood development as a third major factor in the origins of later disease. Large-scale epidemiological observations have led to the hypothesis that environmental factors acting during vulnerable periods of early development can have dramatic and lasting effects on the propensity to develop disease in adulthood. This is supported by research in developmental biology, evolutionary biology, and animal
and human physiology, which has identified a wide range of potential mechanisms acting at varied times, from pre-conception to early childhood. Encompassed as the Developmental Origins of Health and Disease Hypothesis, these findings have led to a rapidly expanding area of research where better understanding of the processes involved has potentially significant medical and socioeconomic implications.\textsuperscript{5,11,12}

1.1.1 The Longstanding Influence of Early Developmental Experience

A universal feature of living organisms is their ability to modify their final form and function during development in response to environmental demands. This is termed developmental plasticity and is more formally defined as ‘the ability of a single genotype to produce more than one alternative form of structure, physiological state or behaviour in response to environmental conditions.’\textsuperscript{13} Without this plasticity, the survival characteristics of individual organisms would be effectively fixed at conception, with no capacity for adaptation to a post-conceptual environment that was ‘unpredicted’ by their genome. Natural selection alone requires a timescale of at least one generation (usually many more) to adapt to environmental change. Given that the timescale of dramatic environmental change can often be far shorter than this, it seems highly unlikely that natural selection would not have favoured the development of organisms capable of exploiting potential non-genetic mechanisms for adaptation to environmental change within one generation. In fact, such a process has long been recognised. Although Darwinism is often equated with a rigid adherence to the idea that natural selection alone is responsible for adaptation of organisms to their environment, Darwin showed considerable insight into the concept of developmental plasticity. Drawing on his observation that domesticated animals and cultivated plants
showed much greater variability than wild types, he rejected the contemporary view that the reproductive system alone produces such variation:

'Some authors believe it to be as much the function of the reproductive system to produce individual differences, or very slight deviations of structure, as to make the child like its parents. But the much greater variability, as well as the greater frequency of monstrosities, under domestication or cultivation, than under nature, leads me to believe that deviations of structure are in some way due to the nature of the conditions of life, to which the parents and their more remote ancestors have been exposed during several generations.'14

He also identified the reproductive system of the parents as the most likely arena for environmental conditions to impact on offspring variability:

'I have remarked ... that the reproductive system is eminently susceptible to changes in the conditions of life; and to this system being functionally disturbed in the parents, I chiefly attribute the varying or plastic condition of the offspring.'14

Darwin felt that the window for such environmental interference in reproduction was likely to be pre-conceptual and, indeed, evidence for pre-conceptual environmental influences on offspring development has since emerged with, for example, a study of mothers who experienced a severe famine in Holland during the Second World War.'15
As expected, this study showed that starvation in pregnancy resulted in a significant decline in the birth weight of the offspring. Less expected was the finding that birth weight and perinatal mortality were affected in the subsequent offspring of the daughters of women who experienced famine during pregnancy as well. Similar second-generation effects of impaired nutrition during pregnancy have also been demonstrated in animal studies.\textsuperscript{16-20} One possible explanation for the epidemiological observations in Holland may be that the famine affected the germ cells of the female fetuses \textit{in utero} with subsequent effects on the offspring formed from those germ cells. Studies in animals indicate that inheritance of an epigenetic trait induced by the environment prior to conception can be produced experimentally and appears to be mediated by incomplete erasure of deoxyribonucleic acid (DNA) methylation (imprinting) during meiosis, causing the offspring to express certain genes at a level similar to their parents.\textsuperscript{21,22} There are other possible explanations for these observations. For example, development of uterine vasculature and structure, and reproductive endocrine axes may also be altered by an adverse fetal environment.\textsuperscript{23} Thus, famine exposure \textit{in utero} might have detrimental effects on the reproductive physiology of female offspring by these mechanisms, resulting in smaller second-generation offspring.

If these findings seem surprising suggestive of Jean-Baptiste Lamarck's \textit{inheritance of acquired traits},\textsuperscript{24} perhaps it is worth remembering that Darwin was by no means dismissive of Lamarck's work. In the preface to later editions of \textit{The origin of species},\textsuperscript{25} he wrote:
'Lamarck was the first man whose conclusions on the subject excited much attention. This justly-celebrated naturalist first published his views in 1801 ... He first did the eminent service of arousing attention to the probability of all change in the organic, as well as in the inorganic world, being the result of law, and not of miraculous interposition ... With respect to the means of modification, he attributed something to the direct action of the physical conditions of life, something to the crossing of already existing forms, and much to use and disuse, that is, to the effects of habit.'

Although the archetype of Lamarckian evolution was refuted by the work of Darwin and Gregor Mendel,² it is now clear that elements of Lamarck's thinking have been unfairly dismissed. Indeed, there are a number of mechanisms by which acquired traits may be inherited. Not only are there epigenetic modes of inheritance, as already discussed, but also entirely non-genomic mechanisms of inheritance where the development of the offspring is influenced by placental and maternal physiological conditions with permanent effects on the physiology and behaviour of the offspring that may continue to have effect over subsequent generations. Furthermore, it is now clear that the window of sensitivity to these mechanisms varies, is not limited to a pre-conceptual time period and may include any part of prenatal life or early childhood. This phenomenon is frequently referred to as developmental programming.
The clearest evidence that non-genomic maternal characteristics and uterine environment can predetermine adult characteristics of the offspring comes from animal experiments\textsuperscript{19,27-36} where, in contrast to experiments in humans, genetic homogeneity can be assured, reducing the confounding influence of genetic variability. These studies show that significant modifications in the offspring's size at birth, metabolic and cardiovascular function, and behaviour, which have lasting effects on the offspring into adulthood, can be induced by manipulation of the maternal environment. Where more than one generation of offspring was studied, it has been shown that altering maternal nutrition or exercise prenatally, and postnatal nutritional or handling interventions have significant effects on birth weight,\textsuperscript{16,31} glucose tolerance\textsuperscript{20} and function of the hypothalamic-pituitary-adrenal axis (HPAA)\textsuperscript{32} in subsequent generations. Thus, the secular trends for increasing birth weight observed in several populations worldwide\textsuperscript{33-35} or inter-generational effects of low birth weight or raised blood pressure, may be partially explained by non-genomic intergenerational inheritance mediated by these mechanisms.\textsuperscript{10,36}

Not all changes in the offspring induced by the environment may be adaptive, allowing the offspring to increase their likelihood of survival. Indeed, changes in the offspring may simply result from environmental constraint where alternative developmental pathways are unavailable to the fetus. However, phenomena such as brain-sparing,\textsuperscript{37} the process whereby a fetus diverts supply of nutrients and oxygen away from less important organs such as the liver, in favour of brain growth during times of impaired oxygen or nutrient supply from the mother, suggest that fetal development seeks the best trade-off for survival in response to adverse conditions. This concept is elaborated in the Thrifty Phenotype hypothesis\textsuperscript{38} which focuses on the development of insulin resistance and type 2 diabetes. It proposes that an impoverished nutrient environment
in the womb provokes the developing baby to become thrifty, increasing blood sugars to promote brain growth at the expense of glycogen storage in the muscles and muscle growth. This phenotype persists into adulthood, increasing the likelihood of insulin resistance and type 2 diabetes, particularly in the context of later adiposity. Support for this theory comes from maternal nutrient restriction studies where altered insulin homeostasis is seen in the offspring. The observations that maternal overnutrition also leads to altered metabolic function in the adult offspring and that the postnatal nutrient environment is important in determining the extent of prenatal nutrient influence on the adult offspring, has led to an extension of the Thrifty Phenotype hypothesis. The Predictive Adaptive Response hypothesis proposes that the developmental choices made by the fetus in response to environmental influences are purposeful and designed to better adapt the fetus to the environment they are likely to encounter in postnatal life. This hypothesis assumes that until our recent evolutionary past, most pregnant mothers would experience an environment and nutrition that was likely to continue during their offspring’s lifetimes, making a mismatch between fetal predictive adaptive responses and later reality unlikely. Modern living has created many more opportunities for such a mismatch to occur and it is proposed that the consequence of these developmental false predictions is the development of adult diseases such as cardiovascular disease or type 2 diabetes mellitus and precursors to these diseases such as dyslipidaemia, impaired glucose tolerance, or vascular endothelial dysfunction.
1.1.2 Cardiovascular Disease

The World Health Organisation estimates that worldwide, cardiovascular disease caused the death of 14.7 million people in 1990 and 17 million people in 1999 which approximates to 30% of all deaths worldwide per annum. Cardiovascular disease is the principal cause of death throughout the world except for sub-Saharan Africa where it is expected to overtake infectious diseases as the leading cause of death in the next few years. Although traditionally thought of as a disease affecting rich industrialised nations, 80% of these deaths occur in countries with low or modest gross domestic product such as India, China, Russia, Argentina and Poland. As these countries become increasingly affluent, the populations are likely to adopt a more Western lifestyle and therefore a worsening risk profile for cardiovascular disease. Clearly, the rise in detrimental lifestyle factors such as obesity, smoking and lack of exercise will worsen the global epidemic. However, the traditional viewpoint that rapid increases in disease prevalence in developing nations are explained by the combination of these lifestyle changes with an inherited predisposition to cardiovascular disease is questionable. Interventional studies targeting these lifestyle factors have failed to significantly reduce levels of cardiovascular disease. This suggests that although these factors modify risk of developing cardiovascular disease, they do not fully account for it. Furthermore, rapid changes in disease incidence in a short time period are unlikely to be explained by changes in gene frequency in these populations. Therefore, until other factors involved in the pathogenesis of cardiovascular disease are more fully investigated, it is unlikely that successful interventions aimed at curbing this global epidemic will be possible.
1.1.3 Risk Factors Associated with Cardiovascular Disease

Approximately 75% of cardiovascular disease can be attributed to conventional risk factors. Of these, the major modifiable risk factors are hypertension, dyslipidaemia, tobacco use, physical inactivity, obesity, diabetes mellitus, and poor diets that are low in fruit and vegetables and high in saturated fat. Other modifiable risk factors include low socioeconomic status, mental ill-health, psychosocial stress, excessive alcohol use, and use of hormonal medications such as the oral contraceptive pill. Although many of these risk factors are related directly to lifestyle, others, such as hypertension and diabetes have complex aetiologies and many of these risk factors may interact with one another to alter overall risk of disease. Furthermore, risk factors that had been previously thought to be exclusively determined by behaviour may have powerful biological imperatives underlying these apparent lifestyle choices. For example, obesity is undoubtedly the consequence of an inappropriately high-energy diet often accompanied by inactivity, but until recently, these factors were thought to be entirely the consequence of individual lifestyle choices. Now, there is growing evidence to support the view that appetite regulation and physical activity are influenced by the prenatal nutritional environment and may be modulated in early life by postnatal nutrition. In addition, hypercaloric diets in later life amplify the effect of prenatal nutrition on later sedentary behaviour suggesting that these biological and behavioural factors are strongly interlinked. Animal studies have shown that fetal adipose tissue development is altered by maternal nutrition such that undernutrition during fetal life leads to adult obesity, hyperinsulinaemia, hyperleptinaemia, reduced locomotor activity and hyperphagia, particularly where offspring are fed high-energy diets. These effects can be reversed by administration of leptin, suggesting that fat signalling is important in controlling appetite and sedentary behaviour in the offspring. It might be argued that if these processes prove to be significant in human
development, they may provide an explanation for the poor record of large interventional studies that have attempted to reduce cardiovascular risk status by altering lifestyle choices.40,51

Although cardiovascular disease typically occurs in middle-age or later, many of these modifiable risk factors appear in childhood with a worrying worldwide trend towards earlier onset of obesity, smoking, poor diet, and lack of exercise. Physical activity decreases dramatically around age 10, particularly in girls, leading to obesity.52 Obesity in childhood is increasing rapidly, not only in North America and Europe, but also in traditionally lean populations such as the Chinese and Japanese. Worldwide, it is estimated that 18 million children under the age of five are overweight and 14% of 13 – 15 year-old students smoke.41 With this degree of risk, it is unsurprising that features of cardiovascular disease are now also found in young children. However, there has long been evidence that even in children without such risk factors, pathological changes associated with cardiovascular disease appear in early childhood. In post-mortem studies of children after accidental death, features of coronary atherosclerosis such as fibrous plaques and fatty streaks are frequently found, particularly in children whose risk factors include smoking, dyslipidaemia, hypertension, and obesity.53 There is growing evidence that blood pressure status, too, is established in early life.54 Longitudinal studies following individuals though childhood and into adulthood show that individual blood pressure rankings, relative to the peer group, remain fairly stable. This phenomenon is known as tracking. There is a growing awareness that many of the risk factors for cardiovascular disease have their origins in early life and that they may be driven by biological as well as sociological factors. Despite this, relatively few studies have targeted interventions, intended to reduce cardiovascular risk, at children and the majority of human studies looking at the aetiology of these risk factors are
carried out in adults. Although they are potentially informative, studies in adults may not provide an adequate picture of the processes involved in generation of cardiovascular disease due to the confounding influence of adult factors such as the vascular pathology resulting from those processes, the cumulative influence of varied lifetime experiences on, for example, stress response systems, and the marked moderating effects of post-pubertal hormone levels on cardiovascular function and control.

1.1.4 Size at Birth and Risk of Cardiovascular Disease

It has long been known that small size at birth is associated with an increased risk of cardiovascular disease such as myocardial infarction or cerebrovascular disease such as stroke. To better understand these associations, many international studies have examined the relationship between size at birth and a range of conditions known to predispose to the development of cardiovascular disease. Specifically, small size at birth is associated with a cluster of disorders known as the metabolic syndrome which substantially increase the risk of development of cardiovascular disease. These disorders include glucose intolerance, type 2 diabetes, dyslipidaemia, insulin resistance and raised blood pressure.\textsuperscript{55}

Evidence from over 80 epidemiological studies shows that low birth weight is associated with increased blood pressure and conditions related to raised blood pressure such as vascular endothelial dysfunction, glucose intolerance, insulin resistance and cardiovascular disease, in childhood and into adult life.\textsuperscript{9,56-58} Such studies have been fundamental to the advancement of the Developmental Origins of Health and Disease hypothesis but have necessarily relied upon measures of size at birth such
as birth weight to indicate impaired fetal growth. On occasion, this has led to misunderstanding their results to represent a causal role of small size at birth itself in the development of adult diseases. However, as studies of twins, siblings, first cousins, and intergenerational pairs of first births suggest that the heritable component of birth weight is small, it is felt generally to be an imperfect but useful reflection of adverse intrauterine influences to which fetal growth is highly sensitive.

Indeed, whilst variations in genotype are unlikely to explain large variations in size at birth on a population level, it is not entirely clear what the major determinants of size at birth are or at which stages during fetal life they have their greatest impact. Therefore, whilst most studies in humans have had to rely, perhaps necessarily, upon birth weight and other markers of size at birth as predictors of later disease processes in the offspring, animal studies have been more directed at the factors which possibly underlie both the alterations in size at birth and the programming of later disease, such as maternal nutrition. These studies suggest that effects on outcome measures such as postnatal blood pressure, arterial endothelial function, glucose tolerance and HPAA responsivity of prenatal manipulations such as maternal nutrient restriction may be demonstrated in the absence of alterations in offspring birth weight. This has led to questions about the role of birth weight in the developmental origins of later disease. Such studies make it clear that birth weight need not be affected for programming to occur but where birth weight is altered, it is unclear whether this is a coincidental and unreliable effect of underlying processes which also set about the development of later disease by growth-independent pathways or whether the degree to which size at birth is altered is a more direct indicator of a global programming phenomenon.
Interestingly, a recent study of sheep was carried out to address the hypothesis that a reduction of size at birth was not on the causal pathway between maternal malnutrition and adult disease. The pregnant ewes in this study were severely undernourished for 10 or 20 days in late gestation and compared to a group of normally fed ewes. To the authors' surprise, maternal nutrition in late gestation was not a significant determinant of blood pressure, glucose tolerance or insulin-like growth factor 1 (IGF-1) response to growth hormone challenge in the offspring once their birth weight and current weight were taken into account. However, a reduced birth weight did predict raised blood pressure, reduced glucose tolerance and raised IGF-1 levels. They postulate that the timing of their nutritional insult was a key determinant of this result as most of the studies of nutritional programming relate to interventions in early gestation or at the time of conception. However, they also suggested the possibility that size at birth may reflect the underlying programming process more closely than the level of maternal nutrition. Indeed, fetal nutrition need not be strongly related to maternal nutrition at all given the potential modulating effects of the maternal metabolic and endocrine milieu, placental transport capacity or uterine blood flow. This raises the possibilities that birth weight remained a better indicator of the severity of fetal undernutrition than maternal nutrition was or perhaps that other processes which were unrelated to nutrition were responsible for both the reduction in birth weight and the programming phenomena in the offspring. Where does this leave studies of programming in humans? Whilst it is likely that specific factors such as maternal nutrition have a specific programming effect which should be studied, I would suggest that measures of size at birth remain useful as 'catch-all' non-specific epiphenomena of a range of underlying processes. Therefore, until the individual effects of all these processes are catalogued and understood, measures of size at birth
such as birth weight should continue to be examined even if only as an adjunct to more
directed measures of, for example, maternal health or diet.

Whilst birth weight has been useful as a non-specific, and possibly non-sensitive,
indicator of the quality of prenatal growth and development in the human
epidemiological studies, it has helped to provoke controversy. As no single process or
set of processes can be easily identified which may have contributed to both alterations
in birth weight and later disease in the human studies, this has facilitated a wider
criticism of the Developmental Origins of Health and Disease Hypothesis. This is in
spite of the clear demonstrations of programming by specific interventions in animal
studies. One such criticism addresses the strength of the epidemiological associations
between birth weight and risk factors for cardiovascular disease such as blood pressure
status and plasma lipid profile. Despite the now substantial body of evidence
supporting such associations, two recent meta-analyses carried out by the same
research group suggest that the size of these associations (but not their existence), their
clinical relevance and, by extension, the Developmental Origins of Health and Disease
hypothesis are all in doubt.\textsuperscript{71,72} The bombastic nature of their conclusions is not entirely
supported by their analyses, however. Whilst meta-analyses are often a useful adjunct
to original science, meta-analytic approaches must be used judiciously to avoid
frequently encountered pitfalls.\textsuperscript{73} It is well known that one of these frequent pitfalls is
the dilution of effect sizes that occurs when meta-analyses include large studies with
relatively poor quality data. In the majority of the larger datasets drawn upon in these
meta-analyses, blood pressure and birth weight were obtained by self-report rather
than by direct measurement. Their use of a fixed-effects meta-analysis gives undue
weight to such studies by virtue of their size which dilutes the estimate of effect size
found in smaller studies with more accurate methodologies. Modern meta-analysis
generally involves more than just simplistic combination of effect sizes of a set of studies. Tests are used to determine if study outcomes show more variation than that expected from sampling different research participants. If so, additional predictor variables are used in the analyses to account for method of measurement, population sampled or aspects of study design. Some methodological weaknesses in studies can then be corrected statistically. For example, it is possible to correct effect sizes or correlations for the downward bias due to measurement error, rounding errors or restriction on score ranges. There are further difficulties with their conclusions regarding the association between size at birth and later blood pressure: whilst the effect of birth weight on blood pressure across the normal range has always been noted to be modest, their conclusions take no account of the much greater impact of birth weight on risk of hypertension. 

An additional source of controversy has arisen over the interpretation of statistical associations between size at birth and health outcomes in later life. Whilst most investigators have interpreted such associations as evidence for the impact of prenatal growth on processes which affect health in later life, Lucas and others have argued that, because these results sometimes depend on adjustment for measures of current size, they reflect postnatal growth. For example, if there is no correlation between birth weight and blood pressure in a study, but both are positively related to current weight (as is usually the case), adjustment for current weight may reveal a negative correlation between birth weight and blood pressure. Thus, this statistical model reveals an effect of birth weight on blood pressure only in the context of current size. Lucas et al. argue that this is a measure of postnatal growth. However, this would be a poor measure of postnatal growth, at best, because relationships between prenatal and postnatal growth trajectories are unlikely to be well approximated by a linear model with two time
points. Indeed, it is well-known that growth in childhood is characterised by periods of variable growth acceleration that depend on a wide range of factors such as nutrition or emotional support and not, simply, upon size at birth. In essence, such models are more akin to studies where subjects are stratified by current weight to assess the effect of birth weight on outcome measure within each stratum and, therefore, arguably the best statistical approach for understanding the mechanisms relating prenatal growth to later disease.

Such arguments over the relative importance of prenatal versus postnatal growth in the determination of health outcomes are unlikely to be settled by the injudicious application of algebra to the problem. Indeed, I would argue that this approach is as futile as rearranging Ohm's law in an attempt to prove that resistance is more important than current as a determinant of potential difference. Only longitudinal studies where postnatal growth trajectories are assessed at multiple key points during childhood are likely to define the importance of postnatal growth in the origins of later health and disease. However, due to the relative paucity of such data, controversy exists in this area also. Drawing from their data on postnatal growth in premature infants, Singhal and Lucas suggest that early postnatal growth may have a detrimental impact on the risk of insulin resistance in later life. There are two difficulties with this. Firstly, they have drawn conclusions about normal child development from a small study of premature infants using split proinsulin as a marker of insulin resistance. Given the well-known additional risks and pathologies involved in prematurity and the uncertainty regarding the interpretation of split proinsulin, this is likely to be misleading. Secondly, growth in their study was only assessed using weight at birth, at two weeks and in adolescence. Their finding of a positive association between growth in the first two weeks of life and a marker of insulin resistance in later life is interesting
but should not be used to draw conclusions about the effects of growth in infancy as a whole. Indeed, a number of studies suggest that over the first year of life, as a whole, growth is inversely associated with adverse outcomes such as insulin resistance, high blood pressure and cardiovascular disease in later life, and that subsequent accelerated growth in later childhood, leading to obesity, carries additional risk. Furthermore, many such studies suggest that the effects of prenatal and postnatal growth may be separate and, therefore, represent entirely different programming phenomena. In view of this, I would suggest that measures of size at birth remain useful indices of prenatal adversity.

The timing and duration of intrauterine adversity seems to be important and may not be reflected at all in measures of size at birth. Therefore, studies relying on size at birth alone may under-detect evidence of developmental influences on later disease (a type II error), but significant associations in studies using size at birth as a measure of fetal growth are unlikely to be due to some other determinant of size at birth such as genetic factors. It is also important to note that most studies report continuous relationships with size at birth across the normal range suggesting that a universal feature of fetal development, not a process confined to the extremes of fetal growth, is responsible for these findings. Therefore, size at birth in studies of normal healthy populations is believed to largely represent the extent of maternal constraint, i.e. those environmental processes that occur during pregnancy and influence size at birth but are not necessarily extreme enough to cause immediate pathology. Any process which either limits uteroplacental transfer of nutrients, perhaps by impairing adequate placental implantation or function, or modifies the nutrients made available by the mother, is therefore a candidate cause of maternal constraint. Maternal age, body size and obesity, parity and a catalogue of processes that limit fetal nutrient supply are all
considered to contribute to maternal constraint through their influences on both of these mechanisms.\textsuperscript{67} Other environmental factors which contribute to this process include: season of birth\textsuperscript{12} and infection,\textsuperscript{97} two factors which may be linked, although ambient outdoor temperature variation has an independent effect,\textsuperscript{1,3} maternal smoking,\textsuperscript{48} and maternal nutrition which may depend, not only on maternal intake, but also on maternal nutritional reserves and tissue turnover of protein and fat.\textsuperscript{88,90} Of course, fetal nutrient supply need not necessarily be limited to have a detrimental impact but may involve an excess supply of specific nutrients, an unbalanced nutrient supply due to maternal metabolic conditions, or an unbalanced maternal diet. Gestational diabetes, a condition where excess availability of glucose may lead to macrosomia in the newborn and, therefore, excessive birth weight would be one example and also an example of where birth weight is a poor measure of fetal adversity. Indeed, in some studies, a \textit{U-shaped} relationship between size at birth and later non-insulin dependent diabetes or cardiovascular disease has been reported which probably reflects the impact of gestational diabetes.\textsuperscript{91,92} Unlike other environmental factors such as smoking, infection and seasonal ambient temperature, all pregnancies may be influenced by maternal diet and body composition. There is increasing evidence that fetal development is affected by variation of nutrients across the normal range of western diets.\textsuperscript{93} Therefore, maternal nutrition is seen as a promising target for potential interventions to improve fetal development and any consequent risk of later disease.
1.1.5 Maternal Nutrition and Risk of Cardiovascular Disease

In humans, the availability of nutrients to the fetus is determined by the mother's body composition at conception, her nutritional stores and her diet during pregnancy, together with her ability to supply nutrients to the fetus through the placenta. Recent studies have shown that variations in the balance of maternal protein, carbohydrate and green vegetable intake during pregnancy are associated with altered fetal and placental growth and with increased adult blood pressure in the offspring. A follow-up study of men and women whose mothers took part in a survey of diet in pregnancy in Aberdeen showed alterations in placental weight at birth and increased blood pressure in adult life at both extremes of the balance of maternal animal protein to carbohydrate intake in pregnancy. Support for adverse long-term effects of maternal diets with a low ratio of animal protein to carbohydrate has come from the experimental studies in pregnant rats and from follow-up of studies children and adults in the Philippines and Holland. Support for adverse effects of a high ratio of animal protein to carbohydrate has come from follow-up of men and women in Motherwell whose mothers had been advised to eat a high animal protein, low carbohydrate diet in pregnancy. A higher maternal intake of meat and fish during pregnancy, coupled with a lower intake of carbohydrate and green vegetables, was associated with increased adult blood pressure.

Prospective studies in Southampton support the importance of dietary balance during pregnancy and suggest that maternal folate status may be a key determinant of the effects that result. In an initial survey of women studied at a time when most took folate supplements in late pregnancy, high intakes of meat and fish protein in relation to carbohydrate were associated with larger fetal size. In contrast, in a subsequent
survey in which many women took folate supplements in early pregnancy but few took them in late pregnancy, those whose diets were high in meat and fish, but low in folate or green leafy vegetables had babies of lower birth weight. These findings are consistent with a review of trials of protein supplementation in pregnancy, which concluded that high protein density supplements led to lower offspring birth weight.\(^9\)

The observation of adverse effects of high protein maternal diets appears paradoxical, in that fetal growth represents net deposition of lean tissue and therefore an absolute requirement for protein. Whilst an inadequate supply of protein might be expected to impair fetal growth, it is less clear why high protein intakes may be detrimental. One possibility is that meat and fish are rich in essential amino acids, which must either be used for protein synthesis or oxidised.\(^1\) Oxidation consumes non-essential amino acids, whose synthesis requires cofactors including folate and vitamin B6. Low intake of green vegetables, a source of folate, accentuates the effect of high meat and fish consumption on the offspring’s blood pressure. In mothers with a limited capacity to synthesise non-essential amino acids, maternal amino acid oxidation could impair fetal growth as a result of reduced availability of the non-essential amino acids required in large quantities by the fetus. Consistent with this hypothesis, increased maternal amino acid oxidation during pregnancy has recently been associated with impaired fetal growth.\(^9\)

Although the observations from these studies suggest that the balance of maternal meat, fish, carbohydrate and folate intake could have important implications for the offspring’s risk of cardiovascular and metabolic disease, the dietary data available in these studies were crude and secure identification of the optimal balance of nutrients requires studies with more detailed maternal dietary data.
1.2 Stress

It is becoming increasingly clear that an important means by which prenatal adversity, perhaps reflected by small size at birth, may have long-term effects on risk of cardiovascular disease is through adaptation of a diverse range of hormonal and neurological systems that control growth and development. Specifically, the HPAA and autonomic nervous system (ANS) have been implicated which are the key physiological mechanisms by which an organism responds to stress. In seeking to explain the pathways from environmental influences during early life to later disease, therefore, the concept of stress may be important. Not only is there considerable evidence that psychological processes related to stress are associated with adult diseases, including cardiovascular disease, but also that the physiological processes implicated in this association act to mediate the somatic effects of psychological stress and are vulnerable to modification by early life processes. A description of these processes and the evidence for such vulnerability are given in sections 1.3 and 1.4.

For a word that encompasses so many important features of so many areas of academic research, stress is remarkably imprecise and poorly defined. To a physicist, for example, stress is the internal distribution of forces in a material that balance and react to the loads applied to it. Whereas, in the realms of musicology, phonology and the biological sciences, stress has entirely different meanings. Nonetheless, a search on MEDLINE, a publicly available database of articles published in the biomedical literature, yields over 270,000 articles since 1943 that have used the term. This amounts to approximately 2% of the biomedical sciences literature indexed by MEDLINE. For comparison, a search in the same database for all articles related to asthma yields less than 88,000 articles. Therefore, to dismiss the concept of stress on the grounds of
continuing difficulties with definition, would be to dismiss a substantial body of biomedical research.

The concept of stress in the medical, biological and psychological literature arose from the work of Hans Selye who first used the term in 1936 to encompass a wide range of strong external stimuli, both psychological and physiological, that can cause a physiological response which he termed the 'general adaptation syndrome'. Unfortunately, Selye was not well versed in the existing definition of stress used by physicists for centuries to describe the elastic properties of materials. Had he been, he might have chosen the word strain, which physicists use to describe the effect of physical stress on a material. Further confusion arose from his initial use of the term stress to describe the cause of the resulting condition, which he initially called the general adaptation syndrome but later dropped and replaced with the term stress. Thus, as one critic wrote after drawing on citations of Selye's publications, 'Stress, in addition to being itself, was also the cause of itself, and the result of itself.' In order to correct this confusion, Selye introduced the term stressor to describe the cause of stress. The result of this early semantic confusion has been a steady adaptation and dilution of the term by both academics and lay public so that stress now has such an expansive and fluid definition as to be almost meaningless when used on its own. Most researchers looking at the effects of psychological stress on animal and human physiology have approached this problem by adopting a circular definition of stress as any condition where physiological stress responses are present, such as enhanced adrenocortical or adrenomedullary activity, and a definition of stressors as any causative agents which produce such responses. As a result of this, some have called for an abandonment of the term, stress. However, when used with clarity about the factors to which the user is referring, it may be a better alternative to exhaustively listing similar psychosocial
factors but it would be unwise to assume that measurement of similar physiological responses (stress responses) to different stressors implies a common underlying process, i.e. that the stress is the same. To give a hypothetical example, sleep deprivation and maternal separation might both cause similar increases of blood pressure in a group of animals, leading to the idea that the same stress condition is being produced. However, one stressor might act entirely through altered endocrine activity whilst the other might rely upon alterations in ANS activation. Furthermore, the mechanisms by which a stressor produces a stress response might differ between individuals with different characteristics such as species, age, sex, or race. Ultimately, work that falls within the domain of stress research in the biomedical sciences attempts to trace causation from social and psychosocial processes through behaviour and physiology to a disease endpoint. Perhaps then, stress is most useful as an orientating term allowing the reader to appreciate that your work has this aim.

This thesis is focused principally on the activity of two specific neuroendocrine systems that are activated by a variety of psychosocial and environmental challenges that occur frequently during the life of all animals including humans. Activation of these systems leads typically to an array of metabolic and cardiovascular changes which promote the immediate survival of an organism in challenging circumstances but may, if prolonged or frequent, interfere with somatic processes of repair, defence against illness, and development. These systems are the HPAA, which produces cortisol (a steroid hormone) in humans, and the sympathetic adrenomedullary axis, which produces adrenaline and other catecholamines and acts rapidly in concert with direct sympathetic nervous system (SNS) activation and parasympathetic nervous system (PNS) withdrawal to prepare the body for an immediate challenge. In contrast, the HPAA exerts slower, more persistent effects on the body to maintain a steady
metabolic state in the face of more prolonged challenges. Both of these systems have been implicated in the development of cardiovascular disease.\textsuperscript{104}

1.2.1 Stress and Psychosocial Pathways to Cardiovascular Disease

There is extensive evidence to support the concept that psychosocial factors are associated with cardiovascular disease.\textsuperscript{104-106} Although there is a growing body of work which defines specific mechanisms that may mediate these associations, an apparent failure to recognise this has led to a somewhat dismissive attitude towards the idea that psychosocial factors may have a direct causative role in cardiovascular disease and a general assumption that where they are linked to cardiovascular disease, their impact is only mediated indirectly through their effects on risk behaviour such as poor diet, lack of exercise and smoking. Whilst there is good evidence for the existence of these indirect pathways,\textsuperscript{104,105} large epidemiological studies, such as the Whitehall II study,\textsuperscript{107} have shown associations between psychological distress and coronary heart disease that are independent of health behaviours, social isolation, and work characteristics suggesting the coexistence of more direct psychosocial pathways in cardiovascular disease. In fact, there is now clear and convincing evidence that factors in at least five specific psychosocial domains contribute significantly to the pathogenesis and expression of coronary artery disease through modification of risk behaviour and through direct psychophysiological effects. These include depression, anxiety, personality factors and character traits (such as hostility), social isolation (including marital status), and chronic or sub-acute life stress.\textsuperscript{105} In addition to altered health behaviours, direct mechanisms such as platelet and neuroendocrine activation have been implicated as links between these psychosocial conditions and cardiovascular disease. Rozanski \textit{et al.} have suggested that perhaps the multidisciplinary nature of this
evidence has led to an under-appreciation of the strength of the epidemiological and pathophysiological data which may account for the continuing resistance to the idea that the psychosocial realm has a causative role in cardiovascular disease. Table 1 gives a summary of the number, size and strength of findings of the studies reviewed in their paper.\textsuperscript{109} One of the striking features of these disparate psychosocial factors is the commonality of the direct pathophysiological pathways implicated in their association with cardiovascular disease.

1.2.1.1 Psychosocial Factors

Depression is accompanied by hypercortisolaemia,\textsuperscript{106,109} attenuated adrenocorticotropic hormone (ACTH) response to corticotrophin releasing hormone (CRH) administration,\textsuperscript{109} non-suppression of cortisol after dexamethasone administration,\textsuperscript{119} and elevated CRH concentration in the cerebrospinal fluid\textsuperscript{111} which all imply an up-regulated HPAA resistant to negative feedback control. Studies in young children and adolescents indicate that these hormonal axis changes precede subsequent development of clinical depression and disappointing life events.\textsuperscript{112-117} This increase in subsequent negative life experiences suggests that altered HPAA function has a role in the development of abnormal cognitive or social processes associated with disturbed interpersonal behaviour.\textsuperscript{116} Additionally, depression is associated with impaired platelet function indicated by enhanced platelet reactivity and release of platelet products such as platelet factor 4 and \(\beta\)-thromboglobulin.\textsuperscript{118,119} This hypercortisolaemia together with the prothrombotic haematological changes form a theoretical basis for the increased likelihood of atherosclerotic plaque formation seen in depressed individuals. Furthermore, ANS derangement has also been noted with increased sympathetic activation,\textsuperscript{120-122} decreased parasympathetic activity,\textsuperscript{123} impaired
baroreflex control\textsuperscript{124,125} and reduced heart rate (HR) variability\textsuperscript{126,127} being reported in this condition which all predispose to subsequent hypertension and increased risk of arrhythmia and sudden cardiac death.\textsuperscript{128-130}

Table 1. The impact of psychosocial factors on risk of cardiovascular events.\textsuperscript{105}

<table>
<thead>
<tr>
<th>Psychosocial factors</th>
<th>No. of significant studies</th>
<th>End points</th>
<th>Median results from significant studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of subjects</td>
</tr>
<tr>
<td>Subjects without cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>6 / 8</td>
<td>ACM, CD, CHF, CVA, IHD, MI</td>
<td>1990</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td>5 / 5</td>
<td>CD</td>
<td>2271</td>
</tr>
<tr>
<td>Hostility</td>
<td>6 / 11</td>
<td>ACM, AP, CAD, CD, MI</td>
<td>1018</td>
</tr>
<tr>
<td>Social isolation or unmarried</td>
<td>15 / 15</td>
<td>ACM, AP, CD, MI</td>
<td>2754</td>
</tr>
<tr>
<td>Subjects with cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>8 / 8</td>
<td>ACM, CABG, CD, MI</td>
<td>248</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td>4 / 4</td>
<td>AP, CD, MI, VF or VT</td>
<td>155</td>
</tr>
<tr>
<td>Hostility</td>
<td>4 / 4</td>
<td>AP, CABG, CD, MI, PTCA, restenosis</td>
<td>171</td>
</tr>
<tr>
<td>Social isolation or unmarried</td>
<td>10 / 11</td>
<td>ACM, CD, MI</td>
<td>1061</td>
</tr>
</tbody>
</table>

F/U, follow-up; ACM, all cause mortality; CD, cardiac death; CHF, congestive heart failure; CVA, cerebrovascular accident; IHD, ischaemic heart disease; MI, myocardial infarction; AP, angina pectoris; CAD, coronary artery disease; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty.
The direct biological links between hostility and coronary artery disease show a striking similarity to those found in depressive subjects. When compared to non-hostile individuals, hostile subjects exhibit greater HR and blood pressure responses to mental stress and higher ambulatory blood pressure levels during normal daily activities. They have weaker vagal control of HR, increased platelet reactivity, and higher concentrations of circulating catecholamines and cortisol which indicate up-regulated sympathomedullary and HPAA activity. As is the case for depression, these features of hostility are both atherogenic and predictive of sudden cardiac death.

Social isolation or lack of social support often accompanies other psychosocial problems such as depression or hostility but has also been found to be independently predictive of cardiovascular disease. In addition to the findings in humans summarized in Table 1, studies in a variety of animals including primates have shown dramatic increases in the risk of cardiovascular disease as a result of disruption of social networks, isolation, or overcrowding. Again, in addition to influences on high-risk behaviour, social isolation, like hostility and depression, exerts direct pathophysiological effects on individuals which are similar to those described for hostility and depression. These include hypercortisolaemia, reversible increases in HR (a marker of sympathetic activation and parasympathetic withdrawal) and increased urinary adrenaline concentration (a marker of increased sympathetic activation).

In contrast to these other psychosocial factors, the association between anxiety syndromes and cardiovascular disease is relatively less studied and those studies that have been done prospectively show strong associations between anxiety and sudden cardiac death but weak or absent associations with myocardial infarction. This suggests
that anxiety may provoke arrhythmias leading to sudden death but not be involved directly in atherogenic processes. In support of this, there is evidence of reduced heart rate variability (HRV) and reduced baroreflex control in patients with anxiety which indicates altered ANS control of cardiovascular function that would predispose such patients to arrhythmia.

1.2.1.2 Chronic Stress

It appears, therefore, that a range of psychosocial factors may have both direct and indirect causative roles in the development of cardiovascular disease and that increased sympathetic and decreased PNS activation, altered baroreflex control, and up-regulated HPAA activity are almost universally implicated in their direct effects on cardiovascular disease. However, the factors explored so far are psychosocial conditions allied with stress but do not traditionally fall under the rubric of stress. Studies of psychosocial parameters more commonly thought of as stress-related suffer from difficulties of definition that impede repeatable measurement. Some reliable models of chronic stress have been developed, most of which focus on work-related stress. For example, in a prospective study which followed 1928 men for six years using a model of job strain, defined as high job demand with concurrent low decision latitude, job strain was associated with a fourfold increase in risk of sudden cardiac death. Although this finding has been replicated in other studies, there have also been large scale studies which have failed to do so. However, in one of these negative studies (the Whitehall II study), a different model of work-related stress, defined as an imbalance between personal efforts and rewards, was associated with a doubling of cardiovascular risk. There also appears to be a sex disparity in these associations with the majority of more than a dozen case-controlled and cohort studies
in men demonstrating a link between job strain and cardiovascular disease but only three of six cohort studies reporting a similar association in women. The largest of these, the US Nurses’ Health Study prospectively followed over 35,000 women aged 46 – 71 for up to four years and found no association between job strain and cardiovascular disease.

Other studies have examined the impact of recent major life events, such as divorce, job loss, or bereavement and show a marked increase in the incidence of these events in the months running up to a major cardiovascular incident such as myocardial infarction or sudden cardiac death in both previously healthy individuals and those with previous myocardial infarction. Overall, the weight of evidence supports an association between chronic life stressors and the development of cardiovascular disease but the evidence remains controversial. It seems likely that the apparent contradictions in the evidence have arisen from individual differences in responsiveness to the stressor chosen for a particular study which may be sex-based or dependent on other characteristics. Sociocultural factors influence the appraisal of demands and threats in social situations in adult life and these influences are embedded in sex-based coping strategies, for example, resulting in differential stress impacts of the same situation for different social groups. Thus, finding a reliable, repeatable and universal human stressor remains a holy grail of stress research.

Until one becomes available, studies in animals are a good adjunct to human research as they can ensure genetic homogeneity and common environmental experiences, controlling for two of the most likely confounding factors in human studies. Primate species are often similar to humans in the organisation and expression of their social behaviour. A series of studies in Cynomolgus monkeys, which have a similar social
structure to humans, have shown that social dominance in an unstable environment where animals are regularly switched between groups, forcing regular re-establishment of dominance, is a potent stimulus for coronary artery atherosclerosis. \textsuperscript{107-109} β-adrenergic blockade with propranolol prevents this,\textsuperscript{119} implicating the SNS. The atherosclerosis is worsened by a high cholesterol diet\textsuperscript{119} but vasoconstrictor responses to acetylcholine in the coronary arteries were shown to be almost independent of diet and largely dependent on the degree of psychosocial stress suggesting that endothelial function is profoundly altered by chronic stress in this model.\textsuperscript{160}

1.2.1.3 Acute Stress

Perhaps due to its temporally discrete nature, acute psychosocial or mental stress is much easier to model and study. Acting through the same autonomic and endocrine processes that have been described for chronic stress, acute stressors produce a range of physiological effects including tachycardia, hypertension, and vasoconstriction which contribute to myocardial ischaemia, arrhythmias, worsening endothelial function and endothelial injury, platelet activation, and haemostatic changes and haemocentration which contribute to greater plaque vulnerability and risk of thrombosis. A large battery of mental stress tasks have been shown to induce myocardial ischaemia measured by an array of techniques including electrocardiogram (ECG) changes, radionuclide ventriculography, echocardiography, and positron emission tomography.\textsuperscript{108} As for chronic stress, the nature of the stressor is a crucial determinant of the characteristics of the response. For example, some mental stress tasks, such as public speaking and mirror-tracing, are more potent than others, such as Stroop colour/word interference\textsuperscript{161} and mental arithmetic tasks, in stimulation of myocardial ischaemia.\textsuperscript{108} However, these studies show that mental stress induces myocardial ischaemia in up to 50% of patients
with existing coronary artery disease. They also challenge the common perception that extreme emotional distress would be required to precipitate cardiovascular events by demonstrating significant myocardial ischaemia in response to behavioural challenges similar to those that most of us encounter daily.

Such studies cannot address the extent to which these processes are responsible for cardiac events in the general population. Insight on this issue comes from studies of incidence of cardiac disease clustered around large-scale social events and natural or man-made disasters which are assumed to be stressful to the general population. For example, the incidence of life-threatening ventricular arrhythmias in patients with implantable defibrillators increased significantly three days following the World Trade Centre terrorist attack in 2004, both in New York City\textsuperscript{162} and in Florida which was not attacked and is geographically remote from New York.\textsuperscript{163} These effects were not attributable to seasonal or monthly variation in these events which are known to occur and incidence remained raised for a month following the attack. Another example comes from studies of myocardial infarction incidence related to important football matches. For example, when England lost to Argentina on penalty shoot-out during the 1998 World Cup, risk of admission for acute myocardial infarction rose by 25\% for three days. Other conditions such as stroke, deliberate self-harm, and road traffic injuries showed no similar association with this event.\textsuperscript{164} A similar finding was reported for the quarter final between the Netherlands and France in the 1996 European cup.\textsuperscript{165} Another study which followed local football team supporters in the North of England for a five year period showed that this effect is not limited to big international matches.\textsuperscript{166} On days when the local professional football team lost at home, risk of mortality attributable to acute myocardial infarction and stroke increased significantly in men by 28\%. This effect was not seen in women. Perhaps the most intriguing finding
in studies of football-related stress comes from a study of the 1998 World Cup which showed that mortality from myocardial infarction of French men on the day that they won the World Cup decreased significantly. This raises the possibility that the celebration and euphoria surrounding such an event may actually reduce stress-related cardiac deaths. Studies examining the activation of candidate stress pathways such as the SNS, for example, will be necessary to clarify the mechanisms involved in these associations.

Animal studies support a role for acute stress in the pathogenesis of myocardial infarction. Studies of people with familial hypercholesterolaemia (a condition which significantly increases the risk of myocardial infarction) have identified a number of important gene polymorphisms, coding for proteins involved in the metabolic regulation of cholesterol, which cause the condition. This has allowed the development of animal models which are genetically predisposed to myocardial infarction. Two genes which may be knocked out to create such models include the apolipoprotein E (ApoE) gene and the low density lipoprotein (LDL) receptor gene. ApoE, like other apolipoproteins, stabilises and solubilises lipoproteins and acts as the primary ligand for the LDL receptor (which binds to apolipoprotein B as well). ApoE is critical to the formation of very low density lipoproteins and the LDL receptor is the primary receptor for transportation of such cholesterol-carrying lipoprotein particles into cells. Therefore, knockout mice which lack the ApoE gene and the LDL receptor gene become hypercholesterolaemic and develop coronary atherosclerosis. Recent studies have shown that myocardial infarction can be triggered in these mice by both mental and hypoxic stress. Furthermore, it was demonstrated that these pathological changes could be prevented by blockade of the endothelin type A receptor suggesting that the
pathway from acute stress to myocardial infarction involves endothelin receptor signalling.

1.2.1.4 Stress Responsiveness and Cardiovascular Disease

Acute stress responsiveness, measured as HPAA or sympathetic activation, is also linked to a number of risk factors for cardiovascular disease. A number of animal models support this. These include a rodent model of psychosocial hypertension achieved through the design of social environments to increase confrontations over dominance, the borderline hypertensive rat (BHR) model that develops sustained hypertension after weeks of daily exposure to shock avoidance conflict tasks, and a canine model that also involves daily shock avoidance. In each case, regular induction of high blood pressure reactivity to stress leads to later hypertension. However, this effect is conditional on either a pre-existing inherited susceptibility or an additional environmental factor which potentiates the effect of stress. Whilst the BHR, which has one parent that is spontaneously hypertensive, will develop hypertension through exposure to stress or a high salt intake, other strains of rat exposed to the same stress regime do not develop hypertension even when they do demonstrate high levels of blood pressure reactivity to stress. In the canine model, concurrent exposure to high salt and low potassium intake is necessary for the dogs to develop hypertension in response to daily stress. Therefore, models of stress reactivity which do not consider inheritability and environmental covariates may be too simplistic to adequately model the long-term cardiovascular effects of stress.
Although there is controversy as to the role of HPAA and sympathetic activation in the development of hypertension, these neuroendocrine systems have potent pressor effects. Recent evidence from case-control and cross-sectional studies of people without pituitary or adrenal disease show that elevated plasma cortisol concentrations in morning samples are associated with high blood pressure. In addition, data from both adults and children shows that stress responsiveness is associated with carotid atherosclerosis, increased left ventricular mass and in follow-up studies predicts subsequent blood pressure and the prevalence of hypertension. Particularly strong evidence for the association between stress responsiveness and later hypertension comes from a prospective study of over 4100 healthy young men and women who were followed for 13 years in the Coronary Artery Risk Development in Young Adults (CARDIA) study where blood pressure responses to a range of psychological challenges were strongly predictive of an earlier onset of hypertension. This finding was robust to adjustment for a range of covariates including age, sex, race, body mass index (BMI), education and resting blood pressure which stands in contrast to an earlier, smaller, but nevertheless influential study which was limited to the male civil servants who participated in the Whitehall II study. The authors of this study found that blood pressure reactivity to a mental stress task provided minimal independent prediction of follow-up blood pressure at five year follow-up when baseline blood pressure (at the time of the initial study) was controlled for. However, this study was carried out only in men aged 35 – 55 years at entry and from a very specific population (civil servants) whereas the CARDIA study examined men and women aged 18 – 30 at entry with a broader range of racial and social backgrounds. One possible explanation for the disparity in these studies may be that blood pressure reactivity in an older population is less indicative of underlying autonomic cardiovascular control where vascular dysfunction due to localised factors such as endothelial damage may modify
blood pressure responses. Additionally, the CARDIA study used a range of stressors including the cold pressor task, star-tracing and a video game task whereas the Whitehall II study only used a non-verbal cognitive task.

It is well known that different tasks elicit different levels of response in different subject groups. In particular, stressors do not have the same meaning for all sex and ethnic groups. A substantial body of literature supports this, showing that, in comparison to women, men display greater blood pressure responses to challenging achievement tasks such as video games and that relative to whites, blacks have greater blood pressure responses to tasks that typically yield an α-adrenergic response such as cold pressor or star tracing tasks. It is possible, therefore, that the association between blood pressure responsivity during stress and later risk of hypertension varies by sex and ethnicity as well. There is evidence, however, that even in an older age group, blood pressure reactivity predicts the onset of hypertension. When the original Whitehall II stress study was repeated in the same cohort ten years after initial enrolment, with just over three quarters of the original group, and using the same stress task, blood pressure reactivity did predict the onset of hypertension. In a similar study in Finnish middle-aged men, blood pressure reactivity in anticipation of performing an exercise test was found to be strongly predictive of later hypertension.

When these epidemiological studies were begun, the Reactivity Hypothesis for the development of hypertension suggested a fairly simplistic model of the relationship between stress-induced cardiovascular reactivity and later hypertension. The message that emerges from these epidemiological studies is that this simple relationship does not exist and that a range of additional factors such as age and sex are important for determining which stressors affect which groups of people and the impact that this has
on their long-term health. Whilst it is probable that individuals with hypertension will
demonstrate high reactivity to a range of stressors, it is equally probable that an
individual who demonstrates a marked response to a given stressor may not develop
hypertension. Why might this be? A number of recent strands of research provide an
answer. It is now apparent that blood pressure reactivity is affected by markers of long-
term stress exposure such as negative affect\textsuperscript{187} and job strain\textsuperscript{188} and that stress buffers
such as social support networks which include family and friends reduce both short-
term stress reactivity and long-term cardiovascular risk.\textsuperscript{189} Additionally, family history
of hypertension interacts with these markers of chronic stress to determine the link
between stress responsivity and hypertension.\textsuperscript{190} Therefore, innate susceptibility to
stress has an inheritable component which may be genetic or non-genetic, a social
component, and a component which depends on life course experiences of stress.
Future studies will be needed to address the apparent disparity in the influence of
stress responsivity on risk of disease between different groups by taking these factors
into account.

\textbf{1.3 The Hypothalamic Pituitary Adrenal Axis (HPAA)}

In order to better understand how psychosocial factors may lead to the development of
cardiovascular disease, a detailed understanding of the development, organisation and
function of the neuroendocrine physiology that has been implicated is required. The
following sections elaborate these details for the HPAA and the ANS.
Figure 1. Central and peripheral neuroendocrine pathways influencing cardiovascular and metabolic outcomes during stress. ACh, acetylcholine; ACTH, adrenocorticotropic hormone; Adr, adrenaline; AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; C1, C1 adrenergic neurons of the ventrolateral medulla oblongata; CRH, corticotrophin releasing hormone; GABA, gamma-aminobutyric acid; IML, intermediolateral cell column; LC-NA, locus coeruleus noradrenergic sympathetic system; NA, noradrenaline; NTS, nucleus tractus solitarius; POMC, proopiomelanocortin; PVN, paraventricular nucleus; R-A-A, renin-angiotensin aldosterone system; RFN, retrofacial nucleus; VGP, vagoglossopharyngeal nerve. Neuronal connections are represented as monosynaptic for simplicity but may well be more complex.
1.3.1 Organisation, Function and Control of the HPAA

The HPAA is a key component of human and animal physiology that responds to stressful stimuli together with the adrenal medulla and SNS to effect metabolic adaptations to those stimuli. Figure 1 shows a schematic diagram outlining the processes involved in responding to external or internal stressful stimuli resulting in changes in circulating glucocorticoid concentration. These stimuli can be processive in nature, requiring integration and interpretation by various higher centres of the brain before affecting the afferent pathways of the stress response. They can also be systemic in nature, resulting from basic physiological changes in the organism. Sensory components of processive stressors are integrated at the cortical level and relayed to the prefrontal cortex, amygdala and hippocampus where the relevance of the stimulus is considered on the basis of emotional state and remembered experiences. Processed information from these regions is then passed via multiple neurotransmitters to the hypothalamus, preoptic nucleus and bed nucleus of the stria terminalis (BNST) regions where it is interpreted in the context of existing homeostatic status. Once integrated, the final common pathway for processive stressors is via gamma-aminobutyric acid (GABAergic) neurotransmission to the paraventricular nucleus (PVN) to inhibit CRH release. The amygdala is the region primarily responsible for behavioural responses to fear and anxiety. GABAergic or neuropeptide releasing efferents from the amygdala up-regulate the endocrine stress response by inhibition of the inhibitory inputs to the PVN. In human psychological studies, environments where the subject feels out of control or fearful cause high levels of HPAA activation. The hippocampus and the prefrontal cortex are the primary centres which inhibit the endocrine stress response by excitation of the inhibitory inputs to the PVN allowing stress response to be moderated by prior experiences.
Systemic stressors are detected by the viscera and relayed via the brainstem by catecholaminergic pathways to effect CRH / vasopressin release at the PVN. Furthermore, circulating stress markers such as angiotensin II (Ang II) or cytokines can be detected by the circumventricular organs which also effect CRH / vasopressin release at the PVN. These hormones travel in the hypophysial portal circulation to the anterior pituitary where they cause cleavage of ACTH from pro-opiomelanocortin (POMC). ACTH is synthesized in and released from corticotrophs in the anterior pituitary into the systemic circulation where it travels to the adrenal glands. Here, ACTH binds to its receptors on the surface of cells in the adrenal cortex stimulating the release of glucocorticoid. Variable stimulation and inhibition of the PVN by the suprachiasmatic nucleus causes concentrations of glucocorticoid and ACTH in the circulation to follow a circadian rhythm. Glucocorticoid levels rise to a peak around 7 am in humans then decline steadily throughout the day reaching a nadir between 7 pm and midnight before slowly rising again. The ACTH rhythm precedes the glucocorticoid rhythm in phase by 1–2 hours. Nocturnal animals, such as rats, display a similar but reversed circadian rhythm with glucocorticoid levels reaching a peak around wakening times (this being the evening in the case of rats). Complex interactions between the PVN, the arcuate nucleus which responds to leptin and insulin, and the locus coeruleus-noradrenergic sympathetic system which integrates incoming information from the PVN and the arcuate nucleus with visceral status and arousal, suggest additional pathways by which systemic stressors and food consumption may influence activity of the HPAA (Figure 1).
In the majority of mammals, including humans, the primary circulating glucocorticoid is cortisol. In rats and other rodents, it is corticosterone. The symptoms of primary adrenocortical insufficiency first described by Addison in the mid 19th century give some idea of the biological importance of these hormones. His description of 'general languor and debility, remarkable feebleness of the heart’s action, irritability of the stomach and a peculiar change in the colour of the skin' came to be known as Addison’s Syndrome. Glucocorticoid receptors (GR) are expressed on the surface of every normal mammalian cell type. Studies in patients and adrenalectomised animals demonstrate a wide range of actions for glucocorticoids including stimulation of gluconeogenesis, inhibition of glucose uptake and utilization in peripheral tissues, increased glycogen deposition, increased lipolysis and free fatty acid release, stimulation of protein and nucleic acid synthesis particularly in the liver, acceleration of various developmental events, acceleration of liver, pancreas, gastrointestinal tract, and lung maturation, suppression of immune and inflammatory responses, inhibition of cytokine synthesis, inhibition of fibroblast proliferation and extracellular matrix deposition, regulation of calcium absorption and redistribution, and maintenance of normal salt and water homeostasis, cardiovascular function and blood pressure. They also have central neuromodulatory actions with effects on behaviour, appetite control, learning, memory and the HPAA itself. In the amygdala, for example, they enhance memory consolidation and in the hippocampus, they act to inhibit memory recall.

Clearly, these hormones have a wide range of effects on physiological systems, the best studied of which are their effects on carbohydrate metabolism and immune function. Glucocorticoids are catabolic hormones which oppose the actions of insulin, increasing the availability of circulating glucose and fatty acids when an organism is stressed. This is achieved through their action on a number of enzyme systems in the liver, muscle
and fat. In the liver, glucocorticoids enhance gluconeogenesis, the process of glucose synthesis from non-hexose substrates such as lipids and amino acids, by up-regulation of the rate-limiting enzyme in gluconeogenesis, phosphoenolpyruvate carboxykinase.\textsuperscript{194} Glucocorticoids also reduce translocation of glucose transporter 4 to the cell membrane thereby reducing glucose uptake and utilization in skeletal muscle.\textsuperscript{194} Glucocorticoids up-regulate the enzyme, phenylethanolamine N-methyltransferase, in muscle tissue resulting in increased conversion of noradrenaline to adrenaline which acts on $\beta$-adrenoceptors to inhibit insulin-mediated glucose uptake in the muscle.\textsuperscript{195} Lastly, glucocorticoids may increase circulating free fatty acids by inhibiting lipoprotein lipase.\textsuperscript{196}

The HPAA is usually depicted as a classical neuroendocrine feedback loop (Figure 1). However, regulatory inputs to the HPAA are diverse and complex. Undoubtedly, increased levels of circulating glucocorticoid have a central inhibitory effect on activity of the HPAA but the pathways that mediate this negative feedback remain unclear. Studies in rats suggest that the day-to-day basal regulation of the HPAA may not rely upon direct feedback by glucocorticoids but rather via their peripheral metabolic actions. Feeding behaviour, energy balance and central CRH release in adrenalectomised rats can be normalised by ingestion of sucrose suggesting a role for circulating carbohydrates and possibly other energy stores in mediating the feedback actions of increased corticosteroids.\textsuperscript{197,198} The fact that many regions of the brain including the hippocampus, hypothalamus and pituitary richly express GR makes it likely that there must also be a role for a direct action of glucocorticoid on the regulation of the HPAA. During stress in adrenalectomised rats, higher levels of corticosterone actually enhance CRH and subsequent ACTH release suggesting a central driving role for glucocorticoids.\textsuperscript{198} The hippocampus, possibly the most
important region of the brain for inhibitory control of the HPAA during processive stressors, richly expresses glucocorticoid and mineralocorticoid receptors, both of which bind glucocorticoids. So do the amygdala and prefrontal cortex which are also regions involved in control of the HPAA during processive stressors. Selective antagonist blockade of these receptors in these regions enhances HPAA response to processive stressors in rats with intact adrenal glands. Therefore, the feedback effects of glucocorticoids on brain seem to be more focused on the limbic system where emotion and memory are integrated to influence behaviour as well as HPAA and SNS arousal rather than a simple housekeeping feedback loop on CRH production in the PVN and ACTH release at the pituitary gland.

Bioactivity of glucocorticoids depends on their interactions with their transport proteins, receptors and activating or deactivating enzymes. In humans, the majority (>80%) of circulating cortisol is bound to transport proteins, largely corticosteroid binding globulin (CBG). Availability of free cortisol is therefore dependent on concentration and binding affinity of CBG. This has been shown to vary between individuals and even within individuals. For example, like plasma albumin levels, CBG falls with a change in posture from standing to lying. CBG also has a role in the differential delivery of cortisol to tissues through alteration of binding affinity. The action of neutrophil elastase on CBG released by granulocytes cleaves a small (5 kDa) fragment from CBG causing it to release more than 80% of bound cortisol at sites of inflammation. Once released from CBG, cortisol passes into the target cells where it binds to intracellular receptors in the cytoplasm.
There are two classes of intracellular receptor to which it binds, the mineralocorticoid receptors (MR) and the GR. MR have an affinity for cortisol approximately an order of magnitude greater than the affinity that ubiquitous GR have for cortisol and are expressed in specific tissues such as the kidney, colon and brain. In the brain, MR are expressed in key areas such as the hippocampus and amygdala. These receptors are bound to heat shock proteins which hold them in a receptive state. Binding to cortisol induces a conformational change in the receptor/chaperone complex causing the heat shock proteins to disengage. The resulting complex binds to another similar complex forming a homodimer which is internalised into the nucleus by a specific transport protein. In the nucleus, these receptor/ligand complexes recognise palindromic DNA sequences known as Hormone Response Elements, or specifically in the case of glucocorticoids, as Glucocorticoid Response Elements (GREs).

Once bound to these regions of DNA, the complex exerts numerous and incompletely understood effects on transcriptional machinery to alter expression of glucocorticoid responsive genes. Thus, cellular effects of glucocorticoids are relatively delayed and more lasting than, for example, those of the ANS. Although the glucocorticoid receptor complexes function as transcription factors, though DNA binding, to achieve some of their effects, recent studies suggest that many of their effects are independent of DNA binding. Point mutations introduced into the DNA binding sequence of the glucocorticoid receptor gene in mice prevent dimerisation and binding to the GREs. However, mice homozygous for this mutation are still viable, whilst GR knockout mice are not, suggesting that many of the functions of the glucocorticoid receptor are not mediated though DNA binding at all. Finally, pre-receptor metabolism by the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes controls the intracellular availability of glucocorticoid for receptor binding. There are two distinct isozymes
produced by distantly related genes. 11 β-HSD₂ is expressed in specific tissues such as the placenta, kidney, colon, pancreas, gonads, adrenal glands and regions of the brain where it inactivates glucocorticoids to their inert 11-keto derivative. 11 β-HSD₁, on the other hand, is more widely expressed and enhances intracellular glucocorticoid levels by regeneration of circulating 11-dehydrocorticosterone or cortisone.²⁰⁴

1.3.2 Development of the HPAA

The HPAA plays an important part in preparing developing mammalian fetuses for extra-uterine life. It acts in late gestation to enhance maturation of numerous organs including the lungs, liver and gastrointestinal tract. For example, in the lungs, it stimulates production of surfactant to prepare the lungs for inflation after birth and brings about structural developments in the small airways.²⁰⁵ Further evidence for this comes from knockout mice lacking the CRH gene that are born with poorly developed lungs and die of respiratory distress shortly after birth. If these mice are exposed to sufficient levels of glucocorticoid during gestation, they are viable and have normal lung function, fertility and longevity.²⁰⁶ In sheep fetuses, there is an exponential rise in plasma cortisol levels in the last 10% of gestation.²⁰⁷ Premature deliveries precede this rise and commonly result in newborns with respiratory distress. It is now common clinical practice to give artificial glucocorticoids such as dexamethasone to human mothers expecting premature delivery of their babies to improve lung maturation and therefore reduce the risk of respiratory distress.²⁰⁸ However, exposure to high levels of glucocorticoids earlier in gestation has been found to predispose the offspring to cardiovascular and metabolic disease in adult life suggesting that the timing of glucocorticoid activity during gestation is critical.²⁰⁹,²¹⁰ Changes in HPAA activity as measured by ACTH and glucocorticoid concentrations in the plasma during gestation
tend to occur over periods of several days. Function of the HPAA is not the same as it is in adult life where HPAA fluctuations often occur in minutes to hours. Therefore, the HPAA probably serves to control developmental processes during fetal life in addition to any role it may have in the response to acute stress.207

Many species such as humans, guinea pigs and sheep have a mature and fully functioning HPAA at birth. However, in the rat and other experimental animals often used to study developmental plasticity of the HPAA, maturation of the axis occurs, to a significant degree, after birth. The human hypothalamus and pituitary gland are formed by the end of the first trimester and begin to express CRH and POMC messenger ribonucleic acid (mRNA) during the early part of the second trimester. GR are also identifiable at this time. Data on the function of the human HPAA in response to stress during fetal life is limited but by late gestation in the ovine model, a robust stress response, involving release of CRH and AVP into the hypophysial portal system and resultant ACTH and cortisol release, can be stimulated by hypoxia.211

1.3.3 Developmental Plasticity of the HPAA

Changes in the set-point of several hormonal systems may mediate the long-term effects of developmental responses to environmental adversity in early life. In particular, increasing evidence suggests that the secretion of glucocorticoid hormones by the HPAA has an important role in developmental adaptation to a harsh environment.213 In a wide variety of studies of different species where early adversity was experimentally induced, the result is altered neuroendocrine maturation leading to persistent differences in the HPAA both in response to stress and during basal conditions. In these studies, alterations to the set-point of the HPAA have been
induced both prenatally, by nutrient restriction, maternal adversity, non-abortive maternal infection, alcohol exposure, maternal stress-induced glucocorticoid production and exposure to synthetic glucocorticoids and postnatally, by neonatal handling, maternal deprivation, maternal care and infection. In most of these examples, the environmental manipulations during early life are stressful with detrimental effects on neurodevelopment, resulting in increased stress-induced corticosteroid production in the older animal with or without accompanying changes in basal HPAA function. Putative stress-reducing manipulations such as feeding or stroking have also been shown to oppose those effects of stress on later HPAA function. These neuroendocrine differences are frequently accompanied by disorders of behaviour including demonstrations of increased anxiety or emotionality in adverse situations. For example, exposure of pregnant rhesus monkeys to mild psychological stress in mid to late gestation results in reduced birth weight offspring with impaired neuromotor development, increased disturbance behaviour during stress, attention deficit and altered basal and stress-induced HPAA function.

It is thought that the effects of prenatal stressors may be mediated by excessive fetal exposure to glucocorticoid hormone resulting in persisting alterations in HPAA activity. In support of this proposal, prenatal treatment of rats with dexamethasone increases fetal glucocorticoid exposure, leading to permanently increased HPAA activity with increased circulating levels of corticosterone. Studies in animals exposed to prenatal stress suggest that this is probably effected, in part, by lifelong reductions in mineralocorticoid or glucocorticoid receptor density in the hippocampus, which is an important site of negative feedback on the HPAA. However, findings from such studies have been inconsistent. Partial explanation for this may lie with the disparate stress methodologies and animal species used but it is also apparent that sex may be an
important factor in the relationship between prenatal stress and later hippocampal receptor populations.\textsuperscript{230,231} Additionally, the same stressor administered at one stage of gestation can have a profound effect on later HPAA function whilst having no effect at a different gestational age, underlining the critical important of the timing of the stressor during prenatal development.\textsuperscript{232}

Evidence from human studies is comparatively sparse. Among men aged 64 years, born in Hertfordshire, a continuous negative relationship between birth weight and timed fasting plasma cortisol concentrations has been observed. For example, individuals who weighed 5.5lb or less at birth had a mean cortisol concentration of 408 nmol/l whilst those weighing 9.5lb or more, had a mean cortisol concentration of 309 nmol/l. This trend was independent of age and BMI.\textsuperscript{173} Similar relationships between birth size and fasting cortisol concentrations in two other populations in Preston, UK, and in Adelaide, South Australia have been demonstrated.\textsuperscript{233} Detailed studies in both Hertfordshire and South Africa show that low birth weight is associated with increased cortisol responsiveness to synthetic ACTH (Synacthen), a finding strongly suggestive of increased activation of the HPAA.\textsuperscript{234,235} With the exception of the findings presented in this thesis, a contemporary retrospective study in male twins provides the only evidence in humans for a link between fetal growth and later HPAA function during stress.\textsuperscript{236} Little is known in humans about the influence of the mother in programming the HPAA of the offspring. In the late 1960s in Motherwell, Scotland, an obstetrician by the name of Kerr Grieve was advising pregnant mothers to eat 1lb of red meat daily and avoid carbohydrate-rich foods, believing that this might help to prevent pre-eclampsia. A recent study of the offspring of those pregnancies provides the first evidence that unbalanced high protein diets, lacking carbohydrates and green vegetables, are associated with higher fasting plasma cortisol levels in the offspring.\textsuperscript{237}
In an earlier study, the same individuals were also found to have raised blood pressure.

1.4 The Autonomic Nervous System (ANS)

The peripheral nervous system is divided into the somatic nervous system which governs the actions of organs (principally the musculature) under conscious control and the ANS which, as its name implies, is autonomous and therefore, largely not subject to voluntary control. The ANS forms part of a wider system of homeostatic mechanisms, including endocrine systems, which regulate the functions of individual organs to adapt an organism to environmental and metabolic demands. The larger portion of the ANS is an efferent system carrying impulses from the central nervous system (CNS) to peripheral target organs, which include endocrine organs. In fact, the only efferent nerve fibres leaving the CNS which are not part of the ANS are the motor nerves which innervate skeletal muscles.

Important effects of the ANS include: control of HR, myocardial contractility, vasoconstriction and vasodilatation, contraction and relaxation of smooth muscle in various organs, visual accommodation, pupillary size, and the activity of exocrine and endocrine glands. There are some afferent nerve fibres in the ANS which are carried generally by the major autonomic nerves such as the vagus (cranial nerve X), the splanchnic, and the pelvic nerves. However, some afferent pain fibres from blood vessels are carried within somatic nerves. Afferent autonomic nerves act predominantly to convey visceral sensation to the CNS and regulate the vasomotor and respiratory reflexes. For example, baroreceptors and chemoreceptors in the carotid sinus and the aortic arch transmit information about cardiorespiratory state via the
vagus and glossopharyngeal nerves to the nucleus tractus solitarius forming the afferent limbs of the baroreflex and chemoreflex which regulates HR, blood pressure and respiratory activity via efferent autonomic nerves. These reflex arcs with exclusively autonomic afferent and efferent limbs are relatively unusual. Most reflex arcs involving the ANS comprise an afferent limb of either autonomic or somatic nerves and an efferent limb involving both the autonomic and somatic nervous system. For example, afferent signals from chemoreceptors, mechanoreceptors, or pain receptors carried by either the ANS or somatic nervous system from the viscera might stimulate a range of responses including those mediated by the efferent autonomic system, such as smooth muscle contraction in blood vessels, the eyes, lungs, bladder, or gastrointestinal tract, and those mediated by the efferent somatic system, such as coughing or vomiting. Most of these reflex arcs are simple involving only single organs whilst some are more complex, may involve signals from multiple organs and act upon multiple organs, usually under the control of higher CNS regions such as the hypothalamus.

The ANS is divided into three distinct branches, based on their anatomical and functional differences. These are the SNS, the PNS, and the enteric nervous system. This thesis will focus on the SNS and PNS. In both systems, myelinated pre-ganglionic nerve fibres make synaptic connections with unmyelinated post-ganglionic nerve fibres which innervate the effector organ. Such synaptic connections are usually clustered in the same locations, which are called ganglia. Most organs are innervated by both the SNS and the PNS which generally exert opposite effects on the function of those organs. For example, the vagus nerve, a parasympathetic nerve, acts on the sinoatrial (SA) node to slow HR, whilst sympathetic innervation of the heart acts, both at the SA node, and throughout the myocardium to increase HR and contractility. There are exceptions to
this rule, however, such as the salivary glands where both the sympathetic and parasympathetic limbs of the ANS act to stimulate salivation (although the nature of the resultant saliva differs markedly in its viscosity).

1.4.1 The Sympathetic Nervous System (SNS)

The SNS has been seen to be largely concerned with activation of organs to prepare an organism for response to a situation of threat. This response is often referred to as the 'fight-or-flight' response which was coined by the American physiologist, Walter Cannon in 1929 to describe his theory that animals react to threats with a general discharge of the SNS. Later, this idea was adopted by Hans Selye as the initial phase of the stress response or, as he described it at the time, the general adaptation syndrome. The resulting physiological changes often observed in such a situation include increased HR, blood pressure, and cardiac output (CO), diversion of blood flow to the cardiac and skeletal muscles by reducing their vascular resistance whilst increasing vascular resistance in other organs such as the skin and spleen, dilation of pupils, contraction of sphincters, dilation of bronchiolar airways, and mobilisation of energy stored in fat or glycogen (Figure 3). This pattern of physiological changes is clearly beneficial to an organism preparing for either fight or flight, so it is not difficult to appreciate why this model has been favoured. However, this oversimplification of SNS function has led to a number of conceptual difficulties.

The first difficulty comes when the sole function of the SNS is seen to be mediation of the fight-or-flight response. This is problematic because it leads to ideas of 'all-or-nothing' responsiveness of the SNS. It is now known that the SNS is highly discrete in its activation and regulates many different target tissues in the somatic and visceral...
domains of the body in a differentiated manner, indicating the existence of separate sympathetic pathways that are functionally defined by their target cell types. This is supported by experimental investigations of lumbar sympathetic outflow to skin, skeletal muscle and viscera and thoracic sympathetic outflow to the head and neck which show that each target organ and tissue is innervated by one or two separate pathways which consist of sets of pre- and post-ganglionic neurons with distinct patterns of reflex activity. Using microneurography in skin and muscle nerves in conscious humans and direct recordings from skin, muscle and visceral nerves in anaesthetised animals, these studies have shown that each type of sympathetic neuron exhibits a discharge pattern that is characteristic for its target cells and, therefore, its function. Thus, the divisions of the peripheral SNS such as lipomotor, sudomotor, cardiomotor, secretomotor, cutaneous vasoconstrictor, or muscle vasoconstrictor neurons all exhibit patterns of activity which reflect the differing organisation and function of higher centres in the spinal cord, various brainstem regions, the hypothalamus and the forebrain that govern them. These patterns of activity vary according to origin of the afferent inputs to any given SNS division and the nature of the stimulus, which is by-no-means limited to a psychological threat and, in experimental conditions, may include a range of noxious stimuli such as hypoxia, hyperthermia, vibration and other mechanical stimuli. Thus, recordings from any single peripheral autonomic neuron show a surprisingly varied set of burst patterns which can be related to both peripheral afferent and centrally generated events. Examples of such centrally generated events that are frequently related to peripheral nervous system discharge patterns include: respiratory, circadian and other body rhythms, and command signals generated in the forebrain.
The second major difficulty with this model arises in the name itself. 'Fight-or-flight' implies to the non-specialist that fight is the first behavioural option taken by an animal during a situation of threat which is not the case. Since the 1920's when this terminology was adopted, ethologists working with non-human primates have established that there are four clear stages of stress-response behaviour that proceed sequentially in response to increasing levels of threat. The sequence begins with the 'freeze response' which refers to a state of hypervigilance where the animal 'stops, looks, and listens'. In a situation where an animal is prospective prey, this behaviour has obvious survival advantages as predatory mammals rely predominantly upon retinal and visual cortex detection of movement to identify prey rather than upon colour information. The next behaviour in the sequence is flight, not fight, which only occurs after all attempts at flight have been exhausted. Whilst the significance of this distinction may not be obvious for the example of a cornered prey animal, it undoubtedly has significance for understanding the exaggerated stress responses seen in human subjects when sociocultural pressures which may demand a fighting response in, for example, a combat situation conflict with the natural tendency of an individual to first attempt flight. Finally, there is a further behaviour that occurs when these initial three options are exhausted. This involves tonic immobility and usually occurs during direct contact with an attacker. Thus an animal under attack can play dead, perhaps leading the predator to relinquish their hold or attack and presenting a further opportunity for the prey animal to escape. Thus this fright response makes up the last part of a 'freeze, flight, fight or fright' sequence which is clearly more complex than the term 'fight-or-flight' implies.
1.4.1.1 Organisation and Function of the SNS

The SNS innervates smooth muscles and glands in the head, blood vessels, piloerector muscles, sweat glands, and the thoracic, abdominal and pelvic viscera. In essence, the whole body is innervated by the SNS. Cell bodies of pre-ganglionic SNS neurons are situated in the intermediolateral cell column (lateral horn) of the thoracolumbar spinal cord between T1 and L2/L3 in humans (Figure 2). These preganglionic neurons pass through the ventral roots and emerge in the ventral rami of the mixed spinal nerves before travelling via the white communicating rami to the sympathetic trunk which is located bilaterally along the full length of the vertebral column. The white communicating rami are so named due to their white shiny appearance in life. This is
due to the myelinated preganglionic fibres which traverse them. Where each ramus joins the sympathetic trunk, paravertebral ganglia containing the cell bodies of postganglionic SNS neurons are found.

Depending on the target tissue, there are a number of alternative neuronal pathways from the paravertebral ganglia (Figure 3). The majority of preganglionic neurons synapse in the paravertebral ganglia with postganglionic neurons which rejoin the ventral rami via the grey communicating rami before distribution to effector organs by the spinal nerves. The grey communicating rami are grey, rather than white, because postganglionic sympathetic neurons are unmyelinated. Some preganglionic neurons do not synapse in the paravertebral ganglia. Instead, they travel up or down the sympathetic trunk to synapse with postganglionic neurons in other ganglia such as the cervical, lumbar, or sacral ganglia which do not receive communicating rami directly from their adjacent spinal cord segments, or they travel in the greater splanchnic nerve and synapse directly with chromaffin cells in the adrenal medulla (Figure 1 & Figure 3). Finally, they may pass through the paravertebral ganglia to synapse in prevertebral ganglia such as the celiac or mesenteric ganglia. The sympathetic supply to the head and neck arises in the first two thoracic spinal cord segments (T1 / T2) and travels up the sympathetic trunk before synapsing in one of the superior, middle, or inferior cervical ganglia. Each of these ganglia gives rise to cardiac branches, branches to blood vessels, sweat glands and hair follicles in the head and neck, and vascular branches which follow the arterial tree to target organs in the head such as the salivary glands or the smooth muscles of the eye which control pupil size.
Figure 3. The sympathetic nervous system (innervation of the major organs). Sympathetic innervation not shown includes that to blood vessels, spleen, piloerector muscles, sweat glands (cholinergic neurotransmission), adipose tissue, thyroid gland, ovaries, testes and uterus. Typical effects on target organs are listed. Ach, Acetylcholine; NA, Noradrenaline.
Neurotransmission in the SNS involves a number of neurotransmitters and receptor classes. The primary neurotransmitter of preganglionic sympathetic neurons is acetylcholine and the primary neurotransmitter of postganglionic sympathetic neurons is noradrenaline except for postganglionic neurons innervating sweat glands or piloerector muscles which secrete acetylcholine. The adrenal medulla is also innervated by cholinergic neurons but these are effectively pre-ganglionic and the chromaffin cells, with which these neurons synapse, share a common embryonic origin with postganglionic sympathetic neurons. Postganglionic sympathetic neurons express nicotinic receptors on their surface which are coupled to ion channels and rapidly activate the neuron when acetylcholine is bound to them. Although these acetylcholine receptors, in common with those of neuromuscular junctions, may be activated by nicotine and are structurally similar to nicotinic receptors in the somatic nervous system, there are a number of differences. For example, they can be blocked by mecamylamine, which has no action at the neuromuscular junction, but not by α-bungarotoxin, which is a potent blocker of nicotinic receptors at neuromuscular junctions. The sympathetic acetylcholine receptors in the sweat glands and piloerector muscles, like parasympathetic target organ receptors, are muscarinic. Greater detail about this receptor class and its subtypes is given in section 1.4.2.1, where they are discussed in the context of parasympathetic neurotransmission.

The adrenergic receptors, on target tissues where noradrenaline is the neurotransmitter, are divided into two main classes: α-adrenoceptors and β-adrenoceptors. They are coupled to secondary messenger systems within target cells by G-protein complexes. Together, these classes have five subtypes: α₁, α₂, β₁, β₂, and β₃. α₁-adrenoceptors are found in the smooth muscle of the blood vessels, bronchi, gastrointestinal tract, uterus, and bladder. Activation of these receptors results
generally in excitation of the smooth muscle cell and consequent contraction of the muscle. However, this is not the case in the gastrointestinal tract where activation causes smooth muscle relaxation except for the sphincters which contract. 

$\alpha_2$-adrenoceptors are also found in the smooth muscle of the blood vessels where their activation causes vasoconstriction but noradrenaline preferentially binds to the $\alpha_1$ subtype. $\beta_1$-adrenoceptors are found in the heart where their activation increases HR, contractility, and conductivity and they are found in the sphincters of the gastrointestinal tract where their activation causes relaxation. $\beta_2$-adrenoceptors are also found in the heart where their activation has similar effects to those of $\beta_1$-adrenoceptor activation. Noradrenaline preferentially binds to $\beta_1$-adrenoceptors but $\beta_2$-adrenoceptor activation becomes more important in patients with heart failure where $\beta_1$-adrenoceptor expression is reduced. Activation of $\beta_2$-adrenoceptors in blood vessels causes vasodilation but this effect is normally overwhelmed by the effect of noradrenaline on $\alpha$-adrenoceptors. $\beta_2$-adrenoceptors are also expressed in the smooth muscles of the bronchi where their activation causes bronchodilation. Finally, $\beta_3$-adrenoceptors are expressed in adipose tissue where their activation leads to thermogenesis and lipolysis, releasing glycerol and free fatty acids into the circulation.

Noradrenaline regulates its own release by acting on receptors in the presynaptic nerve terminals of its releasing neurons. Here, activation of $\alpha_2$-adrenoceptors inhibits further noradrenaline release whilst $\beta_2$-adrenoceptor activation facilitates it. Other factors which may have a role in regulation of sympathetic neurotransmission at the synaptic level include rate of neurotransmitter production, breakdown and reabsorption, and the action of collocated neuropeptides. The catecholamines, noradrenaline, adrenaline, and dopamine, are part of a larger family of biogenic amines which includes serotonin and histamine. Catecholamines are synthesised from tyrosine by the rate-limited
enzymatic action of tyrosine hydroxylase. This first stage in noradrenaline synthesis is inhibited by noradrenaline itself and accelerated by Na$^+$ and Ca$^{2+}$ ions. The result of this enzyme reaction is L-dopa which is rapidly converted to dopamine by L-aromatic amino acid decarboxylase which is then rapidly converted to noradrenaline in neurons by dopamine-β-hydroxylase. In some cells of the adrenal gland, this process finishes with the production of noradrenaline. However, in the majority of adrenal cells, phenylethanolamine N-methyltransferase converts noradrenaline to adrenaline.

Two non-specific enzymes which are also found in non-neuronal tissues are responsible for the breakdown of catecholamines. In the synapse, most noradrenaline (approximately 70%) is actively transported back into the presynaptic terminal where it is recycled or broken down by mitochondrial monoamine oxidase-A and dehydrogenases. Noradrenaline which escapes this process by, for example, being transported into the post-synaptic cell is metabolised by catechol-O-methyltransferase and the inactivated breakdown products are released into the circulation. Some noradrenaline also arrives in the circulation by passive diffusion from synaptic clefts.

In many adrenergic neurons, collocated neuropeptides are released together with noradrenaline. For example, postganglionic neurons from the stellate ganglion, which innervate organs such as the heart, contain neuropeptide Y whilst adrenergic neurons elsewhere may contain other neuropeptides such as somatostatin or no neuropeptides at all. Lacking active uptake mechanisms, neuropeptides are removed slowly from synaptic terminals and therefore have a long duration of action. In general, when collocated with a fast neurotransmitter, their effect seems to be to gradually potentiate or diminish the post-synaptic response to that neurotransmitter. They may also have local hormonal effects in addition to this neuromodulatory role. The highly specific
nature of neuropeptide release within different divisions of the SNS is linked to their discrete target-specific functions and adds weight to the argument that viewing the SNS as a homogeneous 'all-or-nothing' system is highly misleading.

Although discrete sympathetic regulation of tissues is provided by direct innervation, these receptors also respond to circulating catecholamines which are produced by the adrenal medulla in response to activation by the splanchnic nerve (approximately 80% adrenaline and 20% noradrenaline). The effect of these circulating hormones depends on the proportion of adrenoceptor subtypes expressed by tissues. Adrenaline activates $\beta$-adrenoceptors more strongly than $\alpha$-adrenoceptors whilst the opposite is true for noradrenaline. For example, during exercise, adrenaline causes vasodilation in skeletal muscles due to the high proportion of $\beta_2$-adrenoceptors compared to $\alpha$-adrenoceptors in the vascular smooth muscle in that tissue whereas the opposite is true of the splanchnic vasculature. However, the general effect of these circulating catecholamines is to influence metabolic rate of tissues by activating adenylyl cyclase via $\beta$-adrenoceptors which increases glycogenolysis. Thus, the sympathoadrenomedullary axis can increase availability of substrates for oxidative metabolism on demand (e.g. during cold stress or exercise).

1.4.1.2 Development of the SNS

Development of the mammalian nervous system does not terminate at birth but continues well into postnatal life. Therefore, sensory experiences in and ex utero are important determinants of the final post-developmental characteristics of an individual's nervous system. There are many examples of experimental data which support this concept. The archetype of these comes from experiments in the 1970's
where unilateral surgical eye-closure in kittens, which deprived one half of the visual cortex of incoming sensory information, led to permanent deficits in response to sensory information in the adult cats when this procedure was reversed after the early critical period of development. Thus, despite having an apparently intact visual sensory pathway, perception of visual information for the affected eye was permanently damaged.244,245 Similar findings were made in a variety of mammalian species including primates.246 The exact nature of the alterations in neurodevelopment induced by these experiments is still not fully understood. The cerebral cortex of mammalian species including humans undergoes rapid synaptogenesis during early postnatal life. This is followed by a substantial loss of synapses (~50%) that continues through adolescence and then a steady but much slower decline in synapse numbers which persists throughout adulthood. Whilst sensory experiences undoubtedly influence this process, activity-dependent synaptic plasticity appears to vary with timescale of manipulation and experimental systems, complicating understanding of these processes.246 In general, however, it is thought that activity stimulates synaptic formation whilst inactivity, perhaps due to sensory deprivation, decreases it. Furthermore, the extent and duration of the effect of early sensory experiences on the lifelong process of synaptic loss has only recently received attention. In a recent study of mice, long-term early sensory deprivation, caused by whisker trimming, prevents net dendritic spine loss in the somatosensory cortex by preferentially reducing the rate of ongoing spine elimination without affecting the rate of new spine formation.246 Importantly, this effect persisted throughout the life of the animals although it was most marked during adolescence.
Whilst dendritic spine elimination is likely to be one important process involved in the early determination of later SNS function, there are a number of stages during the development of the SNS that represent likely periods of vulnerability to external influences. These include the early stage of cell differentiation, where growth factors play a role in determining cell-type switching, SNS nerve proliferation and apoptosis where substantial numbers of nerve cells are removed, and dendritic and nerve-ending expansion during organ growth. The sympathoadrenal progenitor cell type arises from multipotential stem cells in the neural crest during embryogenesis. These either differentiate into adrenaline and noradrenaline-producing chromaffin cells, in the presence of glucocorticoids or into adrenergic neurons in the absence of glucocorticoids and the presence of growth factors such as fibroblast growth factor 2 and nerve growth factor (NGF). Thus, hormonal and growth factor concentrations, which may vary between individuals for innate or environmental reasons, are important determinants of neuropoietic balance. A small proportion of the postganglionic adrenergic neurons (which release noradrenaline as their primary neurotransmitter) undergo a further differentiation into cholinergic neurons which primarily innervate the sweat glands. Early development of the SNS, like that of the CNS involves an abundant overproduction of nerve cells. Those that establish contact with their target tissues survive whilst those that fail to do so, undergo apoptosis. This accounts for the loss of approximately 80% of the initial SNS neuron population. Survival of neurons during this process is dependent on target tissue production of neurotrophic factors such as NGF. Regardless of eventual body size attained in humans and other mammals, the number of post-ganglionic SNS neurons which remain after this process of apoptosis is fixed. Therefore, processes which influence proliferation of nerve cell endings and dendritic spines are likely to be of great importance in determining variations between individuals after this stage.
1.4.1.3 Developmental Plasticity of the SNS

It is likely that the lasting effects of prenatal stress on the HPAA are closely linked with alterations in sympathoadrenal activity, as the two systems act in concert. In a study of 103 preterm infants, antenatal exposure to dexamethasone was found to predict lower plasma catecholamine concentrations 12 hours after birth than those seen in unexposed infants, but similar plasma cyclic adenosine monophosphate (cAMP) levels. In rats, too, prenatal exposure to dexamethasone was found to predict reduced noradrenergic innervation of target tissues such as the heart, as assessed by regional noradrenaline concentration, and reduced noradrenaline turnover (an indirect measure of neuronal activity). Because the cellular mechanisms of action of dexamethasone include enhancement of β-adrenergic receptor-mediated cAMP generation, the same group carried out similar studies in rats to assess the effect of prenatal dexamethasone on cell transduction systems. These suggested that prenatal dexamethasone exposure produces a dose-dependent enhancement of β-receptor mediated stimulation of adenylate cyclase activity and that this enhancement was at the level of adenylate cyclase itself, and not due to effects on receptors or G-protein coupling to enzymatic activity. These findings are supported by a study in sheep where fetal exposure to hydrocortisone was also associated with greater adenylate cyclase activity in the myocardium following birth. Thus the normal levels of plasma cAMP, despite lower plasma catecholamine concentrations, seen in human preterm newborns might be accounted for by up-regulation of cellular responsiveness to adrenergic stimulation at the enzymatic level. Although noradrenergic cardiac stimulation appears to be reduced in the offspring in these studies, measures were only taken around birth or, at most, into young adulthood. As described below, prenatal adverse interventions generally predict greater SNS activity in adulthood.
This apparent contradiction may have a number of explanations: It is possible that up-regulation of adenylate cyclase activity persists into adulthood whilst the lower catecholamine concentrations seen in neonatal or young animals exposed to corticosteroid may represent an initial adaptation to that exposure which does not persist into adulthood. Thus, eventual hyper-responsiveness to SNS stimulation might result. Alternatively, it should be remembered that these experiments involve a range of animal species where findings have not been consistent, making it difficult to draw parallels with human development, and that they rely predominantly upon dexamethasone as a proxy for adrenocortical hyperactivity. Given the well-known differences in pharmacological properties of dexamethasone, in comparison to endogenous corticosteroids, this may also be problematic. What is clear, however, is that these experiments all demonstrate that the development of the HPAA and the SNS is intimately linked and that prenatal modifications in HPAA function are likely to alter later SNS function.

Recent experiments suggest that the raised blood pressure in prenatally stressed animals may involve modifications of SNS function and may be due to an increase in the magnitude of the stress response. Weinstock and colleagues have reported experiments studying rats exposed to noise and light stress during pregnancy. At five months of age, the offspring showed heightened SNS activation in response to footshock. The effects of prenatal stress in rats are not limited to the peripheral SNS. Brain catecholamine turnover in the cerebral cortex and locus coeruleus is increased in prenatally stressed animals and associated with increased stress-induced behaviour in the adult rats.
Further evidence for developmental plasticity of SNS structure and function comes from studies of rats where lowered environmental temperature during rearing has been shown to permanently increase sympathetic innervation and activity in adipose tissue. Dietary manipulations during pregnancy may also have an impact on SNS development. In the rat, for example, protein restriction through pregnancy and lactation results in increased circulating adrenaline and noradrenaline concentrations and increased expression of $\beta_1$ and $\beta_3$ receptors in the offspring. Another potent stimulus for altered SNS development is hypoxia and this has been used in a number of animal models of fetal adversity. In the chick embryo, chronic hypoxia causes sympathetic hyperinnervation of the peripheral arterial system. In the ovine model, surgical removal of the majority of uterine caruncles in the ewe prior to conception can be used as a model of placental insufficiency, resulting in restricted nutrient supply and hypoxia in the fetus. Fetal adaptations observed in such experiments include increased circulating catecholamine and cortisol concentrations, decreased fetal body growth and relative sparing of brain growth. These hypoxic, placentally restricted fetuses demonstrate greater hypotensive responses to pharmacological $\alpha$-adrenoceptor blockade in late gestation and the magnitude of those responses depends on the degree of hypoxia, suggesting that the maintenance of fetal blood pressure and the redistribution of CO to the brain may depend on peripheral vascular sympathetic hyperinnervation. This adaptation predates the development of significant growth restriction in the fetuses and whilst it probably has short-term benefits in terms of brain growth, it may have adverse consequences for neonatal and adult peripheral vascular function.
Studies in rats show that the effects of hypoxia on the SNS are widespread and long-lasting. Offspring of pregnant rats exposed to 10% oxygen between days 5 and 20 of embryonic life are small at birth and as adults, demonstrate exaggerated blood pressure responses to stress and evidence of disordered baroreflex function.\cite{1}

Neurochemical analysis during their early postnatal development showed that levels and utilization of catecholamines were reduced in the sympathetic ganglia, in target organs, in the adrenal glands and in the A2 cell group of the nucleus tractus solitarius (where baroreceptor information is integrated), but were increased in the locus coeruleus. Once the rats reached adulthood, reduced ANS activity was restricted to cardiac-related structures such as the stellate ganglion, heart and adrenals. Therefore, this data stands in contrast to the evidence from the ovine model, where prenatal hypoxia leads to greater SNS activity. Clearly though, the insult involved in this study was severe and lasted for the majority of intrauterine life, which may not be an appropriate model of natural fetal growth restriction.

In another model of intrauterine adversity in the rat, where uterine arteries were ligated on day 13 of their 22 day gestation, effects on peripheral arterial sympathetic function were found to be regionally specific which may explain some of the disparities in previous findings.\cite{2}

The authors examined adrenergic vascular function in the renal, mesenteric, femoral and saphenous arteries but intrauterine adversity only affected the renal artery. Concentration response curves for phenylephrine (an \( \alpha \)-adrenoceptor agonist) showed reduced sensitivity but greater maximal contractile responses in the renal artery. As \( \alpha \)-adrenoceptor density and binding affinity (assessed by radio-labelled prazosin) was not altered by intrauterine hypoxia, these findings are likely to represent augmented intracellular signalling. However, maximal contractile responses to noradrenaline (stimulates both \( \alpha \) and \( \beta \) adrenoceptors) were significantly reduced and
relaxation induced by isoprenaline (a predominantly β₂-adrenoceptor agonist) was increased. This suggested greater renal β-adrenergic vasodilation in the prenatal hypoxia group which stands in contrast to observations of decreased β-adrenergic vasodilation in aging or hypertensive adult animals. Whilst the observed effects persisted until four weeks of life, the long-term consequences of altered renal vascular adrenergic function in terms of adult cardiovascular health were not examined in that study.

Another study using a similar model of placental restriction in rats showed that whilst the resulting intrauterine growth retardation was not associated with increased cardiovascular responses to stress in the offspring, there was an association with increased SNS activity. For reasons of experimental convenience, few studies in animals have addressed the possibility of a sex disparity in the effects of early adversity on SNS development, but in this study, only female growth restricted rats showed evidence of increased SNS activity whilst males did not. Thus, the picture of associations between prenatal insults and later SNS function emerging from animal studies is not without its apparent contradictions which may be accounted for by variations in the species or sex, type of insult, timing or duration of insult, or methodological approach used.

It could be argued that none of the current models of placental insufficiency or malnutrition in animal studies are appropriate analogues for the relatively mild degree of adversity that may be experienced by human fetuses in the Western world where nonetheless, many epidemiological studies show evidence of associations between fetal development and adult cardiovascular health. Unfortunately, human data relating to early life influences on sympathoadrenal function are limited. Fetuses with intrauterine
growth retardation have higher HRs, reduced HRV, elevated catecholamine concentrations in cord blood, and higher HR and reduced HRV as newborns. In a study of 266 men and women aged 50, resting pulse rate was inversely related to birth weight, a finding that has been confirmed in 2648 African school children. Although resting HR is also associated with cardiac parasympathetic tone, it functions as an imperfect index of sympathetic cardiac activation and these associations are supported by a number of studies using more specific measures of sympathetic function. In a study of 630 healthy children, 24-hour blood pressure variability was significantly and inversely correlated with birth weight, after adjustment for sex, age and height. In a study of 114 adolescent twin pairs, pre-ejection period (PEP) shortening (a well-known marker of cardiac sympathetic stimulation) was shown to account for 63% – 83% of the association between birth weight and adolescent blood pressure. In a recent study, direct recordings of muscle bed sympathetic nerve activity (MSNA) were shown to be increased in a group of 20 adults who were small for gestational age (SGA) compared with 12 appropriate for gestational age (AGA) controls. This finding contradicts an earlier, smaller study where MSNA was recorded in 13 SGA young adults and 13 age, sex and BMI matched AGA controls which showed a weak association between intrauterine growth retardation and MSNA in the opposite direction. Whilst studies of SNS developmental plasticity using MSNA may be informative in the future, neither of the currently published studies is large enough to be conclusive. Furthermore, examination of SNS function at a non-cardiovascular site may not be representative of SNS regulation of the cardiovascular system.
1.4.2 The Parasympathetic Nervous System (PNS)

In contrast to the SNS, the PNS is concerned largely with restorative functions usually occurring when an organism is at rest. It facilitates digestion and absorption of nutrients and excretion of waste products, reduction in cardiovascular activity with lower HR and blood pressure and a range of other localised effects which are summarised in Figure 4. The SNS, through adrenal medullary release of catecholamines can have global effects on the body. No analogous system exists for the PNS which exclusively has localised effects through direct innervation of target organs.
Figure 4. The parasympathetic nervous system (innervation of the major organs). Parasympathetic ganglia are represented as discrete but are usually very close or embedded within target organs.
1.4.2.1 Organisation and Function of the PNS

In mammals, two brainstem regions regulate peripheral parasympathetic functions. The dorsal vagal complex, comprised of the dorsal motor nucleus and its connections, controls parasympathetic activity below the level of the diaphragm, whilst the ventral vagal complex (VVC), encompassing the nucleus ambiguus and the retrofacial nucleus, controls functions above the diaphragm in organs such as the heart and lungs, thymus and other glands and tissues of the neck and upper chest, and specialized muscles such as those of the oesophageal complex. The VVC only appears in mammals and is associated with both positive and negative regulation of HR, bronchial constriction and vocalisation. VVC inhibition is switched off in states of alertness, leading to a decrease in cardiac vagal tone and a resulting increase in HR in response to environmental challenge. This allows mammals to prepare rapidly for potentially adverse situations with higher HRs and metabolic changes without the need for sympathoadrenal activation. Cell bodies of preganglionic neurons of the PNS are also located in the sacral region of the spinal cord (S3–S4). Thus the parasympathetic preganglionic neurons emerge as part of cranial nerves III (oculomotor), VII (facial), IX (glossopharyngeal) and X (vagus) and from the sacral region to form part of the splanchnic nerve. The parasympathetic ganglia are usually located close to the target organ or may be embedded within it. In contrast to the SNS, parasympathetic innervation is characterised by long preganglionic and short postganglionic nerves.

The PNS innervates a number of organs and viscera (Figure 4) including the eye, salivary glands, heart and lungs, gastrointestinal tract, genitalia and other viscera. Parasympathetic innervation of blood vessels is confined to vasodilator nerves supplying salivary glands, the exocrine pancreas, gastrointestinal mucosa, genital erectile tissue, and cerebral and coronary arteries. Other blood vessels, the spleen,
sweat glands and pilomotor muscles in the skin are innervated exclusively by the SNS. The PNS has exclusive control of focus of the eyes by the ciliary muscles and of pupillary constriction by the constrictor pupillae muscle of the iris. Sympathetic stimulation dilates the pupil of the eyes by its action on the dilator pupillae muscle of the iris. Thus, the iris provides an example of functional antagonism rather than dual antagonistic innervation of a specific smooth muscle but with these exceptions, the PNS does provide antagonistic innervation of tissues innervated by the SNS.

Ganglionic neurotransmission in the ANS is cholinergic with postganglionic sympathetic and parasympathetic neuronal cell bodies expressing nicotinic acetylcholine receptors. These receptors are ligand-gated ion channels which open upon binding to acetylcholine and rapidly activate the cell. With the exceptions mentioned in section 1.4.1.1, SNS neurotransmission at target tissues is not cholinergic but adrenergic. However, as mentioned, some sympathetic neurotransmission and all parasympathetic neurotransmission at target tissues is cholinergic and acts via muscarinic acetylcholine receptors which are activated by muscarine and inhibited by low concentrations of atropine. Like adrenoceptors but unlike nicotinic cholinergic receptors, this family of receptors are coupled to G-proteins which, upon activation of the receptor, modulate intracellular levels of the secondary messengers: inositol triphosphate or cAMP which activate various cellular processes including increasing the availability of Ca²⁺, either by causing its release from the endoplasmic reticulum or by opening ion channels in the cell membrane. From a molecular biological perspective, there are five known types of muscarinic receptor. However, from a functional point of view, they are divided into three classes: M₁ receptors, which are mainly located on neurons in the CNS and on peripheral neurons and are involved generally in excitation of target tissues. For example, vagal stimulation of gastric acid
secretion is mediated by $M_1$ receptors; $M_2$ receptors, which are found in the heart and on the nerve terminals of both CNS and peripheral neurons, cause the negative inotropic, chronotropic and dromotropic responses to vagus activation; and $M_3$ receptors, which are located in secretory glands and on smooth muscle. Activation of these receptors generally has an excitatory effect leading, for example, to contraction of visceral smooth muscle. The relative activation of these receptor classes may be of clinical relevance in a number of conditions including coronary heart disease. Vascular endothelial $M_2$ receptors are coupled to the formation of nitric oxide (NO) which is a potent vasodilator. In coronary vascular dysfunction where NO production is disordered, vasoconstriction caused by the action of acetylcholine on $M_3$ receptors in vascular smooth muscle becomes more dominant with implications for coronary blood flow.

The principal parasympathetic neurotransmitter, acetylcholine, is synthesised from acetyl coenzyme A (a Kreb's cycle intermediate) and choline (a component of membrane lipids derived from dietary sources) by choline acetyl transferase which is present in excess in cholinergic neurons. Therefore, the rate of acetylcholine synthesis depends largely on the availability of choline. Acetylcholine is removed from the synapse (or neuromuscular junction) by acetylcholinesterase found on the external surface of the cell membrane, concentrated at post synaptic sites. Additionally, free choline is, in part, taken up by a specific transporter in the presynaptic terminal where it may be recycled.
1.4.2.2 Development of the PNS

Development of the PNS is less well studied than that of the SNS. However, the principles of neuron overproduction followed by apoptosis and variable synapse or dendritic spine formation that were described in section 1.4.1.1 for the SNS, apply to nervous system development in general and are, therefore, likely to be important determinants of parasympathetic structure and function as well. Like SNS neurons, PNS neurons derive from multipotent progenitor cells from the neural crest. Neurotransmitter choice and, therefore, commitment to either cell lineage are determined by the complex integration of instructive cues from both intrinsic transcription factors and extrinsic growth factors. ANS activity increases progressively during fetal life and maturation of the PNS occurs earlier and is more complete than that of the SNS before birth. Thus, infants born prematurely have significantly lower high-frequency (HF) HRV (a marker of parasympathetic activity) than infants born at term and, perhaps surprisingly, this effect persists when they reach their predicted term age.

Of all the functions of the PNS, control of HR via the vagus nerve is arguably its most vital. Studies of human embryos show that cholinergic receptors in the heart are functioning from the 4th post-conceptual week. Muscarinic cholinergic responses to acetylcholine and related agents can be detected from this stage onwards, coinciding with the onset of the beating heart in life. Similar studies of SNS function show that β-adrenergic responses to noradrenaline, adrenaline, and related sympathomimetic agents appear later, in the 5th post-conceptual week. As development continues, maximal responses to both cholinergic and adrenergic stimulation increase. In this early stage of development, other agents such as triiodothyronine and prostaglandins also appear to have a role in regulation of cardiac function. Despite this early...
responsiveness to autonomic neurotransmitters, the embryonic myocardium is not innervated by the ANS until much later in development. *In vitro*, muscarinic cholinergic neurotransmission is demonstrable between 10 and 12 weeks of development and β-adrenergic neurotransmission follows later at between 13 and 14 weeks. Pharmacological experiments *in vivo*, suggest that functional autonomic control of cardiac function does not appear until even later.²⁸ Fetal tachycardic responses to atropine administration *in utero* do not appear until between 15 and 17 weeks and bradycardic responses to β-blockers do not appear until between 23 and 28 weeks. Thus, it seems that parasympathetic control of the developing human heart becomes functional and plays a role in governing cardiac function much earlier than sympathetic control does. From this, it might be assumed that the time periods of vulnerability to lasting alterations in autonomic function, induced by environmental influences, are likely to differ between these two limbs of the ANS. However, although this appears true for cardiac autonomic innervation, the relative pace of sympathetic and parasympathetic development may depend on target tissue. For example, human fetuses show circulating noradrenergic responses to painful blood sampling from the intrahepatic vein from as early as 18 weeks of gestation.²⁹

1.4.2.3 Developmental Plasticity of the PNS

Very little data exists on the developmental plasticity of the PNS. What little data there is, relies upon methods for estimating parasympathetic cardiovascular function through various spectral analytical examinations of heart period (HP; the time interval between adjacent heart beats) variability. As mentioned in section 1.4.2.2, human PNS activity increases steadily throughout fetal life before reaching a level comparable to that in adolescence at around 40 weeks of gestation. In particular, there appears to be a
maturational shift between 33 and 35 weeks of gestation, involving accelerated myelination of vagus nerve fibres. It is perhaps unsurprising, therefore, that a recent study of premature delivery suggested that prematurity has consequences for later PNS function, with the degree of prematurity predicting lower levels of parasympathetic activity and relative PNS immaturity at the infants' theoretical full-term ages. However, despite attempts to ensure that all infants in their study had good Apgar scores at delivery, no acute cardiorespiratory illness or congenital conditions, and were free of incubator support and medications which might affect cardiac or respiratory function by the time of testing, the authors do not address the possibility that routine neonatal intensive care, which is likely to be more invasive with greater prematurity, might also be responsible for their findings. In either case, the study provides evidence of developmental plasticity of the PNS albeit with the limitations just mentioned and the necessary reliance on a non-invasive indirect measure (spectrum analysis of HP) to indicate parasympathetic activity.

Very low birth weight (<1500 grams) premature neonates have been compared to their full-term normal birth weight peers and found to have significantly lower baseline vagal activity. Additionally, in a group of premature, low birth weight (<2500 grams) neonates with a wide range of the expected medical complications of prematurity, lower birth weight and higher scores on a neonatal morbidity scale (Hobel neonatal risk scale) in the early stages of their stay in neonatal intensive care predicted significantly lower baseline cardiac vagal activity assessed weeks after birth, when the neonates were clinically stable.
Intrauterine growth may have an impact on parasympathetic development that is independent of gestational age. In a recent study using a multifractal measure of short-term HRV, investigators compared fetal HR data from normal pregnancies and those complicated by pre-eclampsia. Multifractal indices of fetal cardiovascular control did not differ between normal pregnancies and those complicated by pre-eclampsia unless there was associated growth retardation. Those fetuses showed evidence of reduced parasympathetic governance of very short-term HR behaviour similar to that seen in adult hypertensive humans and animals where diminished vagal baroreflex sensitivity is often observed. This study suggests the possibility that the abnormal parasympathetic cardiovascular control seen in hypertensive adults may be set up in utero by adverse fetal conditions but further studies are required to establish this. Whilst multifractal analysis provides a means to establish that the HR oscillations seen are abnormal in comparison to those from fetuses from normal pregnancies, and that the frequency of those oscillations is consistent with parasympathetic control, a meaningful translation of measures of chaotic cardiac control into physiological parameters remains elusive. Other recent studies which have examined fetal HRV using spectrum analysis show that intrauterine growth retardation is associated with reduced HRV in comparison to normal control subjects. As this measure increases with parasympathetic maturation and is less influenced by sympathetic activity in fetal life, it is likely that this represents evidence of delayed parasympathetic development in growth retarded fetuses.
1.4.3 The Arterial Baroreflex

One of the primary functions of the ANS is regulation of the baroreflex. The baroreflex is the primary mechanism in adult vertebrates for rapid regulation of arterial blood pressure through modulation of HR and systemic vascular resistance (SVR). Thus, fluctuations in arterial pressure are minimised, to maintain tissue perfusion pressure and, thereby, to meet the metabolic needs of end organs. The baroreflex begins with activation of the baroreceptors, which respond to the arterial distension caused by blood pressure and are situated in the arterial walls of the great vessels exiting the heart and in the region of the carotid sinus. Figure 5 shows a schematic representation of the location and innervation of the baroreceptors in these regions. Carotid sinus baroreceptor are innervated by the sinus nerve (a branch of the glossopharyngeal nerve which synapses in the brainstem) and baroreceptors in the ascending aorta are innervated by the aortic nerve which combines with the ascending vagus nerve (Figure 5). Increased stretching of arterial walls containing baroreceptors augments the firing rate of the receptors and nerves, and recruits additional afferent nerves. These afferent parasympathetic nerves relay the information to reflex networks in the nucleus tractus solitarius of the medulla (Figure 1). From the medulla, efferent signals are relayed to vasomotor areas of the brainstem and to central autonomic nuclei, such as the dorsal motor nucleus of the vagus and the nucleus ambiguus. Thus, in response to a fall in blood pressure, parasympathetic efferents cause an increase of HR whilst sympathetic efferents increase myocardial contractility and conductivity, increase peripheral vascular resistance and decrease venous compliance. The opposite condition occurs during a rise in blood pressure. Through these mechanisms, the ANS acts rapidly to minimise acute fluctuations in blood pressure such as those produced by orthostatic changes. Without these mechanisms, simple actions such as standing from sitting would
precipitate dramatic changes in blood pressure due to gravitational effects on venous return.

In a normotensive, healthy individual, the receptors of the carotid sinus respond to pressures ranging between 60 and 180 mmHg (Figure 6). The baroreceptors in the aortic arch have a higher threshold pressure and are less sensitive than the carotid sinus receptors. Therefore, the carotid sinus receptors are usually the dominant arterial baroreceptors. Maximal carotid sinus sensitivity occurs near the normal mean arterial pressure (MAP) for an individual. However, baroreceptors also respond to the rate of pressure change as well as to the steady or mean pressure. Thus, at a given MAP, decreasing the pulse pressure also decreases the baroreceptor firing rate. This effect is important during conditions such as hemorrhagic shock where the combination of reduced pulse pressure and reduced MAP causes even greater baroreflex responses.

A common finding in hypertensive individuals is a set-point for their baroreflex which is shifted such that it centres around higher than normal arterial pressures. Furthermore, as indicated in Figure 6, this is usually accompanied by a reduction in both baroreceptor sensitivity and the operating range of the baroreflex, resulting in a reduced ability to control fluctuations in arterial pressure. As baroreceptor sensitivity is a key factor in determination of the set-point of the baroreflex and therefore, resting blood pressure, it may have a role in the development or maintenance of hypertension. In general, studies of tissue preparations or short-term studies of surgically altered animals have suggested that the baroreflex set-point appears to reset quickly in response to changes in resting blood pressure which has led most authors to the opinion that it can only be involved in the regulation of short-term blood pressure variability. However, recent evidence suggests the baroreflex may, in fact, have roles
in the long-term regulation of SNS activity and the development of hypertension after all.\textsuperscript{28} Lohmeier \textit{et al.} have examined the effects of chronic carotid sinus stimulation (CSS) in dogs and observed marked differences during CSS in dogs with Ang II-induced hypertension as compared to control animals.\textsuperscript{28} In the control animals, CSS lowers MAP over several days suggesting that baroreflex resetting is minimal or absent in this model of chronic baroreceptor activation in an intact animal. Furthermore, in animals with Ang II-induced hypertension, significant long-term attenuation of the effects of CSS on MAP were observed, supporting the idea that reduced baroreflex sensitivity is involved in the development of hypertension.

Several lines of evidence suggest mechanisms which may underlie this observation. For example, studies of long-term renal sympathetic nerve activity (RSNA) have shown that baroreflex resetting does not always occur during hypertension, at least not with regard to RSNA.\textsuperscript{28} Additionally, Thrasher \textit{et al.} have recently developed a new surgical method to create chronic unloading of arterial baroreceptors in dogs which leads to hypertension.\textsuperscript{28} In this method, aortic baroreceptor nerves are cut and the carotid sinus is isolated from systemic arterial pressure. Baroreceptor unloading is induced by ligation of the common carotid artery, proximal to the innervated sinus which results in increases of arterial pressure to a mean level 22 mmHg greater than that observed in control animals. When the ligature is removed, normal carotid flow and, therefore, normalisation of arterial pressure results. In this model, a number of observations suggest that SNS activity is increased during baroreceptor unloading. These include greater HR, greater plasma renin activity in the presence of raised arterial pressure, and reduced or normal sodium excretion. The latter observation is surprising because the increased arterial pressure and, therefore, renal perfusion pressure, should have resulted in a pressure natriuresis which was not observed. This suggests that the
excretory ability of the kidneys was impaired which would be consistent with a baroreflex-mediated increase in RSNA.

In addition to the arterial baroreceptors, there are a number of other mechanisms by which the brainstem reflex networks which control arterial pressure may be modulated. The low pressure system, consisting of the atria of the heart and the great veins returning to the heart, also contains baroreceptors which feedback information on filling pressure on the heart and thus, indirectly measure blood volume. Baroreceptors in the left ventricle measure ventricular load and chemoreceptors in the carotid bodies, aortic body and medulla respond to arterial CO$_2$ tension by monitoring H$^+$ concentration (pH) in the blood with efferent limbs of the chemoreflex affecting both HR and respiratory rate and volume. Finally, atrial natriuretic peptide (ANP) provides an example of a non-neuronal mechanism by which blood pressure may be regulated. In response to raised plasma sodium concentration or volume-induced wall stretching in the atria, ANP is released and acts on the kidneys and vasculature to increase sodium excretion, reduce renin levels, and inhibit the pressor effects of catecholamines and Ang II.
Figure 5. Location and innervation of the principal arterial baroreceptors in the vasculature of the upper thorax and neck.
Figure 6. Effect of varying arterial pressure on carotid sinus baroreceptor firing rates in three hypothetical cases with differing baroreceptor sensitivity. Circular markers A, B & C represent the baroreflex set-points for cases with resting mean arterial pressures of 95, 120 & 150 mmHg, respectively. Alterations in arterial pressure affect carotid sinus firing rate which acts through the baroreflex to return arterial pressure to the set-point. Black lines represent the maximal slope relating arterial pressure to baroreceptor activity (maximal baroreceptor sensitivity) which occurs at the set-point. Case A is normotensive whilst B & C represent cases of progressively greater hypertension. Note that the set-point in hypertensives is shifted toward a higher blood pressure and that the slope (sensitivity) of the hypertensive baroreceptors is decreased. Note also that the operating range of the baroreceptors in B & C is narrower than in A.
1.4.3.1 Development of the Arterial Baroreflex

While the functional significance of the baroreflex is clear in adults, its importance in embryonic or fetal life is less clarified. What data there is on the ontogeny of the baroreflex comes primarily from sheep and chickens. Both of these species possess a functioning baroreflex during fetal or embryonic life. The degree to which this prenatal baroreflex is an important component of cardiovascular regulation during development, as opposed to simply becoming functional in anticipation of adult life, is still debatable and may be species specific. In chickens, the baroreflex appears very late in fetal life and has a lower gain that that found in adult life, suggesting no significant role in cardiovascular control during development. On the other hand, baroreflex function appears in fetal sheep at about 60% of gestation. Early studies in the sheep suggested that baroreflex function is relatively impaired at this stage and only increases significantly in postnatal life. However, the marked disparity between measures of baroreflex sensitivity elicited by three different methods used in the fetal sheep in one of these studies suggests the possibility that methodological difficulties may confound these early results. Subsequent studies with more refined measurement techniques have shown that through the last third of gestation in the sheep, arterial pressures steadily increase and that this is accompanied by reduced baroreceptor sensitivity together with a shift in the set-point of the baroreflex. Similarly, significant baroreflex function appears in alligators at around 70% of gestation and becomes increasingly important in regulation of cardiac function from 80% of gestation to hatching. Thus, it appears that different species demonstrate differing degrees to which the baroreflex plays a role in prenatal cardiovascular regulation and that there is some uncertainty about the ontogeny of baroreflex function even within a single animal model. Human prenatal baroreflex function is, therefore, unlikely to be illuminated without human studies which are currently limited. However, studies of
premature and term human neonates suggest baroreflex sensitivity increases significantly with late gestational maturation\textsuperscript{294,295} and comparisons between newborns and adults suggest that further increases occur during postnatal life.\textsuperscript{296}

1.4.3.2 Developmental Plasticity of the Arterial Baroreflex

Limited experimental evidence from recent studies of animals does suggest that baroreflex function in postnatal life may be susceptible to the influence of prenatal adaptations. In a study of rats, isocaloric protein restriction (9\%) during gestation through alteration of maternal diet, significantly shifted baroreflex set-points in the adult offspring towards higher pressures without significantly affecting fetal growth of the offspring.\textsuperscript{297} Similarly, 50\% nutrient restriction in sheep during early gestation (days 1 to 30), produced offspring that, at one year of age, demonstrated increased pulse pressure and reduced baroreflex sensitivity. However, a study of prenatal stress in rats failed to demonstrate a significant difference in baroreflex function between the prenatally-stressed offspring and controls despite the former showing both greater blood pressure responsivity during restraint stress and reduced recovery following stress in adulthood.\textsuperscript{298} Therefore, it is possible that development of baroreflex function is influenced only by specific adverse environmental factors in prenatal life, such as malnutrition, but more studies are required to establish this.
1.5 Size at Birth and Neuroendocrine Cardiovascular Control in Humans

In the preceding sections of this introduction, I have presented evidence that small size at birth and the high-prevalence of the metabolic syndrome and cardiovascular disease in adult life are linked. Furthermore, there is evidence that specific neuroendocrine systems such as the HPAA and ANS are involved. However, this evidence is limited and largely based on animal studies. Therefore, there is a need to address the question of how fetal adversity, often reflected by the extent of prenatal growth, affects these systems and resultant cardiovascular function in humans. Of particular note is that the few studies addressing this question in humans have tended to be small, focused on adults, and have generally evaluated only one specific marker of neuroendocrine or cardiovascular function, usually at rest. Resting function of these systems may be informative but as described in section 1.2.1.4, differences between individuals are frequently not seen during rest and become exposed when the control systems are activated. As described in section 1.2, a major activator of these neuroendocrine systems is psychosocial or mental stress. Therefore, the studies presented in this thesis were designed to address endocrine, autonomic and cardiovascular function at rest and during stress in both adults and children.
1.6 Hypotheses

The prenatal environment influences the development of physiological systems whose primary role in postnatal life is to respond to environmental stressors. Specifically, the HPAA and the ANS effect such responses by controlling metabolic and cardiovascular physiology. Therefore, this thesis addresses the hypothesis that prenatal adaptations in these systems in response to adverse conditions, reflected by small size at birth, have long-term effects on autonomic and endocrine function, which are apparent in childhood and adulthood. Following from this, a secondary hypothesis is that such adaptations have effects on measurable endocrine, autonomic and cardiovascular responses to stress, as well as function during rest, which are compatible with increased risk of developing hypertension and cardiovascular disease.

In subsequent chapters, I present evidence from three allied studies (the latter two being from the same subject group) in which the associations between markers of size at birth and later autonomic, cardiovascular or endocrine function at rest and during stress, were examined. Particular attention has been paid to potential sex differences in these associations, given the known sex-disparity in such associations described in animal studies (see Sections 1.3.3 and 1.4.1.3). For clarity, a methodological section for each of the three studies is presented in their respective chapter. A broader discussion of methodological issues is given in Chapter 2.

Chapter 3 examines data from a study of stress responsiveness in young adults born in Adelaide, South Australia carried out by colleagues. Their preliminary findings showed that HR and blood pressure responses to mental and psychological stressors were greater in women who were small at birth compared to those who were large at birth.
No similar association was found in men. In parallel with their simple analysis of HR and blood pressure, I developed more advanced analytic techniques to address function of the baroreflex and specific limbs of the ANS. This secondary analysis is the first study to show associations between size at birth and baroreflex function in humans, and the first to examine all of these parameters in relation to size at birth in the same population.

Chapter 4 and Chapter 5 describe a new study which I carried out looking at both HPAA, autonomic and cardiovascular variables at rest and during stress in a group of healthy 8-year-old children. These children formed part of a longitudinal cohort study of mothers and babies born in Southampton between 1994 and 1996. This study is the first of its kind to address prenatal influences on both cardiovascular and endocrine function in the same group in childhood.
Chapter 2. METHODOLOGICAL ISSUES

In section 1.2, I have discussed the difficulties that exist with defining stress and drawn together evidence from disparate fields to suggest that the mechanisms by which stress affects cardiovascular physiology may have been underestimated as potential causes for disease. Whilst the interdisciplinary nature and semantic difficulties of stress research have almost certainly contributed to the degree to which stress as a risk factor for heart disease has been relatively under-investigated in comparison to other acknowledged risk factors, there may be a more prosaic explanation. The study of stress physiology, particularly in humans, is inevitably limited by the degree to which the methods themselves influence the physiology being studied. Whilst this is true of any form of observational science, it is particularly difficult to examine stress systems, whose primary function is to respond to environmental challenges, without the method of observation substantially altering the system under study. For example, venepuncture stimulates both the HPAA, causing a rapid increase in circulating ACTH and cortisol concentrations, and the ANS, increasing HR, blood pressure, and circulating catecholamines and neuropeptide Y.

It is not just painful and invasive clinical techniques which are difficult to use successfully in stress research, however. Indeed, the novelty of an environment or experience alone can be enough to stimulate the HPAA and ANS. For example, relocation to a new environment (such as a visit to a research clinic) provokes significant increases in HPAA activity in both humans and animals and simple non-invasive procedures such as blood pressure measurement provoke increased HR and blood pressure in some individuals, particularly on initial use. Furthermore, the magnitude of these effects is sufficient to be predictive of cardiovascular death and,
therefore, likely to be comparable to, or even exceed, the magnitude of the effect under study. For example, in over 500 middle-aged men followed for 20 years, the act of blood pressure measurement alone was used as a stressor to identify subjects at risk of cardiovascular death. Men with a high blood pressure reactivity ('white-coat' effect) of >30 mmHg had a 2–3 times greater mortality rate than those that had a small or absent response.\textsuperscript{304} Even unintended environmental factors may be stressors. For example, a study of the effects of typical ambient community noise exposure in healthy children showed that noise exposure led to higher resting blood pressure, greater HR reactivity to stress, and higher overnight urinary production of cortisol.\textsuperscript{305}

These are just a few specific examples of factors which may confound attempts to examine the function of stress systems in most individuals. However, there are also likely to be many more factors that are based on individual experience. Thus to minimise the impact of these factors, a successful study design might require an epidemiological approach with larger subject groups than would be appropriate for studies of less complex physiology. Additionally, great attention must be paid to the use of minimally invasive techniques and standardisation of the subjects' experiences of the experimental environment. As discussed elsewhere in this thesis, it is also likely that studies of younger subjects will reveal common mechanisms of stress reactivity whereas studies of older subjects might obscure such mechanisms as these subjects differ too widely in their past experiences of stress, disease and social support.

This chapter details the methods that I have used in my studies of stress physiology. Care was taken to utilise stressors which are as near to universal as is possible and the methods by which stress responses were measured were minimally invasive and well-tolerated by children. It is also worth noting that past studies of cardiovascular

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reactivity, in particular, have focused largely on HR and blood pressure responses. These measures of cardiovascular function are an integrated reflection of several underlying physiological determinants such as blood volume, vascular function, haematological status, thermoregulation and ANS control. As such, they are limited in their ability to discriminate the underlying autonomic processes that effect global cardiovascular responses. Autonomic reactivity to a stressor is effected either by reduced parasympathetic outflow, increased sympathetic activation or a combination of both and may differ by target organ. The resulting increase in HR and blood pressure reflects the variable effect of these autonomic changes on both cardiac and peripheral vascular targets. Therefore, my studies are relatively unusual in that I have used methods that allow a more refined understanding of how underlying autonomic and endocrine processes might generate such blood pressure and HR changes in response to stress.

2.1 Stress Induction

Although the ANS almost invariably reacts to environmental changes, it is a significant experimental challenge to provoke an adrenocortical response reliably. A recent meta-analysis of over 200 studies which examined laboratory-based psychological stress tests concluded that reliable and strong stimulation of this axis requires a number of key elements to be present. Consistent with their proposed theoretical model, this analysis suggested that motivated performance tasks elicit cortisol responses if they are uncontrollable or characterised by social-evaluative threat (i.e. the task performance could be negatively judged by others and that the judgement would relate to an aspect of self-identity that was important to the subject). Thus the greatest stress responses
results when all three elements were present. The best exemplar of such a stressor is
the Trier Social Stress Test.

2.1.1 The Trier Social Stress Test for Children (TSST-C)

Both the adult and child version of the Trier Social Stress Test are well designed to
stimulate response of the HPAA. The child version of this test differs from the adult
version largely in the manner in which the subjects are treated which is neutral rather
than negative. In the child version, the panel of judges are not asked to be hostile or
negative in their approach to the children and the verbal and mathematical tasks are
age-appropriate. In my study of children, they were asked to prepare a five minute
story. They were given the beginning of a story as a stimulant of their imagination
which read as follows:

‘On Saturday, I went to my friend’s house to play. Their playroom is full
of brilliant toys. I like their castle the best. It is massive! We made up a
game about the castle, a bad witch, a princess and a dragon. Suddenly,
there was a loud bang, a puff of smoke and …’

The children then told their story for a panel of three adult judges whom they had not
met prior to the task. There was also a video camera directed at the child and a
microphone on a stand next to the child’s face to increase the sense of performance
(Figure 7). Following the public speaking task, the children were asked to perform a
serial subtraction task \((204 - 3 = 201 \ldots 201 - 3 = 198 \ldots 198 - 3 = 195 \ldots)\) which was
restarted from the beginning if the child made an error. I made a small modification to
the original protocol to increase motivation of the children by offering toys as a potential reward for high performance. They were told that the ‘best performance seen so far’ would secure their first choice of a range of toys and that a less adept performance would secure their second choice. Of course, they always received their first choice. Uncontrollability was a feature of the tasks as the children were unaccompanied by their parents and interacted exclusively with the previously unmet panel of adults whilst carrying out the tasks.

Figure 7. A participant undergoing the Trier Social Stress Test for Children. Whilst a series of bioamplifiers record continuous electrophysiological measures and a wrist tonometer measures arterial pressure, this 8-year-old boy delivers his story for a panel of previously unmet ‘judges’.
2.2 Salivary Cortisol

As venepuncture causes an HPAA response, measurement of HPAA axis activity in response to the TSST-C requires a non-invasive measure of cortisol. Salivary cortisol is ideal for this. Concentrations of cortisol in the saliva are a valid and reliable reflection of the level of unbound hormone in the blood.

Techniques for the collection of saliva are minimally invasive and non-painful and therefore stimulate little or no response in study participants. There are few methodological difficulties with the collection of saliva for cortisol assay. However, care should be taken to avoid collection of samples where subjects have recently brushed their teeth or have loose teeth leading to bleeding. Therefore, subjects were instructed to collect morning samples prior to brushing their teeth and a brief dental examination ensured that loose teeth were not bleeding prior to sample collection in the clinic. There is evidence that caffeine and high protein meals can stimulate the HPAA. Therefore, sample times were carefully organised to precede meals or to follow them by at least 1½ hours and subjects were requested to avoid caffeine-containing drinks such as cola on the day of sampling.

Salivary cortisol concentrations were measured using a time-resolved immunofluorescent assay (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay; DELFIA). This assay has a lower limit of detection of 0.4 nmol/l and an inter-assay coefficient of variation of 5–10% between 2 and 15 nmol/l. Time-resolved fluorometry provides a simple, sensitive and robust alternative to assays which use radionuclide labels. When light hits a fluorophore molecule, it is absorbed and emitted with less energy to produce fluorescence at a longer wavelength. Basic
fluorometry relies on the fact that the emission intensity of a sample containing fluorophores is a measure of their concentration. The greater the difference between the wavelengths of the excitation light and the light emitted by fluorescence (Stoke’s shift), the more sensitive the measure is. Time-resolved fluorescence is an improvement on basic fluorescence quantification because it uses rare-earth lanthanides such as europium that provide a long-lived fluorescent signal. Therefore, their fluorescence may be measured after a time delay which eliminates interference from short-lived non-specific background fluorescence from the sample matrix and the microplate and improves sensitivity. DELFIA relies on non-fluorescent chelates that can be converted to highly fluorescent forms using a developing solution (usually by a marked alteration in pH).

2.2.1 Assessment of Baseline Levels

Many previous stress studies have used pre-stress cortisol measures from the clinic setting as a baseline for comparison with levels taken during or following stress. This makes the assumption that levels taken at or shortly following arrival in a novel environment are representative of resting physiology. As discussed in the opening paragraphs of this chapter, there is good evidence in animals and humans that relocation to a novel environment may act as a stressor and induce increased HPAA activity. Therefore, to obtain a better assessment of baseline HPAA activity, I asked the children to collect a salivary cortisol profile at home on a day when they were taking no part in activities and were resting. Linear interpolation of these data can then be used to construct a set of comparative values for values obtained in a clinical situation and area-under-the-curve analysis can be used to assess overall differences in cortisol production between home and clinic environments.
2.2.1.1 Postal Delivery of Saliva Samples

In order to facilitate assessment of home cortisol levels, I devised an easy to use instruction sheet with colour-coded sections for each time of the day when sampling would occur together with colour-coded tubes for saliva sampling, straws to deposit saliva and sugar-free gum to stimulate salivation (see Appendix B). This colour-coding minimised the risk of mislabelling saliva collection tubes. The completed saliva collection was returned by post. A study of seventeen adults showed that cortisol estimated in samples which were split and then frozen within one hour or exposed to widely varying temperatures and degrees of motion for five days did not differ (paired \( t\)-test; \( t = 1.56, n.s. \)) between these treatments and were highly correlated (\( r = .92, P < .001 \)). This simulation of the worst extremes that a postal trip might involve indicated that cortisol in saliva samples is stable when exposed to widely varying temperatures and degrees of motion over extended periods and therefore unlikely to be significantly altered by postal delivery at ambient temperatures.

2.3 Mobility in Psychological Stress Tests

To my knowledge, I have carried out the first study where continuous blood pressure and electrophysiological data was obtained during the TSST-C. In general, where autonomic or cardiovascular data has been obtained in the past, it has been done with one-off measurements. The advantage of continuous data is that multiple measures reduce the probability of error, minimise the influence of outlying, erroneous measures which are also more easily identified and ensure that variably-timed peak stress responses are captured.
Many stress protocols such as the TSST-C require relocation of a subject from a restful environment to a stressful one. As a result, such studies in the past have not generally recorded continuous measures of cardiovascular function due to difficulties of equipment mobility. Laptop computers (upper left) use < 20V internally and are therefore safer and less precarious than cathode ray tubes and desktop machines which have the potential to deliver a high voltage shock (i.e. during a water spillage). The Vasotrac continuous blood pressure tonometer (upper right) and the BIOPAC bioamplifiers and analogue-digital converter were mounted in restraining blocks. All leads going from the station to the subject were bound in a flexible sheath and attached to the side of the trolley by a strong elastic connector. This allowed for some shock absorbance to prevent damage to the equipment in the case of the subject moving too far from the station. The subject-end of this set of leads was clipped to the waistband or belt of their clothing, preventing strain on electrodes and skin from the weight of the leads. On the lower level of the trolley (not pictured), an uninterruptible power-supply provided power when mobile and when not mobile, this was charged from mains electricity via an isolation transformer. All cabling was carefully tied to the trolley to prevent accidental snagging during mobile use. When mobile, the children followed the trolley to prevent falls.

The TSST-C, in common with many psychological stress protocols, requires subjects to experience the stressor in a different room from that in which they prepare and spend the rest of their time. This poses a considerable experimental challenge, requiring complex recording equipment to be safely mobile. Although there are wearable devices in development, there is currently no miniaturised device or set of devices which would
allow the continuous recording of ECG, thoracic impedance and blood pressure in children. Therefore, I devised a mobile equipment station (Figure 8) for this purpose.

Figure 9. An event marker for accurate timing of key occurrences during the experimental protocol. Many psychological experiments require accurate timing of events such as the beginning and end of different segments of a protocol or elements of subject behaviour. A bank of switches was connected to an array of resistors and powered by the BIOPAC 12V output allowing ten 1V levels (up to ten types of event) to be recorded on a single channel of the BIOPAC. Experimental error is common with event-marking and even experienced investigators forget to appropriately mark events on occasion. It is often possible to infer from existing markers where a missing marker should be (events are often separated by a fixed time interval). Data from the study in Adelaide had only a single marker type making this impossible in some cases. Although missing markers was an infrequent problem in my study, the multiple marker types facilitated reconstruction of the missing events in all cases. I wrote software in MATLAB (The Mathworks Inc., Natick, MA, USA) to automatically identify the square-wave switch pulses in the output signal, their level and their time of onset to the nearest millisecond. Thus events were precisely time-aligned with the physiological signals.
2.3.1 Accurate Event Timing

In addition to the physiological data that was continuously recorded, I devised a multi-event recording device using push-button switches (Figure 9). This allowed events such as the onset and ending of rest or stress periods or changes in posture to be marked directly in the same recording as the physiological data.

2.4 Electrocardiography

ECG was recorded using a modified lead II electrode arrangement (Figure 18) to maximise R-wave amplitude. To minimise artefact, pre-gelled silver chloride electrodes (Blue Sensor – Ambu Ltd, St. Ives, UK) were used and placed in areas of minimal muscularty.

2.4.1 Waveform Detection

The primary step in signal processing of the cardiovascular data is to reliably identify QRS complexes in the ECG. I implemented two algorithms to achieve this. The majority of my data was processed using the Pan-Tompkins algorithm\textsuperscript{311} which I implemented in the C programming language for fast performance. This identifies complexes on the basis of slope, amplitude and width. Band-pass filtering of the signal and variable time and amplitude-based thresholds in this algorithm allow it to reliably identify QRS complexes even in the presence of severe artefact. However, I also implemented an algorithm based on the Hilbert transform in MATLAB (The Mathworks Inc., Natick, MA, USA) which performs slightly better in the presence of severe noise contamination but is slower to execute.\textsuperscript{323} This algorithm achieved an average detection rate of 99.87%, a sensitivity of 99.94% and a positive prediction of...
99.93% when tested with data from the MIT-BIH arrhythmia database with a
detection error rate of less than 0.8% in every study case. This algorithm was
required in a small percentage of cases where the Pan-Tompkins algorithm failed to
detect QRS complexes due to high levels of noise and low amplitude signals. Benitez et al. compared the Hilbert transform algorithm to a range of other high performance
algorithms including the Pan-Tompkins algorithm on a noisy ECG sequence of over
2000 QRS complexes from the MIT-BIH Arrhythmia database. Table 2 shows this
comparison which highlights the superiority of the Hilbert transform algorithm on
noisy data.

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>FP</th>
<th>FN</th>
<th>Failed Detection</th>
<th>Errors (%)</th>
<th>Se (%)</th>
<th>+P (%)</th>
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<tr>
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<td>99.88</td>
<td>99.73</td>
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<td>0.54</td>
<td>99.84</td>
<td>99.61</td>
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<tr>
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<td>13</td>
<td>28</td>
<td>1.09</td>
<td>99.50</td>
<td>99.42</td>
</tr>
<tr>
<td>Topological mapping326</td>
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<td>4</td>
<td>45</td>
<td>1.75</td>
<td>99.84</td>
<td>98.43</td>
</tr>
<tr>
<td>Optimised filter and dual-edge threshold327</td>
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<td>21</td>
<td>56</td>
<td>2.18</td>
<td>99.19</td>
<td>98.66</td>
</tr>
<tr>
<td>Linear adaptive filter324</td>
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<td>22</td>
<td>62</td>
<td>2.41</td>
<td>99.15</td>
<td>98.47</td>
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<tr>
<td>Band-pass filter and search-back328</td>
<td>53</td>
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<td>75</td>
<td>2.91</td>
<td>99.15</td>
<td>97.98</td>
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<tr>
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<td>22</td>
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<td>3.46</td>
<td>99.15</td>
<td>97.46</td>
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<tr>
<td>*Filter bank329</td>
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<td>16</td>
<td>69</td>
<td>3.22</td>
<td>99.26</td>
<td>97.58</td>
</tr>
</tbody>
</table>

FP, false positive; FN, false negative; Se, sensitivity; +P, positive predictive value.
Comparisons were carried out on a highly noise-corrupted ECG (MIT-BIH record 105)
containing 2572 QRS complexes. *This result was reported for 2139 beats only.
I implemented an algorithm to identify potentially erroneous beat detections on the basis of sudden changes in inter-beat intervals which most often occur when the beat identification algorithm has made an error or when ectopic beats or arrhythmias occur (Figure 10). This has a detection rate of > 99.9% and a false detection rate of approximately 0.3%. In order to ensure that all beats were correctly identified, I wrote an ECG editing tool which allows each record to be scrutinised and beat detections to be edited as required (Figure 10). Therefore, all beats in all recordings were correctly identified at the end of this process regardless of which initial algorithm was used for their automated detection. Data from the impedance cardiograph was also discarded for beats where the ECG complex was discarded. Although this was occasionally due to severe noise contamination, the most common reason for this was an abnormal cardiac cycle due to an ectopic beat.

Detection of other features of the ECG waveform such as the T wave and its termination require more sophisticated analysis as these features have a lower frequency and amplitude than the QRS complex which are more similar to the frequency and amplitude of common sources of noise. The most capable published method relies on wavelet decomposition of the ECG signal to identify these features at varying scales (frequencies). Wavelets are discussed in greater detail in section 3.1.1.1. I implemented this method which proved to be highly reliable for determination of the T wave endpoint with a sensitivity of > 99.2% and a positive predictive value of > 98%. Maximal error in determination of the T wave endpoint was two samples (2 ms). Performance remained high in the presence of significant noise contamination but manual oversight and editing was used when necessary.
Figure 10. ECG beat detection and editing using semi-automatic analysis. This figure shows a screen capture of the MATLAB application which I wrote to view ECG recordings, remove ectopic beats from the analysis and edit erroneous beat detections. Although this shows ECG data, I wrote a modified version which allowed the same tasks to be carried out using continuous arterial pressure waveforms from the Portapres (see section 2.5.1). The top window shows heart period (the interval between adjacent beats), the middle window shows the complete ECG recording and the lower window shows a local magnification of the data allowing the waveforms to be scrutinised. The position of this magnification in the complete recording is indicated as a thick green bar in the middle window and as a red bar in the top window and the event markers (beginning and end of rest and stress periods) are shown as thin red lines in the middle window. Red circles in the middle window show where the automatic error-detection algorithm found sudden discontinuities in adjacent heart periods which are likely to represent misdetections or ectopic beats. These can be clearly seen in the top window as large spikes. In the lower window, green triangles indicate correct detections of R waves and red diamonds indicate detections of beats which surround questionable heart periods. The culprits in both cases here are erroneous flagging of P or T waves as R waves. The editing application was used to correct these. The pink squares were used to edit RR intervals.
2.5 Blood Pressure Measurement

Continuous accurate non-invasive blood pressure measurement in psychological stress studies is possible but the available technologies all have limitations. Oscillometric techniques which rely upon inflation of a cuff worn about the upper arm exert high pressures (> 200 mmHg) in order to reliably measure MAP from the brachial artery. The time required for inflation and deflation of the cuff and the discomfort (particularly in children) induced by this limit maximal rates of estimation of blood pressure to 1 – 2 measures per minute with such devices. Extended use of these devices is also limited by the cumulative discomfort that they cause. This thesis presents data measured using two alternative technologies: finger arterial pressure plethysmography and radial artery tonometry. The former causes minimal discomfort initially although this increases with extended use and is particularly advantageous because it produces a continuous arterial waveform whilst the latter is extremely comfortable even with extended use but relies upon an intermittent technology with a maximal rate of approximately 1 measure per 10 seconds. The finger artery measures are not greatly disturbed by motion artefact but unfortunately are not possible in young children because the finger cuffs required are not available in a suitable size. The tonometry measures provide accurate, self-calibrated measures in both adults and children but the technology is sensitive to motion artefact and careful positioning of the sensor.
Figure 11. The Portapres finger arterial blood pressure plethysmograph. This sophisticated device uses the volume-clamp method of Jan Peñáz. A small cuff applies an infrared sensor and transmitter pair to opposite sides of a finger which measure transmission of infrared light through the finger. This oscillates with the pulsatile blood flow in the finger. Initially, the cuff is inflated through the normal range of blood pressures. The inflation pressure which produces maximal amplitude oscillations in the infrared signal corresponds to mean finger arterial pressure. Thus, the mean value of infrared light at this cuff pressure may be taken as a set-point calibration. Once this calibration process is completed, a servo-controlled feedback mechanism maintains constant light transmission through the finger by rapid inflation or deflation of the cuff. Thus, the inflation pressure required to maintain the volume clamp is representative of the instantaneous arterial pressure waveform. Due to the complex nature of the finger cuffs, the minimum diameter cuff is too large to fit the fingers of young children.

2.5.1 Finger Arterial Plethysmography

In their study of adult blood pressure and HR responses to a range of psychological stressors, Ward et al. used a Portapres device (Figure 11; Finapres Medical Systems, Amsterdam, Netherlands) to measure blood pressure. In Chapter 3, I present my additional analysis of their data which uses spectrum analysis to determine baroreflex function. Such analysis is not possible with intermittent blood pressure data and so this device is an essential ingredient in the determination of spontaneous baroreflex sensitivity. Although there is some evidence that arterial pressure measurement in the
finger may be inaccurate when there is significant vasoconstriction (i.e. during exposure to cold, during hypotension etc.), a review of over 40 comparisons between this technology and invasive or intermittent non-invasive standard techniques suggest that in the majority of circumstances and particularly in the healthy ambulatory subject, it performs with a high degree of accuracy – systolic arterial pressure (SAP):
mean bias = -0.8 mmHg, SD = 11.9.

2.5.2 Radial Arterial Tonometry

Of the few alternative technologies which exist for continuous or near-continuous measurement of blood pressure, only one technology is suitable for use in children. The Vasotrac radial arterial tonometer (VasoTrac APM205A – MedWave Inc., St. Paul, MN, USA) uses a cushioned gel-filled chamber (Figure 12) which is applied to the skin over the site of the radial artery at the flattened region of the head of the radius. This sensor is intermittently pressed onto the radial artery using a servo mechanism and this sweeping increase and decrease of pressure allows the sensor to find the point of maximum energy transfer between the artery and the sensor. Thus, an accurate determination of blood pressure may be obtained without requiring an external form of calibration. In sixteen children aged between 8 and 12, the Vasotrac showed excellent agreement with invasive arterial pressure monitoring with absolute mean differences (95% confidence intervals) of 4.0 mmHg (3.0 – 5.0 mmHg), 4.3 mmHg (3.1 – 5.5 mmHg), and 3.5 mmHg (2.5 – 4.0 mmHg) for systolic, diastolic and mean arterial pressure, respectively. Comparisons of these two techniques in adolescents and adults have been similarly encouraging.
Figure 12. The sensor assembly of the Vasotrac radial arterial tonometer. This disposable sensor is applied to the wrist where the radial artery overlies the flat head of the radius. A compressible side wall conforms to the curve of the wrist at this site but cannot distort laterally. Thus, no pressure gradients are allowed to occur across the surface of the diaphragm, maximising accuracy of pressure measurements and reducing the influence of tissue pressures (movement artefact) on the measurement of arterial pressure. A fluid gel beneath the diaphragm couples the diaphragm to a piezoresistive sensor bridge which is uncoupled from the sensor housing. Thus pressures on the side wall do not influence the pressure measurement in the gel-filled chamber. A servo in the wrist device tightens and releases a wrist strap which uses a cantilever to apply and release pressure to the sensor assembly. This is then continuously swept up and down over the radial artery, intermittently measuring arterial pressure at the point of maximal energy transfer (maximal amplitude during the sensor sweep), which corresponds to the mean arterial pressure. Thus, no calibration is required to measure accurate blood pressures but this sensor sweep ensures that the maximum rate of measurement is no greater than once every ten seconds (approximately). However, prolonged use of this device is extremely comfortable in comparison to all other blood pressure devices due to the area and compressible nature of the face of the sensor and the fact that the pressure applied is never substantially greater than mean arterial pressure.

2.5.3 Limitation of Motion Artefact

The Vasotrac radial artery tonometer appears to be relatively tolerant of motion artefact in comparison to other tonometers by virtue of its sensor design and sophisticated computer control which senses motion and pauses or abandons measurement sweeps during this motion. However, it was my experience that during stress, the children moved so dramatically and frequently, particularly with motions of the hands, that it was difficult or impossible for the device to obtain clear radial arterial
waveforms. To address this issue, I designed a wrist splint which held the hand and wrist firmly but gently in a position of mild extension (Figure 13). This substantially improved the ability of the Vasotrac to measure blood pressure during the stress tasks.

Figure 13. A heat-moulded plastic wrist splint to minimise the influence of movement on radial arterial pressure measurements. Although the Vasotrac is considerably more motion-tolerant than other blood pressure tonometers, movement of the hand and wrist frequently results in a failure to reliably detect blood pressure waveforms. The self-calibration sweep of the sensor assembly takes some time to complete and therefore, unlike other tonometers, a missed recording is costly in terms of time between measurements. Children, in particular, move during stress. Therefore, there was a requirement for a mechanism to minimise this problem. I designed a wrist splint using heat-mouldable plastic, memory foam padding and soft Velcro straps to comfortably hold the subjects' hands still during the experiment. A channel at the back of the splint prevented it from impinging on the strap of the Vasotrac. The thumb was separately restrained and finger straps and a palm block held the fingers and hand in place.

To further reduce motion artefact and to make this assembly comfortable for prolonged wearing, I designed a sling (Figure 14) to support the arm in relaxed flexion.
Figure 14. A cuff and splint system to hold the arm and hand still during ambulatory radial artery tonometry. For subject comfort and to further minimise the influence of movement on blood pressure readings, the arm and wrist splint were supported by a fabric cuff suspended from the neck. This prevented excessive hydrostatic errors in the blood pressure estimate due to vertical arm movement although small movements were still possible. Additionally, the weight of the wrist device and splint would have been uncomfortable in prolonged use without support. The splint was designed to hold the hand of an 8-year-old child comfortably with the wrist in slight extension. In this illustration, the system is modelled by an adult whose hand did not fit well to the splint and who is holding her wrist in more marked extension to emphasise the shape of the splint.

2.5.4 Hydrostatic Adjustment for Arm Height

Although the sling generally held the wrist in a fixed position relative to the heart, changes in posture such as sitting or standing, sudden movements of the arm during speech and occasional slippage of the sling could contribute hydrostatic errors in estimation of the blood pressure. To compensate for these, I devised a pressure sensor and water-filled tube system (Figure 15) which allowed the pressure at the wrist to be referenced to a fixed point (the insertion of the deltoid tendon) for the entirety of the
experiment. Thus, any error in pressure estimation at the wrist due to arm movement relative to that point was removed.

Figure 15. A device for hydrostatic correction of radial arterial pressure readings for alterations in the distance between the heart and the wrist. Although the arm was held in place with a cuff, there was still potential for arm movement relative to the heart. This device was secured to the measurement arm such that the pressure sensor was attached to a common reference point on the upper arm at the level of the insertion of the deltoid tendon (heart level). For pressure sensing, a commonly available disposable arterial line kit (TruWave Disposable Pressure Transducer, Edwards Lifesciences LLC, Irvine, CA, USA) was rewired for connection to the BIOPAC. One end of the water-filled tube was sealed whilst the other was left open to the air and attached to the wrist at the level of the radial artery allowing a continuous measurement of the pressure difference between the wrist and heart level. The tubing was flushed, refilled, checked for air bubbles and recalibrated daily. The output signal was filtered with a low-pass filter and found to track height changes quickly and accurately.
2.6 Frequency Analysis of Cardiovascular Signals

Since the early 18th Century, physiologists have noted the existence of regular oscillations of HR and blood pressure. Initially, Stephen Hales, an eminent respiratory physiologist in London and Albrecht von Haller, a Swiss anatomist and physiologist, noted oscillations in both parameters occurring at the respiratory rate whilst over a century later, Siegmund Mayer, a German physiologist working in Vienna, reported additional oscillations at frequencies slower than the respiratory rate which he suggested were related to vasomotor activity. Since then, a wealth of studies, most recently employing sophisticated power spectrum analysis (frequency analysis) using computers, has made it apparent that these fluctuations are not merely undesirable noise but rather represent a rich source of information about the mechanisms of cardiovascular control. This control relates principally to the regulation of blood pressure and the homeostatic mechanisms which act perpetually to maintain blood pressure at a steady level. Achievement of this aim requires the action of the ANS, including the baroreflex, on both the heart and vasculature and it is likely that, as for many active control systems, a small degree of oscillation is required for these control systems to act efficiently.

Through monitoring of beat to beat HR and blood pressure, as described elsewhere in this chapter, these fluctuations can be quantified either by simple non-specific measures such as a calculation of variance (or standard deviation) of their average values or, more recently, by frequency domain analysis. When the latter technique is employed, it is apparent that there at least three significant cardiovascular oscillations with a period shorter than one minute. The first of these occurs at frequencies around 0.2 to 0.4 Hz, a frequency range similar to that of normal respiratory activity, and is
defined as HF. The next has a frequency close to 0.1 Hz (the classic Mayer wave in the context of blood pressure) and is defined as low-frequency (LF). The lowest frequency oscillation occurs at 0.02 – 0.07 Hz and is defined as very low-frequency (VLF).

However, because this latter oscillation appears to relate to a variety of physiological processes such as thermoregulation, endothelial factors and the function of the renin-angiotensin system, it is frequently disregarded in studies of autonomic cardiovascular control. Furthermore, the frequency and amplitude of these oscillations can vary markedly with environmental and behavioural conditions so the VLF, LF and HF definitions are never more than an approximation.

Figure 16 shows an example of a power spectrum (right panel) demonstrating the two dominant oscillations (LF and HF) in an HP series (left panel; the reciprocal of instantaneous HR). Although these peaks in the power spectrum have initially attracted the most attention from researchers, it has become apparent that a peak in the spectrum may, in fact, relate to the actions of more than one cardiovascular control mechanism and that a single cardiovascular control mechanism may contribute to different peaks. Furthermore, it is now clear that both blood pressure and HRV includes non-rhythmic fluctuations in addition to the better described rhythmic ones. These appear in the spectrum, not as peaks, but as a general increase in power spread across a broad frequency region. This is apparent in studies of both animals and humans. For example, in a study of non-anaesthetised cats, removal of baroreceptor restraint of sympathetic activity by sino-aortic denervation was accompanied by systematic changes in both blood pressure and HRV across several broadband, non-peaked frequency regions. In humans, sleep is a condition where SNS activity is almost universally reduced whilst PNS (vagus nerve) activity is typically increased. Studies of sleeping humans have shown that non-peaked, broadband frequency regions
Figure 16. Frequency analysis of a physiological time series (heart period). The panel on the left shows the natural oscillations that occur in cardiovascular signals such as heart period (the interval between adjacent heart beats) or blood pressure over time. At rest, over a short time period (minutes), there are two dominant oscillations which are difficult to perceive in the time domain. The panel on the right shows a spectrum analysis of the signal on the left (autoregressive spectrum) which reveals these dominant oscillations in the frequency domain with one peak around 0.1 Hz (low frequency) and one peak at the respiratory frequency (high frequency). The magnitude of these peaks (power) relates to the variance of the oscillations at that frequency.

of the spectrum are altered during alterations in ANS activity in addition to changes in the peak regions. Thus, it is now more common to examine the power in broad frequency bands of the power spectrum rather than to focus on specific peaks.

2.6.1 Methods of Spectrum Analysis

There are numerous methods by which the relative contributions of component frequencies to a signal can be estimated. These include the fast Fourier transform (FFT), autoregressive (AR) modelling (see section 2.6.3 for a description of AR modelling), and variants of the wavelet transform (WT). Whilst the first two provide a complete transformation of the signal into the frequency domain, discarding information about the time at which those frequencies occur, the latter retains information about both frequency and time content of the signal at the expense of
precision in either domain. A more detailed discussion of the differences between WT-based methods and other forms of spectrum analysis is given in section 3.1.1.1. The spectrum which results from the FFT is derived from all of the information in the signal and therefore includes all of the signal variance broken down by frequency. By contrast, the AR method imposes a best-fitted model on the raw data which includes assumptions about the number of important frequency components in that data (the order of the model includes terms for the DC component, the principal oscillations and noise). Any component of the signal which does not fit the model is then discarded as noise. Therefore the AR method is more appropriate when specific control processes are being assessed or modelled as it deals only with the peak (rhythmic) components of the spectrum. It also has advantages where the frequency and amplitude of these peaks (which may vary) is being tracked over time because the AR method has a frequency resolution that is not as dependent on the length of data being analysed as the FFT is. Therefore, quite short segments of data can be used. The FFT has an advantage, however, when broadband powers are of interest as these are frequently not modelled adequately by the AR method unless a high model order is used. However, in practice, all of these methods produce comparable results with most physiological data.

2.6.2 Interpretation of Cardiovascular Spectrum Analysis

Despite the large number of studies addressing blood pressure and HRV, the interpretation of spectra derived from these data still provokes considerable debate. The following sections attempt to summarise the issues surrounding the interpretation of these spectra but as this is, of itself, the sole subject of many journal articles, theses and books, an exhaustive discussion is beyond the scope of this thesis.
2.6.2.1 Interpretation of Heart Rate Spectra

Whilst the vagus nerve appears to be able to modulate HR rapidly at frequencies up to 1 Hz, it seems that sympathetic cardiac control is band-limited at a much lower cut-off frequency and is capable of modulating HR only at frequencies below 0.15 Hz. This partial separation of the actions of the two limbs of the ANS in frequency domain is supported by a number of studies. For example, broadband electrical stimulation of the vagus nerve in dogs produces HR responses with minimal dampening up to at least 0.7 Hz whilst similar stimulation of the right stellate ganglion is followed by HR responses with a delay of approximately two seconds and a dampening that leads to a minimal response above 0.15 Hz. Pharmacological blockade of the PNS using atropine eliminates HR fluctuations above 0.15 Hz in dogs and humans whilst fluctuations below 0.15 Hz are partially unaffected whereas blockade of the SNS using propranolol reduces HR fluctuations below 0.15 Hz whilst leaving those above 0.15 Hz largely unaffected. Thus, oscillations in HR (or HP) above 0.15 Hz (HF) seem to be largely effected by modulation of cardiac vagal nerve activity.

As respiratory frequencies are most often above 9 breaths per minute (0.15 Hz), respiratory fluctuations in HR are likely to be primarily mediated by parasympathetic efferent pathways. Therefore, respiratory sinus arrhythmia (RSA) is often used as a measure of cardiac vagal modulation. However, as respiratory activity may occur below 0.15 Hz, RSA may not always accurately reflect only vagus nerve activity as sympathetic modulation of HR can also occur at these frequencies. Finally, not all modulation of HR above 0.15 Hz occurs due to ANS activity. There is evidence that a small RSA effect persists after complete pharmacological autonomic blockade or surgical denervation of the heart (as a consequent of transplant surgery). This is presumed to occur with alterations in sinus node discharge rate caused by mechanical
stretch of the atria due to respiratory activity. However, this effect is very small in comparison to nervous system-mediated effects and is generally disregarded. As powers at frequencies below 0.15 Hz relate to the activity of both limbs of the ANS, they are likely to be less reliable indices of the modulatory activity of either one, although in many behavioural and clinical circumstances, high correlations between LF band power and other indices of sympathetic activity have been observed. \(^{360,361}\)

### 2.6.2.2 Interpretation of Blood Pressure Spectra

There is conflicting evidence on the origins of HF oscillations in blood pressure. Whilst some studies suggest that these oscillations arise as a consequence of oscillations in CO mediated by vagus nerve modulation of HR at this frequency,\(^{361}\) other studies point out that HF power of blood pressure oscillations is not substantially reduced in patients with denervated donor hearts.\(^{369}\) This has led to the suggestion that these oscillations are caused by the mechanical effects of respiration on the pressure gradients, size, and functions of the heart and large thoracic vessels.\(^{358}\)

There is likely to be little or no influence of HRV upon blood pressure oscillations in the LF or VLF regions because combined atropine and propranolol administration eliminates only a small fraction of blood pressure variability below this frequency. Therefore, it is likely that power in the LF region (0.05 to 0.15 Hz) is largely determined by the strength of fluctuations in vasomotor tone and SVR. Power of blood pressure oscillations in this frequency band have been shown to increase with laboratory stimuli which increase sympathetic cardiovascular influences, such as head-up tilt or mental stress, and decrease with conditions which decrease sympathetic cardiovascular influences, such as sleep or \(\alpha\)-adrenergic blockade.\(^{345,353,362}\) It is reasonable
to assume that a measure of LF power might be a better indicator of sympathetic vasomotor activity than a similar analysis of HR because, unlike for HR, there is no influence of the vagus nerve on LF power of blood pressure fluctuations. However, as for all other parameters derived from spectrum analysis of cardiovascular variables, it may not be a consistent marker of sympathetic vasomotor regulation in all behavioural or environmental circumstances.

In summary, until better measures of autonomic cardiovascular regulation in humans appear, spectrum analysis of HR and blood pressure variability is likely to continue to be a useful if somewhat controversial tool in human physiology.

2.6.3 The Relationship Between Blood Pressure and Heart Rate

One approach to the difficulties with interpretation of individual markers of cardiovascular control is to model the relationship between the fluctuations of two or more cardiovascular parameters such as blood pressure and HR. Such multivariate models may operate in the time or frequency domain and allow the function of the physiological control systems (such as the baroreceptor-HR reflex) which connect these parameters to be studied. Early frequency-domain methods attempted to assess baroreflex sensitivity by computing the squared ratio between the spectral powers of the HP and SAP or the modulus of the cross-spectrum between these parameters in the LF and HF frequency bands where the two signals showed a significant coherence. Experiments in non-anaesthetised cats demonstrate marked changes in these models when sino-aortic denervation was performed, validating their use to reliably index baroreflex sensitivity.
However, such approaches ignore a key concern with regard to the relationship between HR and blood pressure. These parameters operate in a closed-loop fashion with HR influencing blood pressure through a mechanical feed-forward effect on CO and blood pressure influencing HR via a feedback mechanism (the baroreflex). Although simple cross-spectrum analysis of these two parameters can determine the phase relationship between them at different frequencies, this allows no inference regarding causality to be drawn, given the closed-loop nature of their interaction. In the LF band, blood pressure oscillations have a leading phase on associated oscillations in HP (usually measured as the interval between R waves on the ECG). In the HF band, blood pressure and HR fluctuations are in phase. This may suggest a delayed and fast neural feedback operating in the LF and HF bands, respectively.\(^{368}\) However, it should be noted that in the LF band, whilst blood pressure has a phase lead on associated changes in HP, HR (in phase opposition to HP) has a phase lead on blood pressure. Therefore, the same relationship between the two signals can be seen either as the effect of baroreflex feedback, in the first instance, or as the effect of mechanical feed-forward mechanisms, in the second instance. Indeed, although some animal studies have validated simple cross-spectrum analysis for measurement of baroreflex sensitivity, a study in dogs suggested that the overall pattern of the phase diagram between blood pressure and HP was consistent with a simple model of the feed-forward effect and was insensitive to both pharmacological and surgical manipulations of autonomic activity.\(^{369}\) Therefore, more advanced mathematical approaches have been proposed which allow for the two limbs of the closed-loop system to be examined independently.\(^{370,371}\)
In the study described in Chapter 3, I used an approach based on a multivariate autoregressive moving-average (ARMA) model. An AR model may be defined by the equation:

\[ x(t) = \bar{x} + \varphi_1 \cdot x(t-1) + \varphi_2 \cdot x(t-2) + \ldots + \varphi_p \cdot x(t-p) + \xi(t) \]

where \( x(t) \) is the time series, \( \bar{x} \) is the mean of the series, \( \xi(t) \) represents normally distributed random errors and \( \varphi_1 \) to \( \varphi_p \) are the parameters of the model. AR models are simply a linear regression of the current value of the series against one or more prior values of the series. The number of those values, represented by \( p \) in the equation above, is called the order of the model. Such models forecast future values of a time series purely on the basis of past values of the time series.

Moving average models are defined by the equation:

\[ x(t) = \bar{x} + \xi(t) - \theta_1 \cdot \xi(t-1) - \theta_2 \cdot \xi(t-2) - \ldots - \theta_q \cdot \xi(t-q) \]

where \( x(t) \) is the time series, \( \bar{x} \) is the mean of the series, \( \xi(t-i) \) represent random disturbances of one or more prior values of the series and \( \theta_1 \) to \( \theta_q \) are the parameters of the model. The concept behind moving average models is that random disturbances are propagated to future values of the series and therefore such models predict future values purely on the basis of past random disturbances. Fitting moving average models requires iterative, non-linear fitting techniques.
The power of ARMA models is that they incorporate both AR terms and moving average terms. When applied to cardiovascular data, a multivariate AR model of order \( p \) expresses current HP as a weighted mean of \( p \) previous values of HP, \( p \) previous values of SAP, and white noise. This approach imposes a causality constraint (HP can only be predicted by past values of HP and blood pressure) and accounts for non-baroreflex related changes in HP because it is predicted not only by blood pressure but also by its previous values. The weightings are estimated iteratively to minimize the error between the actual HP values and the values predicted by the model using a recursive least squares algorithm. From the estimated weightings, the causal transfer function (similar to a ratio) between SAP and HP is estimated and the baroreflex sensitivity indices are computed for the different frequency bands. Baroreflex gain is then estimated as the mean gain of the transfer function in the LF band for frequencies where coherence is high (> .5). The method used in this thesis is an adaptive version of the multivariate AR model which differs from the standard version only in the way in which the weightings are estimated. In the standard approach, weightings are estimated once for a given epoch of HP and SAP data yielding an average measure of the transfer function for that time period. In the adaptive approach, estimations of the weightings are made for each sample of the HP signal, taking into account the previously estimated weightings and the previous values of HP and SAP, yielding a continuous measure of the transfer function.
Figure 17. Time-frequency analysis of the relationship between systolic arterial pressure and heart period using a bivariate adaptive autoregressive model to determine baroreflex function. The causal transfer function (indicating baroreflex gain) between systolic arterial pressure and heart period is shown with colour representing the range of low to high values in a blue, green, yellow, red, red/brown sequence. Men and women show a high gain in both the low and high-frequency bands at rest (0.05 – 0.15 Hz and 0.15 Hz to 0.4 Hz, respectively), representing strong baroreflex control of blood pressure. However, this diminishes during three stress tasks (Stroop, Mirror and Speech – see Chapter 3 for a full description), particularly at their onset, representing reduced baroreflex control of blood pressure during stress.

Figure 17 shows how the transfer function between SAP and HP at frequencies up to 0.5 Hz evolved over time during a study of stress in men and women from Adelaide, Australia (see Chapter 3) by graphing the mean of their values by sex. The red/brown regions highlight strong causal links (high transfer function or gain) between SAP and HP in both the LF and HF bands which are more apparent at rest. These strong links diminish (low gain is indicated by blue/green regions) in both the LF and HF bands during the three stressful tasks (see Chapter 3 for a complete description of the study), particularly in the early part of each task. As the baroreceptor-HR reflex mediates the causal link between SAP and HP in this direction, a reduction in this transfer function reflects a reduction of baroreceptor reflex gain or sensitivity.
2.7 Thoracic Impedance Cardiography

Thoracic impedance cardiography has now been around for over forty years. Yet despite a considerable literature on the technique and advances in the signal processing required and the technology underlying it, it has struggled to gain acceptance in a clinical environment. To some extent, this is justified as the technique has limitations which appear to particularly affect its accuracy when it used in the critically ill or individuals with heart defects such as valvular insufficiency or septal defects. Given that a substantial proportion of the early validation studies were performed in exactly these types of patients, perhaps it is not surprising that the disappointing results were so influential on the opinions of clinical users.

Despite these setbacks, the technology has enjoyed a strong interest in academic research which continues to this day and improvements to the methodology have led to a slow return of interest in impedance cardiography in specific clinical settings. For example, it is now being used successfully in a clinical outpatients setting to advance management of hypertension and hypertensive stroke, to improve the tuning and performance of multi-chamber pacemakers in patients with heart failure, and to assess the effect of various temporary pacing strategies on cardiac performance during cardiac catheterisation prior to implantation of a permanent pacemaker.

A meta-analysis of 154 studies of the validity of impedance cardiography showed a correlation of .82 (95% CI of .80 – .84) with reference methods such as thermodilution or the indirect Fick method for determination of CO. Indeed, this figure almost certainly underestimates the accuracy of impedance cardiography in healthy subjects for two reasons: The meta-analysis confirmed the expected worse performance of the
technique when used with critically ill subjects and a substantial portion of the studies were of such subjects. It is also well-known that there is no true reference method for determination of CO. All of the other methods have a similar level of error to impedance cardiography. As the meta-analysis effectively assigned all of the error in agreement between the two methods to impedance cardiography, it is likely that this underestimated its accuracy. The authors of the meta-analysis concluded that it is sufficiently accurate, particularly in healthy subjects, for use in research. It is particularly suitable for use in an epidemiological setting where the likely effect of errors in the estimation of cardiovascular parameters which might be significant for the clinical management of individuals would merely be to attenuate estimates of the strength of associations with other variables.

Although under certain circumstances, reliability of impedance cardiography for determination of stroke volume (SV) and CO may be a concern, it is a well-established means by which various events within the cardiac cycle can be accurately timed. Lababidi et al. described a range of fiducial points on the thoracic impedance cardiograph which are coincident with these. Figure 25 shows a representative recording of the impedance cardiograph with the three key fiducial points marked. Comparisons with direct aortic pressure waveforms show that timing of systolic ejection using impedance cardiography is highly accurate (r = .986) across a wide range of HRs. It is suggested that the sudden reorientation of blood cells as heart valves open and close is responsible for the notches seen in the impedance waveform and, therefore, timing of these events is likely to be extremely accurate. In practice however, it has been my experience that reliable identification of these events requires sophisticated signal processing particularly when the recordings have a high level of movement and respiratory artefact.
Figure 18. Electrode positioning and circuitry for thoracic impedance cardiography. The green circles show the positions of the modified lead II ECG electrodes. There are nine thoracic impedance electrodes pictured. Electrodes in the same horizontal plane are connected together to form an approximation of a circumferential band electrode. A high-frequency alternating current is applied to the outer sets of electrodes (purple) whilst the voltage is determined across the inner sets of electrodes (blue). The electrodes were placed in the frontal plane with the exception of two of the abdominal electrodes (lower purple) which were spaced equally from the other two on the front of the abdomen and of the head electrode which was placed in the centre of the forehead. Vertical placement of the head and neck electrodes was determined by anatomical landmarks whilst the electrodes at the level of the xiphisternum (lower blue) were placed 17% of the subject's height below the neck electrodes, defining the same proportion of thorax in all subjects (red shaded region). The abdominal electrodes were placed 2/3 of the distance between the head and neck electrodes below these.
Although the understanding and processing the signals is often necessarily sophisticated, the measurement of the thoracic impedance cardiograph is relatively simple. Figure 18 is a schematic representation of this method. Essentially, a high-frequency (50 kHz in my study) very small (400 μA RMS) alternating current is passed longitudinally through the thorax. An additional set of electrodes are then used to measure the voltage developed across a defined volume of the thorax and impedance is obtained using Ohm’s law.

### 2.7.1 The Parallel Cylinder Model

In order to determine blood volume changes in the thorax from thoracic impedance measurements, Kubicek et al. used a simplified model of the thorax as a cylinder homogeneously filled with blood. This was later extended with the blood and tissues of the thorax modelled as two parallel cylinders. The total longitudinal impedance of this model is:

\[
Z = \frac{Z_b \cdot Z_t}{Z_b + Z_t}
\]

where \( Z \) is the longitudinal impedance of the model, \( Z_b \) is the impedance of the blood volume, and \( Z_t \) is the impedance of the tissue volume. Differentiation of this equation with respect to \( Z_b \), gives the relationship between the impedance change of the thorax and the impedance change of the blood volume:

\[
dZ = \frac{Z^2}{Z_b^2} \cdot dZ_b
\]
A simplified cylindrical model of the thorax containing uniform blood and tissue compartments for determination of thoracic impedance. In this model, the impedance of the thorax is divided into two parts: that of the tissues and that of the fluids. In the absence of breathing, all components forming the impedance of the thorax are constant except for the distribution and quantity of blood. The amount of blood in the thorax changes as a function of the cardiac cycle. During systole, the right ventricle ejects a volume of blood (the stroke volume) into the lungs and blood returns from the lungs to the left atrium. Measurement of thoracic impedance reflects the distribution of blood in the thorax during the cardiac cycle and can be used to infer cardiac stroke volume. \( A_b \) and \( A_t \) represent the cross-sectional area of the blood and tissue components of the model and \( l \) is the length of the cylinder (height of the thoracic segment) being measured.

Given the cylindrical geometry of this model of the thorax (Figure 19), the impedance of the blood component with resistivity \( \rho_b \) is:

\[
Z_b = \frac{\rho_b \cdot l}{A_b}
\]
where $\rho_b$ is blood resistivity, $A_b$ is the cross-sectional area of the blood cylinder (Figure 19), and $l$ is the length of that cylinder.

By solving for the blood volume in this equation and differentiating, the relationship between changes in blood volume $v_b$ and the blood volume impedance is found:

$$dv_b = d(l \cdot A_b) = -\frac{\rho_b \cdot I^2}{Z_b^2} \cdot dZ_b$$

where $v_b$ is the blood volume. By solving the second equation for $dZ_b$ and substituting it into this equation, the dependence of a change in blood volume on a change in thoracic impedance is derived:

$$dv_b = -\frac{\rho_b \cdot I^2}{Z_b^2} \cdot dZ$$

This equation represents the relationship between net changes in blood volume in the thorax and changes in thoracic impedance. However, it should be remembered that during systole, the net change in blood volume is the result of at least two main blood flows. The aim of the technique is to estimate SV which is equal to the volume of blood that the right ventricle ejects into the lungs. However, the other major blood flow during systole is the return of blood from the lungs to the left atrium. Therefore, to derive a value for SV, Kubicek et al. made a number of assumptions about the relationship between net thoracic blood volume changes and SV which are highly simplified and may be unreliable. The basis of their method for determination of SV is that in the earliest part of systole, ejection of blood from the heart is the primary
source of volume shifts within the thorax, with significant flow from the lungs to the left atrium occurring only later in systole. Given this assumption, SV might be determined by measuring the maximal rate of change of thoracic impedance which occurs in the early part of systole and should relate principally to flow of blood from the heart into the major vessels. It is assumed that if no blood were to flow away from the thorax during systole, the impedance of the thorax would decline at this maximal rate. Thus, a linear extrapolation over the ventricular ejection time yields the value of thoracic impedance were these conditions to be true and, therefore, an estimate of SV. Thus the SV can be determined from the thoracic impedance waveform as follows:

\[ SV = \rho_b \cdot \frac{l^2}{Z_0} \cdot t_e \cdot \left| \frac{dZ}{dt} \right|_{\text{max}} \]

where \( \rho_b \) is the resistivity of blood (\( \Omega \cdot \text{cm} \)), \( l \) is the height of the thoracic segment measured (cm), \( Z_0 \) is the basal thoracic impedance (\( \Omega \)), \( t_e \) is the ventricular ejection time, and \( \left| \frac{dZ}{dt} \right|_{\text{max}} \) (\( \Omega \cdot \text{s}^{-1} \)) is the absolute maximum rate of fall of thoracic impedance during systole (coinciding with the C point; Figure 25).

2.7.1.1 Limitations of the Model

Even with a cursory examination of the Kubicek model, it is apparent that it has a number of limitations. Efforts to identify the source or sources of the measured changes in impedance during the cardiac cycle have yielded varied results. Early theoretical models of the thorax suggested that the lungs, and therefore the right ventricular circulation, were the dominant source of the signal. This was supported by the observation that blood flow in the pulmonary circulation correlates well with
thoracic impedance measures of CO in children with ventricular septal defects.\textsuperscript{385} However, more recent studies in animals and humans suggest a range of possibilities.\textsuperscript{386} With the general consensus being that changes in blood volume in the vena cava, atria, ventricles, aorta, thoracic musculature, and lungs all contribute to the signal.\textsuperscript{387} A critical analysis of the various hypotheses concerning the origin of the cardiac impedance signal concluded that it was due to cardiac haemodynamics only and reflects changes in both blood volume and blood velocity.\textsuperscript{388} Thus, rapid movement of blood during ventricular ejection was postulated as the primary source of the signal during systole whereas the changing volume (mainly of the atria and great veins) was postulated as the dominant source of the diastolic portion of the impedance curve. Thus perhaps one of the greatest limitations of impedance cardiography is that the source of the signal is not accurately known.

The two-compartment model is clearly a gross simplification of thoracic anatomy and the assumed cylindrical geometry is also a highly simplified approximation which might be expected to vary in accuracy according to body habitus. As a result, alternative models have been used which attempt to deal with these factors by modelling the thorax as a truncated cone adjusted according to parameters such as age, sex, weight and height.\textsuperscript{389,390} However, unlike the Kubicek model, these models are likely to be erroneous in the presence of pulmonary oedema\textsuperscript{391} and meta-analysis of validation studies comparing these approaches suggests that, if anything, these more recent models are less accurate than the original one.\textsuperscript{379}
The change of blood conductivity with change in velocity has been entirely neglected in this model. Indeed, it is now well-established that alignment of red blood cells reduces the resistivity of blood in parallel to the direction of flow.\textsuperscript{392-395} A study in dogs showed that \textit{in vivo} resistivity of blood is far from constant and that the contributions of variations in blood conductivity and volume to thoracic impedance changes which are synchronised with the cardiac cycle are of comparable magnitude.\textsuperscript{396} Furthermore, the contribution of the volume variations is the sum of the volume variations in the contributing intrathoracic vessels whilst the effect of variations in orientation are added up in proportion to the relative volumes of the contributing vessels. Thus, it is likely that high speed flow of blood in the aorta and pulmonary artery at the onset of systole is indeed a significant contributor to the early part of the thoracic impedance waveform and this effect may partly underlie the observation of a linear relationship between the maximal rate of change of thoracic impedance and blood flow in animal experiments.\textsuperscript{382,397} There has been considerable attention in the literature given to the problem of choosing a value for blood resistivity in the Kubicek equation. Given the dynamic nature of blood resistivity, it is unlikely that the approach that some researchers have taken of measuring resistivity of blood directly or calculating it from measures of haematocrit would improve accuracy of the method. Indeed, studies in animals have shown that rearranging the Kubicek equation to make resistivity the outcome variable and then measuring all the other components using an electromagnetic flow-meter and impedance cardiograph whilst haematocrit is manipulated yields a constant value for ‘blood resistivity’ (\(\rho\)) in this equation.\textsuperscript{398} This has led to the recommendation that a constant value of 135 \(\Omega\).cm should be used in all subjects unless there is reason to expect a significant abnormality of haematocrit.\textsuperscript{399}
2.7.2 Electrode Placement

Much attention has been given to the placement of electrodes on the thorax and, therefore, to the inaccuracy in between-subject comparisons that could result if the measurements of distance between electrodes are not comparable. There are two important considerations with regard to placement of the electrodes:

Firstly, alterations in the distance between the voltage recording electrodes will alter the measure of basal thoracic impedance and its first time-derivative. This led some authors to suggest that a fixed distance should be used, rather than anatomical landmarks. In fact, small variations in the distance between these electrodes is unlikely to have any effect on the estimate of SV at all. This is apparent both from the equation itself, where it can be seen that SV is proportional to $Z^{-2}$, whereas $\frac{dZ}{dt}$ is proportional to $Z^{2}$. Slight displacement of the detector electrodes changes the measured mean impedance and first derivative signal, but their effect on the computed SV is compensated by the changed value of the mean distance of the electrodes. This is supported by investigations in humans where mean thoracic impedance was altered experimentally by up to 20% using a defibrillator electrode placed under the back of a supine patient. It was shown that despite the large difference in mean impedance induced by this manipulation, there was no alteration in estimates of SV because of the simultaneous influence on $\frac{dZ}{dt}$ which compensated for the change in $Z$. It is also of some interest that removing half of the lower electrode (a band electrode in this case) made no difference to the measurement suggesting that it was placed on an equipotential surface supporting the model's assumption of cylindrical symmetry. Therefore, whilst between-subject standardisation of the distance between voltage
recording electrodes is desirable for comparison of measures derived only from $Z$ or $\frac{dZ}{dt}_{\text{line}}$, it is unnecessary for measures of SV.

The second consideration regarding electrode placement is likely to be more important. One of the assumptions of the model is that there is a uniform distribution of excitation current density across the volume of tissue being examined. Thus variations in electrode positioning relative to one another and relative to the underlying thoracic anatomy might alter excitation current pathways and the resulting measures of impedance. To meet these criteria, at least 3 cm separation is required between the current inducing electrodes and the voltage sensing electrodes.

Initially, researchers met these requirements by using circumferential band electrodes with both upper electrodes placed on the neck. This arrangement has a number of disadvantages, however, particularly with regard to its use in children. Children have a shorter neck than adults making adequate separation of the voltage and current electrodes difficult or impossible in some cases. Furthermore, the band electrodes can cause considerable discomfort especially when placed around the neck. Finally, spot electrodes can be placed in areas less prone to movement artefact and therefore offer better signal-to-noise ratio than recordings made using band electrodes. As a result of these considerations, various spot electrode arrays have been compared with band electrodes. In my study, I have used an optimal spot electrode array (Figure 18) which produces estimates of SV that compare as well with reference method estimates as those obtained using band electrodes ($r = .9$) and is better tolerated by young children.
2.7.2.1 Accurate Height Measurement on the Surface of the Body

For the reasons just discussed, it is prudent to standardise the volume of thorax defined by the voltage sensing electrodes to be a fixed proportion of each subject. In my study, I used 17% of subject height measured down from the base of the neck. In practice, this results in a lower voltage electrode level very close to the xiphisternum level which has also been recommended as a landmark for placement of the lower voltage electrodes. Thus, scaled by the subject size, the same section of thorax was recorded in each case. Additionally, the distance between the current and voltage electrodes was scaled in proportion to the subject. Thus, the distance between the lower current and voltage electrodes was set at two thirds of the distance between the neck and head electrodes (Figure 18). In order to achieve this, it is necessary to place electrodes with a high level of accuracy. This was achieved using a device which I invented and which is pictured and described in Figure 20. This is a laser device which uses a projected laser point to measure the height of electrode positions on the curved surface of the body. Figure 21 shows the device in operation. With careful observation of the requirements of a level floor and of a subject held in the standard posture for accurate height measurement, I found that this device could repeatedly measure the vertical distance between the thoracic electrodes with an error of less than 1% whereas straight measurements using a tape measure were not possible due to the position of the arms and the curved surface of the body.
Figure 20. A laser device for determining the height of points on the curved surface of a standing subject. This consists of a drip-stand modified such that when sited on a level surface (checked with a spirit level), the vertical pole is precisely 90 degrees to the horizontal plane. On this pole, a horizontal arm travels and can be fixed in place with a screw. This arm was precisely 90 degrees to the pole. Thus, the arm travels parallel to the floor at all times. At the end of the arm, a modified laser-pointer is mounted and adjusted with a fixable universal joint to be precisely level. This was checked with a spirit level and by moving the stand to a series of positions on the floor whilst confirming that the beam cast by the laser pointer remained at a fixed height.
Figure 21. Demonstration of the laser-levelling device in use. In practice, the device was used on a level hard floor (laid with self-levelling compound). The subject's height was measured whilst they stood on a stadiometer having been positioned appropriately for height measurement with their head in the Frankfurt plane and their neck supported by an assistant. With the subject still in this position, the height of the electrodes which were fixed according to anatomical landmarks was determined by casting the laser point on the landmarks and then onto the ruler of the stadiometer. A computer calculated the positions of the other electrodes and these positions were 'read off' the stadiometer before the beam was cast onto the skin and marked with a skin marker.
2.8 The Pilot Study

The study of children presented in thesis was novel both in design and execution. Although most of the protocols and technologies had been used separately in the past, they had not been combined in the same way before and there was no data on how children might respond to the TSST-C in terms of the cardiovascular variables which I had chosen to study and only a very small amount of data on HPAA responsivity in this age group. Additionally, at the time of study design, there was no data in adults or children relating measures of prenatal growth to stress responsivity. Therefore, I carried out a pilot study in 20 healthy children (9 boys and 12 girls) recruited from a local school. No perinatal data was available for these children. Because the protocol for the pilot study was essentially identical to the protocol presented elsewhere in this thesis, I will not repeat it here. The principal differences were the age range of the children (7 – 10 years) which was less tightly bounded than in the main study and the use of paper data recording and manual timing. For the main study, I designed a computer program running on a laptop using Microsoft Access forms and computer timing to ensure accurate and direct data entry.

The pilot study allowed the technologies to be refined and the complex protocol to be accomplished smoothly before the onset of the main study. The pilot study confirmed that the approach was valid with the children demonstrating consistent haemodynamic and endocrine responses to the TSST-C of a similar magnitude to those later seen in the main study. This allowed an estimate of an appropriate sample size for the main study to be made.
2.8.1 Power Calculations

Despite the limitations of using birth weight as a marker of prenatal adversity, it remains a useful marker of prenatal growth and is often predictive of future health. Therefore, power calculations were made for linear regression models with birth weight as the single predictor and either salivary cortisol response or blood pressure response as the outcome measure. Although other variables were intended as outcome measures in the final study, blood pressure response was used as an integrative measure of the underlying autonomic, cardiac and vascular variables for the purpose of power calculation. The standard deviation for birth weight of term babies in the cohort was 0.52 kg (N = 507).

I obtained regional health authority information for the original cohort. Of 507 subjects who were last seen at nine months of age, 374 (74%) were still living within the regional health authority area. Therefore I based power calculations on a range of possible recruitment levels within this total. In the pilot study, the TSST-C provoked a mean SAP response of 29 mmHg (SD 8 mmHg) and a mean cortisol response (measured from clinic baseline to clinic peak levels) of 7.2 nmol/l (SD 6.1 nmol/l). Table 3 shows power calculations based on these estimates of effect size. Given these values, recruitment at any level approaching 40% or above was deemed satisfactory.
Table 3. Power calculations at the 90% level for 40, 60 & 80% recruitment rates from a possible total of 374 children.

<table>
<thead>
<tr>
<th>TSST-C response variable</th>
<th>Sample Size (subjects)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 (40%)</td>
<td>225 (60%)</td>
</tr>
<tr>
<td>Salivary cortisol (nmol / l)</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Systolic arterial pressure (mmHg)</td>
<td>4.0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Values are beta coefficients that could be detected with 90% power in a linear regression model at the .05 significance level with birth weight in kilograms as the predictor, having a standard deviation of 0.52 kg, and the outcome variables expressed as increments between levels at rest and those during stress.
Chapter 3.  **CARDIOVASCULAR CONTROL IN ADULTS.**

As described in detail in Chapter 1, there is now substantial epidemiological evidence that small size at birth is associated with raised blood pressure, coronary heart disease and related conditions. Animal and human studies suggest that the enhanced biological responsiveness to stressors seen in individuals with reduced fetal growth provides an important mechanism to explain their subsequent development of disease. In a recent study, Ward et al. demonstrated that low birth weight is associated with enhanced blood pressure and HR responses to psychological stressors in women but not men. Given emerging evidence that blood pressure reactivity to psychological stressors predicts later hypertension, this provides a potential explanation, in women, for previously described associations between reduced fetal growth and raised blood pressure in later life. This chapter describes further work that I have done with the data from Ward et al.'s original study to examine autonomic cardiovascular control in relation to size at birth in adults. The aim, therefore, is to refine the findings of the previous study to better understand which modes of cardiovascular control are moderated by prenatal conditions, leading to the previously observed blood pressure and HR associations with size at birth.

Enhanced cardiovascular reactivity to a psychological stressor, such as the Stroop colour-word conflict task, is predominantly mediated by increased sympathoadrenal activity. As discussed in section 1.4.1.3, there is evidence that the early environment is linked with altered SNS function in later life. In 1997, an association was described between size at birth and resting pulse rate, a crude index related to sympathetic activity. This finding is supported by a number of studies using more specific measures of sympathetic function. In a study of 114 adolescent twin pairs, PEP shortening (a
well-known marker of cardiac sympathetic stimulation) was shown to account for 63% – 83% of the association between birth weight and blood pressure. Although human studies are often limited to general indicators of prenatal adversity such as size of the offspring at birth, animal studies implicate a number of processes which may account for the findings in human studies. Manipulations of the maternal environment such as exposure to stress, reduced environmental temperature or low protein diet, and the fetal environment such as hypoxia or surgical induction of placental insufficiency produce evidence of increased SNS function at rest and during stress and alterations in target organ innervation in the adult offspring. However, the picture of associations between prenatal insults and later SNS function emerging from animal studies is not without its apparent contradictions which may be accounted for by variations in the species or sex, type of insult, timing or duration of insult, or methodological approach used. Thus, further clinical studies which account for factors such as sex are required to establish the impact that programming of SNS function might have on human health.

In this study, I have used spectrum analysis to derive indices of sympathetic and parasympathetic HR and blood pressure control. The actions of these limbs of the ANS on the heart and vasculature are partially separable in the frequency domain, with distinct low and high frequency bands associated with HR and blood pressure variability. Estimates of spectral power in these bands yield indices relating to autonomic cardiovascular control. I assessed baroreflex function using a model which quantifies the strength with which spontaneous variations in blood pressure influence subsequent variations in HR.
3.1 Methods

For this study, 103 men and 76 women with a mean age of 26.3 (SD 0.4) years, drawn from a larger cohort of adults,\textsuperscript{335} underwent a set of three psychological stress tasks engaging the subjects in different cognitive challenges. The first stressor was the Stroop colour-word conflict task, the second was a mirror-tracing task and the third was a speech task simulating a defence against an accusation of shoplifting. Throughout each session, a continuous recording of finger arterial pressure was made using the Portapres device (see section 2.5.1). An index of socioeconomic disadvantage (IRSD) at birth, based on parents' postcode, and information about their health, socioeconomic status, smoking and alcohol consumption, and mood (Centre for Epidemiologic Studies (CES) Depression Scale) was determined for each subject as previously described.\textsuperscript{336} The study was approved by the ethics committee of the Women's and Children's Hospital, Adelaide and each subject gave written informed consent.

3.1.1 Signal Processing

The blood pressure data (sampled at 100 Hz) was analysed to determine HP and the beat-by-beat time series of SAP. Artefacts and aberrant beats were excluded at this stage (see section 2.4.1). To facilitate processing in the next stages, the data were converted to a regularly sampled series using linear interpolation at 1 Hz for wavelet analysis and at 4 Hz for analysis of baroreflex function.

Power spectrum analysis of HP variability was used to assess ANS activity. Over short time periods, HP variability contains a distinct low frequency component (LF) and a high frequency component (HF). Although their peak frequencies vary, these components are usually defined by dividing the signal into bands of 0.04 – 0.15 Hz and
0.15 – 0.4 Hz, respectively. The precise origin and interpretation of these oscillations remains somewhat controversial. However, the power in the HF band corresponds to RSA and is thought to originate from respiratory-centre gating of cardiac vagus nerve activity, whereas power in the LF band is thought to be determined by both SNS and PNS activity (see section 2.6). Hence, the ratio (LF:HF) is often used as a potentially better index of SNS activity than LF alone. Although LF variability in HP may indicate SNS activity, recent evidence suggests that the origin of this variability is in CNS pacemakers which influence vascular tone via SNS efferents and, therefore, LF variability of SAP may be a more direct indicator of this activity.

### 3.1.1.1 Wavelet Analysis of Heart Period and Systolic Arterial Pressure Variability

It is well-known that the frequency content of the HP and SAP signals is not stationary, and so techniques such as the Fourier Transform (FT) are not ideal. Instead techniques such as WTs, which are able to characterise the time-varying frequencies that occur during the experimental period, are more suited. Recent comparisons of FT and WT analysis of HRV support this. For example, in a study of young men given escalating doses of atropine, both FT and WT showed similar profiles of ANS activity prior to and in response to atropine. However, statistical analysis of the FT data did not show a significant effect of atropine on the ANS whilst analysis of the WT data did. I used an extension of the discrete WT known as the wavelet packet transform (WPT) because it has better frequency resolution across all frequency bands.
Figliola et al. provide a detailed treatment of the use of WT on physiological data. Whereas the FT decomposes a signal into a set of sine and cosine functions, the WT decomposes the signal into a set of wavelets which are functions well localised in both time and frequency. WT begins with a wavelet which has the same width as the signal ('ψ', the mother wavelet). Scaling and translation of this wavelet along the time axis generates a family of daughter wavelets which are correlated with the signal (e.g. the HP) to yield time-localised information about its frequency content. There are many wavelet functions which differ in the manner in which they decompose a signal in the time and frequency domains. I used the Daubechies 4 mother wavelet, which has been previously validated for use with HP variability. At a 1 Hz sample rate, the third scaling level of the WPT tree yields eight frequency bands of equal width (0.0625 Hz) from 0 – 0.5 Hz. The summed powers in the 0.06 – 0.18 Hz and 0.18 – 0.44 Hz bands were used for low-frequency (LF) and high-frequency (HF) variability analysis of the signals.

3.1.1.2 Adaptive Autoregressive Modelling of Baroreflex Function

As discussed in detail in section 2.6.3, I used a bivariate adaptive AR model to estimate baroreflex sensitivity as the gain of the transfer function (a) between the SAP and HP signals in the LF band. This approach was chosen as it models the causal relationship between SAP and HP via the baroreflex as well as the effect that HP has on SAP (via CO), in the closed-loop control system. Unlike most alternative methods, the approach explicitly assumes a cause-and-effect relationship between variables and thus allows limbs of the closed-loop to be examined individually.
3.1.2 Statistical Methods

For all parameters, average values during the five minute rest and stress task periods were obtained. Stress-induced increments in parameters were calculated with respect to the rest period preceding the stress tasks. Parameters with a skewed distribution were log-transformed prior to parametric testing. Results in Table 4 are expressed as geometric means and geometric standard deviations. BMI, phase of menstrual cycle, investigator and resting HR are associated with cardiovascular responses to stress. Therefore, I adjusted for these potential confounders using multiple linear regression. As subjects were measured on multiple occasions, analysis was carried out using a repeated measures approach (generalized least squares random-effects linear regression). This gives an improved estimate, for example, of the relationship between size at birth and overall stress response to the three tasks by accounting for differences between the tasks as a cofactor in the model. Parameters were transformed to their z-score, providing normalized regression coefficients analogous to correlation coefficients.

3.2 Results

Table 4 shows SAP, HR and derived parameters at rest and during the stress tasks. Both sexes show marked increases of SAP and HR during stress compared to rest. Low frequency variability of both heart period (HP) and systolic arterial pressure (SAP), indicators of sympathetic activation, both declined during the Stroop and Mirror tasks but rose above resting levels during the Speech task. High frequency heart period variability (HPf), an indicator of parasympathetic activation, declined with respect to rest across all tasks. The ratio of low to high frequency heart period variability (HP) is often used as an indicator of sympathovagal balance with higher
values representing a shift towards sympathetic activation. Both sexes showed a significant shift towards sympathetic activation during the Speech task with respect to rest but men (Table 4a) also showed a significant shift in the opposite direction during the Mirror task. In both sexes, $a$ was significantly lower during the three stress tasks than at rest, representing diminished baroreflex cardiovascular control as HR and blood pressure increased.

There were no significant relationships between birth weight and the cardiovascular parameters in men (Table 5a). However, in women (Table 5b), low birth weight was associated with greater SAP during the stress tasks and greater stress-induced increases in SAP and HR with respect to resting levels. Both sympathetic activation, indicated by $\text{SAP}_m$ and $\text{HP}_{\text{norm}}$, and parasympathetic withdrawal, indicated by $\text{HP}_{\text{nr}}$, were greater in low birth weight women. Furthermore, low birth weight women demonstrated reduced baroreflex sensitivity ($a$) during the Stroop and Speech tasks, suggesting decreased autoregulation of blood pressure during stress. As evidence for increased sympathetic activation was also present at rest in the low birth weight women, this may represent an underlying characteristic that explains their exaggerated HR and blood pressure responses. The three key findings of increased sympathetic activation ($\text{SAP}_m$), greater parasympathetic withdrawal ($\text{HP}_{\text{nr}}$) and decreased baroreflex control ($a$) during stress in the low birth weight women are illustrated in Figure 22 (lower panel). The absence of similar associations between these parameters and birth weight in the men is illustrated in the upper panel of this figure.
Table 4. Geometric mean (geometric SD) of cardiovascular parameters in adults at rest and during three stress tasks.

(a) Men (N = 103)

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Stroop</th>
<th>Mirror</th>
<th>Speech</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mmHg)</td>
<td>122.4 (14.7)</td>
<td>142.8*** (17.8)</td>
<td>150.2*** (18.0)</td>
<td>163.4*** (18.7)</td>
</tr>
<tr>
<td>SAP$_v$ (mmHg$^2$)</td>
<td>8.3 (1.78)</td>
<td>5.9*** (1.91)</td>
<td>6.2*** (1.66)</td>
<td>16.1*** (1.63)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>69.8 (1.17)</td>
<td>77.0*** (1.18)</td>
<td>76.3*** (1.17)</td>
<td>83.7*** (1.19)</td>
</tr>
<tr>
<td>HP$_v$ (ms$^2$)</td>
<td>1265.1 (2.06)</td>
<td>690.8*** (2.38)</td>
<td>672.6*** (1.96)</td>
<td>1353.8 (2.15)</td>
</tr>
<tr>
<td>HP$_f$ (ms$^2$)</td>
<td>399.6 (2.55)</td>
<td>233.3*** (2.54)</td>
<td>240.0*** (2.41)</td>
<td>290.7*** (2.76)</td>
</tr>
<tr>
<td>HP$_{ratio}$</td>
<td>3.91 (2.08)</td>
<td>3.72 (2.00)</td>
<td>3.46* (2.01)</td>
<td>6.26*** (1.64)</td>
</tr>
<tr>
<td>$a$ (ms.mmHg$^{-1}$)</td>
<td>8.79 (1.60)</td>
<td>6.99*** (1.62)</td>
<td>7.49** (1.64)</td>
<td>7.36*** (1.58)</td>
</tr>
</tbody>
</table>

(b) Women (N = 76)

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Stroop</th>
<th>Mirror</th>
<th>Speech</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mmHg)</td>
<td>122.9 (15.2)</td>
<td>139.0*** (17.1)</td>
<td>144.9*** (18.4)</td>
<td>158.6*** (20.4)</td>
</tr>
<tr>
<td>SAP$_v$ (mmHg$^2$)</td>
<td>5.4 (1.69)</td>
<td>3.7*** (1.84)</td>
<td>4.2** (1.88)</td>
<td>11.7*** (1.59)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>73.8 (1.15)</td>
<td>81.9*** (1.16)</td>
<td>79.7*** (1.16)</td>
<td>88.2*** (1.16)</td>
</tr>
<tr>
<td>HP$_v$ (ms$^2$)</td>
<td>865.1 (2.17)</td>
<td>441.3*** (2.88)</td>
<td>496.1*** (2.19)</td>
<td>972.3 (2.39)</td>
</tr>
<tr>
<td>HP$_f$ (ms$^2$)</td>
<td>499.6 (2.92)</td>
<td>230.5*** (3.38)</td>
<td>301.3*** (3.11)</td>
<td>263.1*** (2.81)</td>
</tr>
<tr>
<td>HP$_{ratio}$</td>
<td>2.21 (1.83)</td>
<td>2.38 (1.98)</td>
<td>2.07 (2.04)</td>
<td>5.08*** (1.59)</td>
</tr>
<tr>
<td>$a$ (ms.mmHg$^{-1}$)</td>
<td>9.96 (1.98)</td>
<td>7.23*** (1.82)</td>
<td>8.07** (1.99)</td>
<td>7.41*** (1.89)</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; $^\dagger$, Arithmetic mean (SD); SAP$_v$, low frequency systolic arterial pressure variability; HR, heart rate; HP$_v$, low frequency heart period (interval between adjacent heart beats) variability; HP$_f$, high frequency heart period variability; HP$_{ratio}$, ratio of low to high frequency heart period variability; $a$, baroreflex sensitivity. $P$-values refer to paired t-test comparisons between parameters during a stress task and at rest. *$P<.05$. **$P<.01$. ***$P<.001$. 

Table 5. Normalized regression coefficients relating birth weight to cardiovascular parameters in adults at rest and during three stress tasks.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Stroop</th>
<th>Mirror</th>
<th>Speech</th>
<th>Rest</th>
<th>Stroop</th>
<th>Mirror</th>
<th>Speech</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>-.05</td>
<td>.03</td>
<td>.05</td>
<td>.05</td>
<td>-.16</td>
<td>-.33**</td>
<td>-.29*</td>
<td>-.35**</td>
</tr>
<tr>
<td>( SAP_{inc} )</td>
<td>.10</td>
<td>.12</td>
<td>.11</td>
<td></td>
<td>-.32**</td>
<td>-.29*</td>
<td>-.34**</td>
<td></td>
</tr>
<tr>
<td>( SAP_{lf} )</td>
<td>-.06</td>
<td>-.02</td>
<td>.04</td>
<td>-.06</td>
<td>-.28*</td>
<td>-.41***</td>
<td>-.34**</td>
<td>-.28*</td>
</tr>
<tr>
<td>HR</td>
<td>-.00</td>
<td>.05</td>
<td>.03</td>
<td>-.01</td>
<td>.05</td>
<td>-.13</td>
<td>-.08</td>
<td>-.07</td>
</tr>
<tr>
<td>( HR_{inc} )</td>
<td>.13</td>
<td>.08</td>
<td>-.03</td>
<td></td>
<td>-.31*</td>
<td>-.25*</td>
<td>-.17</td>
<td></td>
</tr>
<tr>
<td>( HP_{lf} )</td>
<td>.01</td>
<td>-.08</td>
<td>-.09</td>
<td>-.02</td>
<td>-.02</td>
<td>.01</td>
<td>-.04</td>
<td>.05</td>
</tr>
<tr>
<td>( HP_{lf} )</td>
<td>-.09</td>
<td>-.16</td>
<td>-.13</td>
<td>-.01</td>
<td>.15</td>
<td>.28*</td>
<td>.14</td>
<td>.28*</td>
</tr>
<tr>
<td>( HP_{ratio} )</td>
<td>.09</td>
<td>.06</td>
<td>.04</td>
<td>-.00</td>
<td>-.16</td>
<td>-.37**</td>
<td>-.21</td>
<td>-.31*</td>
</tr>
<tr>
<td>( a )</td>
<td>-.00</td>
<td>-.07</td>
<td>-.16</td>
<td>-.02</td>
<td>.12</td>
<td>.43***</td>
<td>.10</td>
<td>.31*</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; \( SAP_{inc} \), change in systolic arterial pressure between rest and stress; \( SAP_{lf} \), low frequency systolic arterial pressure variability; HR, heart rate; \( HR_{inc} \), change in heart rate between rest and stress; \( HP_{lf} \), low frequency heart period (interval between adjacent heart beats) variability; \( HP_{hf} \), high frequency heart period variability; \( HP_{ratio} \), ratio of low to high frequency heart period variability; \( a \), baroreflex sensitivity. *\( P < .05 \). **\( P < .01 \). ***\( P < .001 \).
Figure 22. Key cardiovascular parameters in men (N = 100) and women (N = 68) experiencing stress, grouped by birth weight. Values represent z-scored repeated measures estimates of stress response across the three stress tasks. *P-values* refer to the trend.
Of 76 women in the study, 68 had provided adequate information to calculate the phase of their menstrual cycle. Thirty-seven women were in the follicular phase and 31 were in the luteal phase. Further analysis of the data showed that statistically significant relationships between birth weight and all of the cardiovascular parameters were confined to women in the luteal phase of their menstrual cycle. In subgroup analysis, this finding remained significant regardless of hormonal contraception use. However, formal testing of the interaction between birth weight and menstrual phase showed that this reached statistical significance \( P = .04 \) for blood pressure but not for the other variables.

Table 6. Normalized regression coefficients relating the size at birth and gestational age of women (\( N = 68 \)) to their cardiovascular responses to stressors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Birth Weight</th>
<th>Length</th>
<th>Head Circumference</th>
<th>Gestational Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>-.42**</td>
<td>-.29*</td>
<td>-.37**</td>
<td>-.11</td>
</tr>
<tr>
<td>SAP_{inc}</td>
<td>-.37**</td>
<td>-.33**</td>
<td>-.31**</td>
<td>-.13</td>
</tr>
<tr>
<td>SAP_{ff}</td>
<td>-.41***</td>
<td>-.30*</td>
<td>-.29**</td>
<td>-.20</td>
</tr>
<tr>
<td>HR</td>
<td>-.16*</td>
<td>-.14</td>
<td>-.13*</td>
<td>-.04</td>
</tr>
<tr>
<td>HR_{inc}</td>
<td>-.31*</td>
<td>-.20</td>
<td>-.17</td>
<td>-.09</td>
</tr>
<tr>
<td>HP_s</td>
<td>.00</td>
<td>.00</td>
<td>-.04</td>
<td>.06</td>
</tr>
<tr>
<td>HP_{lf}</td>
<td>.22*</td>
<td>.18</td>
<td>.14</td>
<td>.11</td>
</tr>
<tr>
<td>HP_{ratio}</td>
<td>-.34***</td>
<td>-.26*</td>
<td>-.28**</td>
<td>-.05</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>.34**</td>
<td>.25</td>
<td>.23*</td>
<td>.15</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; SAP_{inc}, change in systolic arterial pressure between rest and stress; SAP_{ff}, low frequency systolic arterial pressure variability; HR, heart rate; HR_{inc}, change in heart rate between rest and stress; HP_{lf}, low frequency heart period (interval between adjacent heart beats) variability; HP_{hf}, high frequency heart period variability; HP_{ratio}, ratio of low to high frequency heart period variability; \( \alpha \), baroreflex sensitivity. Coefficients represent repeated measures estimates of stress response across the three stress tasks. *\( P < .05 \). **\( P < .01 \). ***\( P < .001 \).
To elucidate the pattern of fetal growth that predicts these cardiovascular responses in women, I examined the associations between other neonatal measurements and cardiovascular function during stress. Table 6 shows relationships between size at birth and estimates of the combined effect of the three stress tasks on cardiovascular parameters. These were calculated using a repeated measures approach. Gestational age was not found to be a significant predictor of cardiovascular outcomes. The effects of low birth weight were paralleled by short body length and small head circumference. In further multiple regression analysis, I allowed for the confounding factors previously adjusted for in Table 5 (BMI, investigator, phase of menstrual cycle, resting HR) together with other potential confounding factors (smoking status, oral contraceptive pill use, IRSD, CES depression score and educational achievement). The findings presented in Table 6 remained largely unaltered in this further analysis. Relationships between birth weight, for example, and SAP ($r = .37, P < .05$), SAP increment from rest to stress ($r = .28, P < .05$), SAP$_i$ ($r = .48, P < .001$), HP$_{ratio}$ ($r = .41, P < .001$), and $a$ ($r = .28, P < .05$) during stress were generally strengthened whilst relationships between birth weight and HR ($r = .09, n.s.$), HR increment from rest to stress ($r = .21, n.s.$), and HP$_{hf}$ ($r = .19, n.s.$) were somewhat weakened.

### 3.3 Discussion

In this study, I have shown that the associations between small size at birth and increased HR and SAP responses to psychological stressors in women may have their origins in autonomic cardiovascular control and baroreflex function. The major findings (Figure 22) were that those with low birth weight showed increased measures of sympathetic activation both at rest and during stress (as evidenced by SAP$_i$ in Table 5b & Table 6), reduced parasympathetic activity (reduced HP$_{ratio}$ and reduced baroreflex...
These findings were strongly statistically significant and in multiple regression analysis were independent of potential confounders such as obesity, smoking, socioeconomic status and depression. The similar associations found with other measures of neonatal size and the absence of significant relationships with gestational age suggest that these associations are due to growth restriction rather than untimely birth. In contrast, no significant relationships between size at birth and these cardiovascular parameters were found in the men (Table 5a).

These findings add to the previously published human evidence showing that indices of sympathetic function, including resting pulse rate,413 PEP,270 and MSNA 271 are associated with fetal growth. This is supported by evidence from animal studies showing that, in models of placental restriction in sheep422 and rats,261 the developing SNS is modified such that the adult animals display different SNS function during stress. Indeed, in the study of rats, this association between birth weight and SNS function was limited to females, supporting the sex differences found in my study. Interestingly, many models of the effects of fetal growth restriction on the physiology of blood pressure control show sex differences which are currently unexplained. A striking finding in my study was that the association between birth weight and cardiovascular control in the women was confined to the luteal phase of the menstrual cycle. Several studies43 suggest that hormonal responses to stressors are increased in the luteal as compared with the follicular phase. It is therefore of great interest that some recent animal studies64 showed that menstrual phase influences the effects of early growth restriction on hormonal responses to stressors.
A novel finding in my study was that measures of birth size were associated with altered baroreflex sensitivity in women. This is the first evidence of such a relationship in humans and is supported by a recent report from a study in sheep. Evidence is now emerging that the baroreflex may play a vital role in the long-term regulation of autonomic cardiovascular control. Lifelong effects on baroreflex function resulting from fetal growth restriction might explain some of the associations found between size at birth and adult SNS and PNS function.

As with previous studies using similar techniques, both men and women showed a significant increase in HR and SAP in response to stressors (Table 4). Furthermore, the underlying changes in autonomic and baroreflex parameters were similar for men and women. However, although strong relationships between these parameters and size at birth were present in women, this was not the case for the men. Men and women are known to differ in their autonomic cardiovascular control mechanisms. Women demonstrate a relatively reduced sympathoadrenal response to stressors, as indicated by lower MSNA during orthostatic stress and reduced levels of circulating noradrenaline and adrenaline during stress compared to men. Animal studies support this with evidence that regulation of the SNS in females differs from that in males at several levels. Central control of sympathoadrenal function is different in females. Responses to stressors vary with phase of menstrual cycle and pathways regulating adrenal release of adrenaline are less sensitive to excitatory stimuli and more sensitive to inhibitory stimuli than they are in males.
Despite reports of sex differences in arterial baroreflex sensitivity in humans and animals, no clear consensus has emerged as to the strength or direction of the relationship between sex and baroreflex sensitivity. Some studies have failed to find a difference in baroreflex sensitivity between sexes. Observations that female pro-oestrous rats exhibit greater baroreflex sensitivity while oestrous or dioestrus rats exhibit lower baroreflex sensitivity when compared to males may go some way to explaining the conflicting findings of other studies which do not control for phase of menstrual cycle in the females. Noradrenergic neurotransmission also differs between sexes. Vascular responses to direct adrenergic nerve stimulation in rats is greater in males than females and this sex difference in vascular reactivity is abolished by ovariectomy. In humans, women also demonstrate a greater plasma adrenaline clearance than men after an exogenous dose of adrenaline. In general, it appears that females are better able than males to limit SNS activation and enhance SNS inhibition and that this difference is dependent on phase of menstrual cycle. The observation that the sex difference in cardiovascular responses to stressors is less marked or disappears in older women, likely to be post-menopausal, supports the idea that sex hormones play an important regulatory role governing autonomic function during stress in women. Therefore, one possible explanation of my findings is that growth restriction impairs these protective mechanisms in women resulting in SNS function during stress that is similar to that in men and therefore, increased blood pressure and risk of hypertension.
The men and women who took part in this study were a random sample of healthy individuals recruited from an established cohort. The sample was stratified to ensure that a representative group was studied. A potential limitation of this study is the use of the Portapres to derive HP. A small-scale comparison of finger plethysmography with ECG showed a high correlation between these methods at rest, a minimal reduction in correlation during stress for LF power analysis but a modest reduction for HF power analysis. The Portapres outputs pulse waveforms sampled at 100 Hz and beat times are estimated by identifying the foot of the pulse onset. As the pulse foot is less well defined in time than features of the ECG, this data is therefore less accurate, particularly in the HF band. Therefore, associations between measures of size at birth and spectral parameters are likely to be underestimated in my study, particularly with respect to the HF band. However, the indices of cardiovascular function (Table 4) are consistent with reported values in previous studies of men and women in this age group.

Although both the Stroop and mirror tasks are recognised stimulators of sympathoadrenal function, an unexpected finding was that LF variability of both heart period ($H_P$) and systolic arterial pressure ($S_A_P$), both indicators of sympathetic function, declined during these tasks. It is possible that the youth and relative fitness of the adults who took part in this study may account for this. Young, fitness-trained adults have been shown to demonstrate greater reductions in parasympathetic tone and lower increments in sympathetic tone than their untrained peers in response to mild mental stress. Therefore, it is possible that the two milder stressors in this study (Stroop and mirror) produced this pattern of autonomic response in the majority of the subjects which, given that LF power is affected by both parasympathetic and sympathetic activity, resulted in a reduction of LF power during these tasks. In contrast,
the more stressful speech task, which caused greater blood pressure and HR responses, may have stimulated a more dominant response of the SNS and an overall increase in LF power. Finally, some care should be taken in drawing comparison between the values for LF and HF power in my study with those of other studies. The WPT technique which I used separates these two bands at 0.18 Hz rather than the more typical 0.15 Hz leading to potential differences in the quantification of power in these bands. However, I found that the higher cut-off still fell close to the minimum power between the two spectral peaks and therefore was unlikely to have a significant impact on estimates of power in the two bands.

In summary, this study shows strong relationships between impaired fetal growth and autonomic cardiovascular control, which are restricted to women. There is evidence of modulation of sympathetic, parasympathetic and baroreflex function (Figure 22). Because these findings appear to be affected by the menstrual cycle, it is likely that interactions between fetal growth and adult gonadal hormone secretion mediate these effects.
Chapter 4. **ADRENOCORTICAL FUNCTION IN CHILDREN**

As discussed in detail in Chapter 1, low birth weight is associated with the metabolic syndrome and cardiovascular disease in adult life. These associations are thought to result from adverse prenatal environmental influences which reduce fetal growth and induce thrifty developmental responses in the fetus. These responses may be beneficial if the offspring continue to live in adverse environments but are inappropriate and predispose to disease if the postnatal environment is one of nutritional excess. Animal studies demonstrate that changes in the set-point of several hormonal systems, particularly the HPAA, may have an important role in developmental adaptation to harsh environments. These changes may be induced prenatally by nutrient restriction, maternal adversity and glucocorticoid exposure or postnatally, by neonatal handling, maternal deprivation, and infection, for example.

In humans, although lower birth weight is linked to increased fasting cortisol concentrations, it does not appear to relate to cortisol secretion in the unstressed state. It has been suggested that induction of psychological stress by venesection for fasting cortisol may account for this disparity. Indeed, as discussed in Chapter 2, the anticipatory stress of venesection provokes a rapid increase in circulating cortisol. Therefore, I have examined the relationship between birth weight and HPAA stress responsivity. As discussed in section 2.1, reliable induction of HPAA responses to psychological stimuli requires motivation of the subject to perform well in a task with elements of uncontrollability and social evaluative threat which occurs when an important aspect of self-identity is, or could be, negatively judged by others. Ensuring such factors in adults may be difficult due to the confounding influences of expectation and prior experience. Consequently, I studied children using the TSST-C (see section
2.1.1) and increased motivation by offering toys as a potential reward for high performance. As they were unaccompanied by their parents during the TSST-C, uncontrollability was also increased. It is increasingly apparent that many of these factors alter HPAA activity in a sex-specific manner. Therefore, I studied both sexes separately.

4.1 Methods

I recruited healthy children (68 boys and 72 girls, aged 7 – 9 years – see Appendix B for recruitment letters) who have been followed since 12 weeks of gestation when their mothers took part in a study of children born in Southampton, UK. In that study, the mothers were visited at home by trained research nurses in late gestation (median of 32.7 weeks) and asked about their menstrual history and smoking habits during pregnancy. Their height and triceps skin fold thickness were measured with standard techniques and their first recorded pregnancy weight was used to calculate their BMI. A food frequency questionnaire was administered which assessed the average frequency of consumption of 100 foods or food groups in the three months preceding the visit. The nutrient content of a standard portion of each food was multiplied by its frequency of consumption to calculate average daily nutrient intakes. At birth, the children's weight, length, head circumference and placental weight were measured with standard techniques and duration of gestation was estimated from menstrual history and ultrasound scan data using a standard algorithm. During a follow-up visit to the children's homes in late infancy (median age 11 months), their weight was measured again. Table 7 shows the birth and current characteristics of the subjects by sex.
Table 7. Birth and current characteristics of the 8-year-old participants (median and interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Boys (N = 68)</th>
<th>Girls (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>281 (12)</td>
<td>281 (16)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.62 (0.47)</td>
<td>3.41 (0.66)</td>
</tr>
<tr>
<td>Placental weight (kg)</td>
<td>0.57 (0.13)</td>
<td>0.55 (0.16)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50.8 (3.0)</td>
<td>49.4 (2.5)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>35.7 (1.7)</td>
<td>34.8 (1.9)</td>
</tr>
<tr>
<td>Ponderal index (kg.m⁻³)</td>
<td>27.0 (2.9)</td>
<td>28.2 (3.2)</td>
</tr>
</tbody>
</table>

| **Current characteristics** |              |               |
| Age (years)                | 8.9 (0.5)    | 8.8 (0.7)     |
| Weight (kg)                | 28.9 (8.1)   | 29.5 (5.9)    |
| Height (cm)                | 134.4 (9.2)  | 131.4 (7.1)   |
| BMI (kg.m⁻²)               | 16.1 (2.3)   | 17.2 (3.0)    |
| Body fat by DXA (%)        | 19.2 (7.4)   | 27.6 (8.9)    |

To assess their baseline adrenocortical function, the children were asked to use a home-testing kit to collect salivary cortisol at five time points (see Appendix B) – on awakening, 30 minutes later, 12.30pm (prior to lunch), 3.30pm and 6.30pm (prior to evening meal) – on a restful day (usually a weekend or holiday) when the children were taking no part in activities. On a different day, the children attended a clinical research facility for the TSST-C (see Figure 23 for timing and duration of the protocol) which was timed to occur in the afternoon (1.30 – 2.30pm) when diurnal secretion of cortisol is levelling out. Lunch times were arranged to be at least 1½ hours prior to the test to avoid postprandial effects on cortisol. During their visit, a dual energy X-ray absorptiometry (DXA) scan was performed to measure body composition. Parents
were asked to change their appointment if stressful events or illness occurred in their family in the preceding week. Parents and children gave written informed consent (see Appendix C).

Figure 23. Timeline showing median times and durations of the pre- and post-stress periods, and the Trier Social Stress Test for Children. During the former, the children were asked to rest whilst watching a calming video whilst the latter involved a storytelling task and a serial subtraction task. All four periods lasted five minutes with a one minute break between stress tasks for explanation of the maths task.

The children were asked to stand in front of a video camera and microphone and perform an exciting story of their own invention followed by a serial subtraction task for an audience of three adult strangers. They had five minutes to prepare prior to the stress test which lasted for ten minutes. The original TSST-C protocol was modified to reduce task difficulty appropriately for the younger age group in my study and motivation was increased by offering toys as potential rewards for high performance. Saliva samples were collected at seven time points during their visit (on arrival, one hour later, just prior to the TSST-C, and then at ten minute intervals following the stress test). Cortisol concentrations in the saliva samples were determined as described in section 2.2 using a DELFIA assay. The Local Research Ethics Committee (LREC) approved the study (see Appendix A).
4.1.1 Statistical Methods

Baseline values corresponding to the period of the clinic visit were calculated by linear interpolation of the home data using time of awakening as a common reference point for the home and clinic series (Figure 24, main panels). The difference between the area under the clinic curve and the area under the equivalent time period of the home curve was used to estimate the stress-induced change in cortisol concentration. Because this measure might be biased by variations in the interval between first and last salivary sampling, I divided area under the curve by this interval to yield time-weighted means. As season and time of day affect HPAA function, I adjusted for their confounding influence. Associations with birth weight were also adjusted for gestational age to examine the effects of prenatal growth on the outcome measures and not of untimely birth. Postnatal growth was assessed at 11 months and 8 years of age. In the former case, growth was assessed as the residual weight that was not predicted by birth weight in a linear regression model. Post-infancy growth from 11 months to 8 years of age was assessed as the residual weight that was not predicted by weight at 11 months and birth weight in a multiple linear regression model. By construction these postnatal growth variables were independent of birth weight and, in the case of post-infancy growth, of the growth at 11 months variable. Therefore, multiple regression models which included postnatal growth variables also included the appropriate foregoing measures of growth. Skewed data were log-transformed. Differences in cortisol between sexes or between pre- and post-stress measures were assessed using \( t \)-tests. All other analyses were performed using multiple linear regression and results are presented as normalised regression coefficients, analogous to correlation coefficients.
4.2 Results

Figure 24 shows comparisons between cortisol profiles at home with those obtained during the clinic visit in both boys (upper main panel) and girls (lower main panel). Home profiles did not differ significantly between sexes with the exception of the awakening response which was evident in boys but not girls ($P = .04$ for difference in increment between awakening and 30 minutes later). During the clinic visit, cortisol profiles were similar in boys and girls until the post-stress measures which were greater in the girls ($P < .05$ for last three samples). In both sexes, cortisol had risen in anticipation of the stress test ($P < .001$ for comparison of second and third samples). In girls, the further rise was significant ($P < .001$ for comparisons of third sample with all following samples) whilst boys showed no further increment in cortisol.

Table 8. Normalised regression coefficients showing associations between birth weight and salivary cortisol measures during a restful day at home and during a clinic visit for a stress study in boys and girls.

<table>
<thead>
<tr>
<th></th>
<th>Morning Peak</th>
<th>Evening Nadir</th>
<th>Time-Weighted Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinic†</td>
</tr>
<tr>
<td>Boys</td>
<td>.20</td>
<td>.37*</td>
<td>-.16</td>
</tr>
<tr>
<td>Girls</td>
<td>-.36*</td>
<td>-.05</td>
<td>.35</td>
</tr>
</tbody>
</table>

Morning peak and evening nadir cortisol measures describe salivary cortisol concentrations at 30 minutes post-awakening and at 6.30 pm, respectively. Time-weighted mean values were derived by area-under-the-curve analysis and the 'clinic – home' measure is used as an indicator of adrenocortical stress responsivity. †Time-weighted mean values for the clinic visit were calculated using the cortisol concentration taken one hour after arrival as a baseline and are given for comparison with values where the home baseline was used instead ('clinic – home'). ‡The home baseline measure was calculated as the time-weighted mean cortisol concentration for the period of time at home corresponding to the time span of subjects’ clinic visits. This was calculated using linear interpolation of the home cortisol profiles and is given here to show that ‘clinic – home’ responsivity did not depend on significant associations with home baseline levels. *$P < .05$. **$P < .01$. ***$P < .001$. 160
Table 8 shows the birth weight relationships with home and clinic cortisol measurements. In boys, I found a strong inverse relationship between birth weight adjusted for gestational age and HPAA stress responsivity (Figure 24, *upper inset panel*) when home cortisol profiles were used as a baseline but not when pre-stress clinic levels were used. An elevated cortisol response was also associated with lower placental weight ($r = -0.28, P < 0.05$), smaller head circumference ($r = -0.46, P < 0.01$), lower length ($r = -0.36, P < 0.05$) and lower ponderal index ($r = -0.26, P < 0.05$). In boys, size at birth was not related to the home baseline cortisol values or to morning peak cortisol levels. Evening nadir levels (6.30 pm) were positively associated with birth weight in boys but not with other measures of size at birth (placental weight, $r = 0.23$; head circumference, $r = 0.20$; length, $r = 0.23$; ponderal index, $r = 0.19$, all n.s.).

By contrast, size at birth in girls was not associated with HPAA responsivity (Figure 24, *lower inset panel*; placental weight, $r = -0.02$; head circumference, $r = 0.06$; length, $r = 0.24$; ponderal index, $r = -0.04$, all n.s.) or evening nadir levels (placental weight, $r = -0.02$; head circumference, $r = 0.15$; length, $r = -0.03$; ponderal index, $r = -0.01$, all n.s.). However, morning peak cortisol was inversely associated with birth weight in girls but not with other measures of size at birth (placental weight, $r = -0.09$; head circumference, $r = -0.06$; length, $r = -0.15$; ponderal index, $r = -0.19$, all n.s.). On additional multiple regression analysis, all of these findings were found to be independent of social class, educational achievements and markers of obesity such as BMI and percentage body fat derived by DXA.
Figure 24. Geometric mean (± SEM) salivary cortisol profiles during a restful day (dashed line) and a clinic visit (solid line) for the Trier Social Stress Test for Children (red vertical bar) in boys (upper panel) and girls (lower panel). The inset graphs show the relationship between time-weighted mean cortisol responses (comparing home and clinic visit cortisol concentrations) and birth weight adjusted for gestational age. Linear regression and 95% CI lines are shown.
In view of my findings with regard to markers of prenatal growth and given the availability of data from the birth cohort on maternal body composition, smoking habits and diet during pregnancy and on postnatal weight, I carried out a preliminary analysis of the influence of these factors on the primary outcome measures in my study. Table 9 shows the results of this analysis. None of these factors predicted cortisol responsivity to stress and neither maternal body composition nor postnatal growth were associated with morning peak cortisol. However, maternal smoking during pregnancy was positively associated with morning peak cortisol for girls and there was a trend in the same direction for boys ($P < .1$). A similar analysis combining both sexes and adjusting for sex showed a correlation of $0.26$ ($P = .004$) which improved to $0.29$ ($P = .003$) when further adjustment was made for current smoking status of the parents. Given the association in girls between morning peak cortisol and birth weight, I examined the possibility that maternal smoking might account for this. In this additional regression model for girls, birth weight, in fact, predicted morning peak cortisol more strongly ($r = -.39, P = .004$) and smoking was an independent and significant predictor of morning peak cortisol ($r = .32, P = .01$). In further multiple regression analysis, these associations were independent of maternal social class and education.

Finally, there were inverse associations between morning peak cortisol in the boys and all of the maternal dietary measures. This achieved significance for folate and calorie consumption and was strongest for fat consumption. In further multiple regression analysis, these associations were independent of maternal social class, maternal education and smoking habits and of birth weight. There was no association between maternal diet and cortisol measures in the girls.
Table 9. Normalised regression coefficients showing how salivary cortisol measures in children relate to their postnatal weight gain and their mothers' body composition, smoking history, and diet in pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>Boys (N = 68)</th>
<th>Girls (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning cortisol</td>
<td>Clinic – Home</td>
</tr>
<tr>
<td><strong>Maternal Characteristics in Pregnancy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>.08</td>
<td>.09</td>
</tr>
<tr>
<td>BMI</td>
<td>.16</td>
<td>-.18</td>
</tr>
<tr>
<td>TSF</td>
<td>.11</td>
<td>-.13</td>
</tr>
<tr>
<td>Smoking</td>
<td>.24</td>
<td>-.01</td>
</tr>
<tr>
<td><strong>Maternal Diet in Late Gestation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kilocalories</td>
<td>-.29*</td>
<td>.07</td>
</tr>
<tr>
<td>Fat</td>
<td>-.36**</td>
<td>.02</td>
</tr>
<tr>
<td>Protein</td>
<td>-.23</td>
<td>.08</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-.20</td>
<td>.07</td>
</tr>
<tr>
<td>Folate</td>
<td>-.31*</td>
<td>.05</td>
</tr>
<tr>
<td><strong>Postnatal Weight Gain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 11 months</td>
<td>.02</td>
<td>-.08</td>
</tr>
<tr>
<td>1 – 8 years</td>
<td>.09</td>
<td>.21</td>
</tr>
</tbody>
</table>

TSF, Triceps skin fold thickness. Smoking was assessed as a binary variable which was positive if the mother smoked at any time during pregnancy. Maternal diet was assessed by food frequency questionnaire at 33 weeks of gestation (median time). *P < .05. **P < .01.
4.3 Discussion

These data suggest that the effects of the prenatal environment on the HPAA are significant and lasting but sex-specific. In boys, but not girls, lower birth weight or other indices of fetal growth restriction, such as thinness at birth, is associated with greater HPAA stress responsivity and, as this is seen with adjustment for gestational age, this reflects the effect of growth restriction rather than untimely birth. Less expected were the associations between birth weight and morning peak cortisol in girls, and evening nadir cortisol in boys. However, they require confirmation as they were only found with birth weight and not with other markers of size at birth and they are at variance with studies of adults\textsuperscript{448,449} which found no such associations. In animals, females appear to be generally more susceptible to programming of HPAA stress responsivity which also stands in contrast to my findings. However, to my knowledge, there have been no previous studies examining the role of sex in programming of HPAA stress responsivity in humans which may differ between species.

I also found sex differences in the pattern of both home and clinic cortisol profiles. In comparison with girls, boys had a clear post-awakening rise of cortisol, similar anticipation of the stress task and did not show a significant further increment of cortisol following stress (Figure 24, main panels). Whilst there is still much debate about the biological meaning of morning peak cortisol, several authors have suggested that this acts to prepare individuals for expected metabolic and psychosocial demands of the day.\textsuperscript{459} One interpretation of these findings is that, in this age group, the HPAA plays a greater preparatory role in boys and a more reactive role in girls but this requires confirmation in future studies.
There are also some notable differences between the clinic cortisol profiles seen in my study and those from previous studies\textsuperscript{31a,60-62} of similar age groups. Post-stress peaks appear slightly less marked than those in previously published figures (although there are no previously published profiles split by sex). However, in these preceding publications, cortisol profiles have been plotted as mean values rather than median or log transformed values (Figure 24 shows geometric mean values). Given the well-known and marked right-skew that is generally seen in salivary cortisol data, this form of presentation may well have been inappropriate and likely to have been misleading as a small number of subjects with high values would have had the most influence on the mean cortisol profiles. It is also worth noting that there is very little published data on the TSST-C in young children of a similar age to those in my study. Indeed, the number of subjects in my study approaches an order of magnitude greater than any previously studied group and may be more representative as a result. Therefore, it is difficult to draw strong conclusions from comparisons with the previous studies. To my knowledge, only one other research group (Trier, Germany) has published data on the TSST-C in 8-year-old children. The other notable difference between my data and theirs is the marked anticipatory effect seen in my data which is absent in theirs. It is possible that this represents a difference in approach to the study. It was not considered ethical by my peers in the UK to confront the children with the TSST-C without forewarning them that they would be taking part in a challenge and without giving them some idea of the nature of that challenge (storytelling and mental arithmetic). Descriptions of the protocol used in Trier suggest that the children in Germany were much less aware of the nature of the TSST-C until they were asked to perform it and this may account for the absence of any anticipatory effect seen in their data.
An important decision in the design of this study was to use measures of cortisol at home as a baseline for comparison with measures during stress. Previous stress studies have generally used pre-stress cortisol measures from the clinic setting as a baseline. However, there is increasing evidence that HPAA function is altered well in advance of arrival for such studies by the anticipated threat of the visit itself. For example, a study of 9-year-old girls showed that laboratory baseline cortisol measures were 40% greater than measures at home and several animal studies show that relocation alone is a stimulus for increased HPAA activity. This may explain why the association between birth weight and HPAA responsivity was stronger in my study than in the only previously reported study of programming of HPAA responsivity which was carried out in adult male twins. As birth weight did not relate significantly to either time-weighted mean home or clinic cortisol concentrations (Table 8), it is likely that my result represents an association between birth weight and stress responsivity (clinic – home) rather than differences in underlying basal cortisol production.

It has been noted that studies of pre-school children where home baselines were obtained almost universally report significantly lower cortisol levels during the clinic visit than at home whilst in adults and older children, the opposite is generally true. A proportion of high birth weight boys appear to have higher home cortisol levels than those measured during the stress visit (Figure 24, upper inset panel) which is similar to the pattern observed in pre-school children. Although little is know about HPAA maturation, this similarity raises the possibility that an inverse association between rate of HPAA maturation and birth weight might explain my findings in the boys.
In a further analysis, I examined the possibility that the associations between size at birth and measures of HPAA function in childhood might be explained by maternal factors such as body composition, smoking, or diet during pregnancy (Table 9). This analysis revealed two significant findings which are, to my knowledge, entirely novel. Namely, that maternal smoking during pregnancy was found to predict a higher morning peak cortisol in the children regardless of their sex and that increased maternal dietary consumption of macronutrients and folate during late gestation was found to predict a lower morning peak cortisol in the children. In both cases, these findings were independent of measures of size at birth and therefore do not further understanding of the primary findings of this study other than through exclusion of possible explanations. Although these findings are potentially of great interest and are robust to potential confounders such as maternal social class and education, they represent a preliminary and post-hoc analysis that does not directly address the primary hypothesis of this thesis which is focused on prenatal growth. In view of this, a more in-depth analysis of these findings is required, together with confirmation from similar studies, before prudent discussion of their implications is possible.

This study suggests that processes occurring during fetal life, resulting in smaller newborns, have a lasting effect on adrenocortical responses to stress in boys and on basal adrenocortical activity in girls. Given the known associations between small alterations in adrenocortical activity and features of the metabolic syndrome such as raised blood pressure and glucose intolerance, these effects warrant further investigation of their potential impact on the future health of pre-pubertal children.
Chapter 5. CARDIOVASCULAR FUNCTION IN CHILDREN

As described in Chapter 1, emerging evidence supports the concept that adaptations of the ANS may occur during fetal life which predispose individuals to a greater risk of developing cardiovascular disease. It exhibits marked plasticity during fetal adversity, and size at birth has been linked to physiological indices of autonomic function in adulthood. These indices include resting HR, blood pressure and HR responses to psychological stressors, and measurements of PEP.

Most previous studies have addressed the relationship between size at birth and cardiovascular function only at rest and have relied upon integrative measures such as blood pressure or HR, which reflect the influence of the ANS on both the heart and the vasculature. To improve our understanding of these influences requires more detailed measures of cardiovascular physiology, such as CO, SVR and measures of autonomic cardiovascular control, such as PEP. Furthermore, although cardiovascular function at rest may be informative, stress significantly alters autonomic and baroreflex cardiovascular control both in the steady state and during minor cardiovascular challenges such as standing. Thus, studies of the cardiovascular system during stress may reveal between-subject differences in cardiovascular control that are not apparent at rest.

Studies of cardiovascular function during stress may also have greater relevance to risk of future cardiovascular disease. A number of large-scale studies now show that blood pressure responses to stress predict later development of hypertension with the strongest findings in studies of younger subjects. However, family history of hypertension, measures of chronic stress exposure such as negative affect and job
strain,\textsuperscript{189} and the buffering effect of social networks all modulate this association.\textsuperscript{189} This suggests that the cardiovascular control mechanisms which participate in the stress response of an individual are affected by both life course experiences and parental cardiovascular status; the latter reflecting genetic influences or non-genomic mechanisms, such as the influence of maternal hypertension on fetal development.

Therefore, studies in children may have an advantage over those in older subjects, as they are less likely to be affected by life-course experiences and the development of overt, non-congenital cardiovascular disease.

There is now considerable evidence from animal studies demonstrating sex-specific programming of the neuroendocrine systems which are activated during stress and which influence cardiovascular function. These systems include the HPAA\textsuperscript{447} and the ANS. In Chapter 3, I have described my findings that small size at birth predicts greater sympathetic activation, parasympathetic withdrawal and reduced baroreflex sensitivity during stress in young adult women but not men, suggesting that sex has an effect on these associations. These findings are supported by animal studies\textsuperscript{291,298} which show stronger associations between an adverse prenatal environment and increased sympathetic activation during stress in females compared to males.

This study was designed to examine the influence of prenatal growth on sympathetic cardiac activation and peripheral vascular function at rest and during psychosocial stress. This was carried out in healthy children and analysed in a sex-specific manner for the reasons described above.
5.1 Methods

I recruited healthy children (68 boys and 72 girls, aged 7 – 9 years – see Appendix B for recruitment letters) who have been followed since 12 weeks of gestation, when their mothers took part in an earlier study of fetal growth in Southampton, UK. In that study, the mothers were visited at home by trained research nurses in late gestation (median of 32.7 weeks) and asked about their menstrual history, smoking habits, family history of hypertension and alcohol use. Their height and triceps skin fold thickness were measured with standard techniques and their first recorded pregnancy weight was used to calculate their BMI. A food frequency questionnaire was administered which assessed the average frequency of consumption of 100 foods or food groups in the three months preceding the visit. The nutrient content of a standard portion of each food was multiplied by its frequency of consumption to calculate average daily nutrient intakes. At birth, the children’s weight, length, head circumference and placental weight were measured with standard techniques and duration of gestation was estimated from menstrual history and ultrasound scan data using a standard algorithm. During a follow-up visit to the children’s homes in late infancy (median age 11 months), their weight was measured again. Mother’s social class (UK National Statistics Socio-Economic Classification) and the children’s educational achievements in their national standardised assessment tests at age 7 were recorded during their follow-up visit. Table 7 shows the birth and current characteristics of the subjects by sex.

The children attended a clinical research facility for the study and underwent DXA scans to measure body composition. Stress tests were carried out at the same time in the afternoon (for timing and duration of tasks, see Figure 23). To assess cardiovascular...
function, an optimal array of nine spot electrodes (Blue Sensor – Ambu Ltd, St. Ives, UK) for impedance cardiography and two spot electrodes for a modified lead II ECG were applied (Figure 18). The thoracic impedance electrodes were sited using a laser levelling device which, together with careful postural adjustment, allowed repeatable and highly accurate positioning (within a few millimetres) in the standing subject (see section 2.7.2). As discussed in section 2.7.1, the distance between the inner set of these electrodes (l) defines the volume of the thorax in which impedance is measured and may therefore have a marked effect on the values obtained. This distance was fixed at 17% of the subject’s height. A blood pressure tonometer was attached over the site of the radial artery of the non-dominant hand (VasoTrac APM205A – MedWave Inc., St. Paul, MN, USA; Figure 12). As described in section 2.5.2, this intermittently applies a cushioned pressure sensor over the site of the radial artery, partially compressing the artery against the head of the radius, allowing blood pressure estimation approximately every 12 – 15 heartbeats (depending on HR). In comparison to measures obtained invasively from the radial artery, this device performs better than oscillometric devices in adults and children with the advantages of being better tolerated, particularly by children, and allowing more frequent measurements. The non-dominant wrist and hand were splinted on their dorsal aspect to minimize motion artefact in the blood pressure recordings and the arm was supported with a sling (Figure 13 & Figure 14). To control for the influence of movement, blood pressure readings were corrected to a reference level (insertion of the deltoit tendon) using a fluid-filled tube and calibrated pressure-sensing device which continuously measured and adjusted for the ‘hand to heart’ differential (see section 2.5.4 & Figure 15). Analogue signals from these electrodes and devices were digitally sampled at 1000 Hz and recorded by a computer-based data acquisition system connected to a series of bioamplifiers (MP150, EBI100C and ECG100C – BIOPAC Systems Inc., Goleta, CA, USA) calibrated and configured
according to manufacturer’s instructions. Impedance measurement was calibrated with a series of high-precision resistors.

As described in section 2.1.1, the children were asked to perform a public speaking task involving storytelling and mental arithmetic for a panel of three unknown adult ‘judges’ in front of a video camera and microphone (TSST-C). Motivation was increased by offering toys as a potential reward for high performance. This type of stressor is known to be a reliable stimulant of both HPAA and autonomic limbs of the stress response. Prior to and following the stressor, the children rested by watching a calming video. The children were standing during all of these tasks. Finally, the children were given a voucher for toys worth £10. The LREC approved the study and both parents and children gave written informed consent (see Appendices A & C).

5.1.1 Signal Processing

Data recorded by the BIOPAC was analysed using my own software. The onset and peak of the QRS complex and the termination of the T-wave in the ECG were reliably identified even in the presence of marked motion artefact as described in section 2.4.1. Intervals between R-waves (Figure 25) were used to calculate HR after artefacts and ectopic beats were identified and removed from each time series by an automated procedure with manual oversight. QT interval was calculated and corrected for HR (cQT) on an individual basis using a least squares fit of a power function. The thoracic impedance data was filtered using an adaptive filter to remove the influence of body movements and respiration on the cardiac signal. Figure 25 shows fiducial points on the impedance cardiogram and their relative timing to the ECG and cardiac cycle. The B-point in this signal was identified automatically using a linear
extrapolation technique, allowing accurate timing of aortic valve opening. The X-point in this signal was identified by searching for maxima in a 100 ms Gaussian-weighted window of data, which spans the T-wave termination time in the ECG, allowing accurate timing of aortic valve closure.

Figure 25. A representative recording of the electrocardiogram (ECG) and first time-derivative of the impedance cardiogram (dZ/dt) from a single study participant for two heart beats. Following convention, impedance is shown such that a decrease in impedance results in a greater y-axis magnitude. The cardiac cycle and the derivation of cardiac time intervals – RR interval, pre-ejection period (PEP) and left ventricular ejection time (LVET) – are indicated. Hollow circles indicate fiducial points on both signals identified automatically by computer. In addition to the well-known PQRST sequence of the ECG, major time points on dZ/dt are marked. These include the B-point which coincides with aortic valve opening, the X-point which coincides with closure of the aortic valve, and the C-point which marks the maximum rate of decline in thoracic impedance which coincides with peak systolic ejection rate.
PEP was calculated as the interval between R-wave onset and the B-point (Figure 25).\textsuperscript{469} Left ventricular ejection time (LVET) was calculated as the interval between the B-point and the X-point (Figure 25) and systolic time ratio (STR) as the ratio between PEP and LVET. SV was estimated using Kubicek's formula:\textsuperscript{382}

\[
SV = \rho_b \cdot \frac{l^2}{Z_0 \cdot LVET} \cdot \left| \frac{dZ}{dt} \right|_{\text{min}}
\]

where \(\rho_b\) is the resistivity of blood (\(\Omega\cdot\text{cm}\)), \(l\) is the height of the thoracic segment measured (cm), \(Z_0\) is the basal thoracic impedance (\(\Omega\)), and \(\left| \frac{dZ}{dt} \right|_{\text{min}}\) (\(\Omega\cdot\text{s}^{-1}\)) is the absolute value of the maximum rate of fall of thoracic impedance during systole (coinciding with the C point; Figure 25). I used a fixed value of 135 \(\Omega\cdot\text{cm}\) for \(\rho_b\) in accordance with current guidelines which suggest that this yields more accurate results than individual correction of \(\rho_b\) for haematocrit.\textsuperscript{399}

CO and SVR were derived from SV as follows:

\[
CO = \frac{SV \cdot HR}{1000} \quad SVR = 80 \cdot \left( \frac{MAP - CVP}{CO} \right)
\]

I simplified the SVR equation to exclude the central venous pressure (CVP) term which is usually close to zero and unvarying.
To control for the potential confounding influence of associations between size at birth and body surface area (BSA), which is known to relate to SVR, I calculated the systemic vascular resistance index (SVRI) for each subject \(\frac{SVR}{BSA}\). BSA was calculated using a method that has been validated in children.\(^{490}\)

### 5.1.2 Statistical Methods

For all parameters, mean values for the duration of each task (both stress tasks and the pre- and post-stress periods) were obtained. Parameters with a skewed distribution were log-transformed prior to parametric testing. As obesity affects autonomic function, analyses were adjusted for current BMI (adjustment for percentage body fat estimated by DXA yielded almost identical results which are not presented). The majority (80%) of the clinical sessions were carried out by the same investigator. A second investigator (also male) performed the remainder and this was allowed for in my analysis. To exclude the possibility that associations between cardiovascular parameters and measures of size at birth were dependent on variations in the duration of gestation, I adjusted for gestational age in the analyses using multiple linear regression. Postnatal growth was assessed at 11 months and 8 years of age. In the former case, growth was assessed as the residual weight that was not predicted by birth weight in a linear regression model. Post-infancy growth from 11 months to 8 years of age was assessed as the residual weight that was not predicted by weight at 11 months and birth weight in a multiple linear regression model. By construction these postnatal growth variables were independent of birth weight and, in the case of post-infancy growth, of the growth at 11 months variable. Therefore, multiple regression models which included postnatal growth variables also included the appropriate foregoing
measures of growth. I transformed parameters to their z-score, providing normalized regression coefficients analogous to correlation coefficients.

5.2 Results

Table 10 shows the median values of the cardiovascular parameters before, during and following stress for boys and girls. In comparison to pre-stress levels, measures of arterial pressure and HR in both sexes increased during stress and declined post-stress. However, SAP did not fully return to pre-stress levels. In girls, HR and diastolic arterial pressure fell to lower levels than those pre-stress. Significant shortening of PEP, indicating increased sympathetic cardiac stimulation, was present during speech in both sexes and during the maths task in girls. LVET decreased significantly during the maths task in both sexes and increased post-stress in the girls. In both sexes, STR, an index inversely related to myocardial contractility, was lower during and post-stress with the exception of the maths task in the boys. In both sexes, SV and CO fell during stress whilst SVR increased. In boys only, these changes persisted post-stress. Finally, in both sexes, significantly shorter cQT was present during stress. This finding is consistent with recent evidence that the ANS, particularly the SNS, modulates QT interval in humans. Thus, increased sympathetic myocardial activation is likely to be responsible for the shortening of cQT observed during stress.
Table 11 outlines the relationships between the cardiovascular parameters and birth weight. In boys, lower birth weight was associated with higher arterial pressure and this association was strongest post-stress, 25 – 30 minutes after the onset of the stress test. The concurrent association between lower birth weight and greater SVR suggests an explanation for this. As this remained significant with SVRI as well, a confounding relationship between birth weight and current BSA is unlikely. Additionally, lower birth weight boys had a longer LVET which was significant pre- and post-stress.
Table 10. Median (interquartile range) of cardiovascular variables prior to, during and following stress.

<table>
<thead>
<tr>
<th></th>
<th>Boys (N = 68)</th>
<th>Girls (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-stress</td>
<td>Speech</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>101.9 (15.7)</td>
<td>104.7 (13.6)***</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>97.3 (13.7)</td>
<td>119.4 (15.9)***</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>72.1 (10.9)</td>
<td>88.4 (12.2)***</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>55.7 (9.0)</td>
<td>70.5 (9.3)***</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>75.7 (10.9)</td>
<td>73.3 (9.6)***</td>
</tr>
<tr>
<td>LVET (ms)</td>
<td>226.7 (26.7)</td>
<td>226.3 (20.3)</td>
</tr>
<tr>
<td>STR (%)</td>
<td>33.4 (5.0)</td>
<td>33.0 (4.5)**</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>75.1 (24.9)</td>
<td>67.1 (24.2)***</td>
</tr>
<tr>
<td>CO (l.min⁻¹)</td>
<td>7.6 (2.3)</td>
<td>6.9 (1.9)***</td>
</tr>
<tr>
<td>SVR (dyne.sec.cm⁻⁵)</td>
<td>713.4 (230.8)</td>
<td>938.1 (216.1)***</td>
</tr>
<tr>
<td>SVRI (dyne.sec.m².cm⁻⁵)</td>
<td>707.6 (229.2)</td>
<td>979.3 (252.1)***</td>
</tr>
<tr>
<td>cQT (ms)</td>
<td>311.2 (29.7)</td>
<td>307.5 (26.4)***</td>
</tr>
</tbody>
</table>

HR, heart rate; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; PEP, pre-ejection period; LVET, left ventricular ejection time; STR, systolic time ratio (PEP : LVET); SV, stroke volume; CO, cardiac output; SVR, systemic vascular resistance; SVRI, systemic vascular resistance index (normalised by body surface area); cQT, corrected QT interval. Subjects were standing during all four periods and watching a restful video pre- & post-stress. P-values refer to paired comparisons with the pre-stress rest period using Wilcoxon matched-pairs signed-ranks tests. *P < .05. **P < .01. ***P < .001.
Table 11. Normalised regression coefficients relating birth weight to cardiovascular variables prior to, during and following stress.

<table>
<thead>
<tr>
<th></th>
<th>Boys (N = 68)</th>
<th>Girls (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-stress</td>
<td>Speech</td>
</tr>
<tr>
<td>HR</td>
<td>.20</td>
<td>-.06</td>
</tr>
<tr>
<td>SAP</td>
<td>-.44*</td>
<td>-.33</td>
</tr>
<tr>
<td>MAP</td>
<td>-.37</td>
<td>-.29</td>
</tr>
<tr>
<td>DAP</td>
<td>-.32</td>
<td>-.27</td>
</tr>
<tr>
<td>PEP</td>
<td>.07</td>
<td>.10</td>
</tr>
<tr>
<td>LVET</td>
<td>-.41*</td>
<td>-.11</td>
</tr>
<tr>
<td>STR</td>
<td>.30</td>
<td>.17</td>
</tr>
<tr>
<td>SV</td>
<td>.00</td>
<td>.09</td>
</tr>
<tr>
<td>CO</td>
<td>.13</td>
<td>.07</td>
</tr>
<tr>
<td>SVR</td>
<td>-.30</td>
<td>-.26</td>
</tr>
<tr>
<td>SVRI</td>
<td>-.26</td>
<td>-.21</td>
</tr>
<tr>
<td>cQT</td>
<td>-.09</td>
<td>-.04</td>
</tr>
</tbody>
</table>

HR, heart rate; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; PEP, pre-ejection period; LVET, left ventricular ejection time; STR, systolic time ratio (PEP : LVET); SV, stroke volume; CO, cardiac output; SVR, systemic vascular resistance; SVRI, systemic vascular resistance index (normalised by body surface area); cQT, corrected QT interval. Subjects were standing during all four periods and watching a restful video pre- & post-stress. *P < .05. **P < .01.
Figure 26. Mean z-scores (SEM) of key cardiovascular parameters grouped by birth weight in boys and girls. HR, heart rate; SAP, systolic arterial pressure; PEP, pre-ejection period; LVET, left ventricular ejection time; SVR, systemic vascular resistance; cQT, corrected QT interval. Data are shown for PEP and cQT during the maths task and during the post-stress period for the other parameters.
Girls showed none of the associations between birth weight and cardiovascular parameters that were seen in boys. However, lower birth weight girls had significantly shorter PEP, and cQT and a lower STR throughout the tasks, suggesting greater levels of sympathetic cardiac stimulation which were manifest regardless of stress. Figure 26 summarizes the key parameters shown in Table 11 for both sexes grouped by birth weight.

Of the other measures of size at birth, length had a very similar pattern of associations with cardiovascular parameters in childhood to those seen with birth weight, whilst placental weight, head circumference and ponderal index (a marker of obesity at birth) did not (data not shown). To address the possibility that other potential confounders, such as family history of hypertension, social class, education and maternal smoking or alcohol consumption during pregnancy might explain my results, I repeated my analyses with these variables included. In no case were any of these parental variables significant predictors of cardiovascular parameters in childhood and relationships between size at birth and later cardiovascular function were not significantly altered by their inclusion. For example, the relationship between birth weight and post-stress SAP in boys increased slightly ($r = -.63, P < .01$) whilst relationships between birth weight and post-stress SVR in boys ($r = -.42, P < .05$) or resting PEP in girls ($r = .51, P < .05$) were reduced slightly.

In view of my findings with regard to markers of prenatal growth and given the availability of data from the birth cohort on maternal body composition, smoking habits and diet during pregnancy, I carried out a preliminary analysis of the influence of these factors on the primary outcome measures in my study. For these, I chose measures of MAP, SVRI, SV and CO as the principal explanatory variables of
haemodynamic change and PEP as the best descriptor of underlying sympathetic drive. As size at birth in the boys related best to MAP and SVRI in the post-stress period and to PEP in girls regardless of which period was used, I chose the post-stress period for this secondary analysis. Table 12 show the results of this analysis. Although maternal body composition was not associated with cardiovascular parameters in the girls, boys born to short, thin (indicated by low triceps skin fold thickness and low BMI) mothers had higher SVR and lower CO in the post-stress period. By contrast, in girls, but not boys, maternal smoking during pregnancy was associated with higher CO with a trend towards lower SVR ($P = .06$). With regard to maternal diet in late gestation, low consumption of protein and folate was associated with increased sympathetic activity (a positive correlation with PEP) in the boys but not the girls. Increased consumption of carbohydrate was associated with greater MAP in the girls but not the boys. Finally, both sexes showed a similar pattern of associations between cardiovascular function and measures of postnatal growth but these were stronger in the boys. Greater weight gain in infancy was associated with higher SV and CO, and lower MAP and SVRI in the post-stress period. Although post-infancy weight gain continued to be associated with higher SV and CO, the associations with MAP and SVRI reversed in direction. Finally post-infancy weight gain was associated with higher PEP (indicating reduced cardiac sympathetic activation) in the boys, but not the girls. In further multiple regression analyses, all of these associations were independent of birth weight, maternal social class and maternal education.
Table 12. Normalised regression coefficients showing how cardiovascular function of children following stress relates to their postnatal weight gain and their mothers' body composition, smoking history, and diet in pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>Boys (N = 68)</th>
<th>Girls (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>SVRI</td>
</tr>
<tr>
<td>Maternal Characteristics in Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-.06</td>
<td>-.16</td>
</tr>
<tr>
<td>BMI</td>
<td>-.16</td>
<td>-.32*</td>
</tr>
<tr>
<td>TSF</td>
<td>-.27</td>
<td>-.42**</td>
</tr>
<tr>
<td>Smoking</td>
<td>-.05</td>
<td>.04</td>
</tr>
<tr>
<td>Maternal Diet in Late Gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kilocalories</td>
<td>.02</td>
<td>-.00</td>
</tr>
<tr>
<td>Fat</td>
<td>.13</td>
<td>.08</td>
</tr>
<tr>
<td>Protein</td>
<td>.02</td>
<td>-.05</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-.05</td>
<td>-.05</td>
</tr>
<tr>
<td>Folate</td>
<td>.04</td>
<td>.03</td>
</tr>
<tr>
<td>Postnatal Weight Gain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 11 months</td>
<td>-.29*</td>
<td>-.35*</td>
</tr>
<tr>
<td>1 – 8 years</td>
<td>.36*</td>
<td>.33</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; SVRI, systemic vascular resistance index (normalised by body surface area); SV, stroke volume; CO, cardiac output; PEP, pre-ejection period. *P < .05. **P < .01. ***P < .001
5.3 Discussion

In this study, I present the first evidence in children of relationships between size at birth and later cardiovascular function. The sex-specific patterns of these relationships were striking. As highlighted in Figure 26 and Table 11, boys who had a lower birth weight demonstrated higher blood pressure and SVR whilst girls who had a lower birth weight had shorter PEP and cQT. As these associations were seen with measures of size at birth adjusted for gestational age, these findings are due to differences in fetal growth and not untimely birth.

I recruited normal healthy children from a community-based cohort study of mothers and their children. The anthropometric measurements and assessment of gestational age at birth (Table 7) were obtained using standardised techniques including the use of ultrasound confirmation of gestational age in early pregnancy. I used an established stress test that has been validated for use in children which recreates a stressful environment similar to the moderate stressors that most adults and children experience in day-to-day living. Unlike many milder stressors, the TSST-C reliably activates the HPAA, which modulates the actions of the SNS. Therefore, in this context, a more complete picture of cardiovascular function during stress can be obtained. Great care was taken to maintain the accuracy of the cardiac parameters, in particular by accurate placement of thoracic impedance electrodes, which could significantly affect estimation of SV and the use of measures to reduce the influence of motion on estimation of blood pressure.
A potential weakness of this study is reliance upon impedance cardiography to derive measures of SV, CO and SVR. Whilst this has been a source of controversy, a meta-analysis of 154 published comparisons between impedance cardiography and reference measures of CO concluded that it is sufficiently accurate, particularly in healthy subjects, for use in research. Indeed, in healthy children, impedance cardiography compares well to the indirect Fick method for assessment of CO and yields repeatable and consistent measures of reactions to a range of mental and physical stressors. Although, my measures of SV and CO were somewhat higher than those from a study of seated children experiencing mild stressors, this may be because the subjects in my study were standing and experiencing greater stress. Furthermore, although they are supported by these impedance-derived measures, my findings are not reliant upon them and impedance cardiography remains an accurate method for timing of events in the cardiac cycle.

Table 10 shows that the TSST-C resulted in marked responses of all the cardiovascular parameters that I measured, in both sexes. Blood pressure increased with a mean increment of 24 mmHg in the girls and 19 mmHg in the boys accompanied by simultaneous increases in HR. Data from the impedance cardiograph shows that SVR also increased, generating the blood pressure increments, whilst SV and CO fell. At the same time, STR decreased, suggesting greater myocardial contractility whilst PEP and cQT fell, indicating that greater sympathetic cardiac stimulation is responsible for this. Unlike previous studies using milder psychological stressors in children, I did not see a dramatic β-adrenergic response to the stress tasks, as indicated by modest changes in HR and PEP. For example, a previous study in pre-pubertal children of the same age showed a 5.6% increase of CO with a modest 3.6% increase of SVR in response to a reaction time task, a 3% increase of CO with an 8.4% increase of SVR in response to a
mirror-tracing task, and a 1.3% fall of CO with an 18.2% increase of SVR in response to a cold pressor test. The pattern of responses to the TSST-C in my study is even more biased towards vascular response with greatest similarity to the cold pressor test from that study, which also showed a fall of CO during stress. Boys showed an 8.8% fall of CO and a 29% increase of SVR, whilst girls showed a 4.1% fall of CO and a 24% increase of SVR. Therefore, my data adds to this emerging story of a high degree of specificity of cardiovascular responses to the nature of the stressor.

This study also adds to growing evidence of major sex differences in the relationships between reduced fetal growth and physiological alterations that may predispose to cardiovascular disease. The pattern of shortened PEP and cQT seen in girls who were smaller at birth is consistent with up-regulation of β-adrenergic sympathetic cardiac activation at rest and during stress. Chapter 3 describes my finding that young women who were small at birth not only had greater sympathetic activation, but also enhanced blood pressure responses, greater parasympathetic withdrawal and reduced baroreflex sensitivity during stress. The common finding of these studies, therefore, is an association in females between small birth size and greater sympathetic cardiac stimulation. However, these studies differed in that birth weight was also associated with blood pressure in the women but not in girls. A possible explanation for this may be that lower birth weight females have enhanced sympathetic activity from a young age but that abnormal baroreflex function leading to exaggerated blood pressure responses to stress takes time to develop. Longitudinal studies of a single cohort into adulthood would be required to establish this but, given the known associations between blood pressure reactivity and hypertension, such sympathetic hyperactivity may be an important mediator of the known associations between small size at birth and hypertension in females.
In contrast to the girls, boys who were smaller at birth had higher arterial pressures, particularly following the stress test. However, like the girls, they did not have significantly different HR, SV or CO. Therefore, the finding of greater SVR in the boys who were smaller at birth suggests that their greater arterial pressures were generated by peripheral vascular constriction rather than increased CO. These patterns suggest that boys who were smaller at birth have a predominantly α-adrenergic, vascular (as opposed to myocardial) response to stress. Given that a predominance of vascular rather than myocardial activity is associated with early hypertension, this suggests a potential mechanism by which they may develop hypertension. There is evidence that a predominantly vascular response to stress is linked with prolongation of the response. This is consistent with my findings in boys (Table 11) which were most marked during the post-stress period. A possible explanation for this may be enhanced adrenocortical activity in the boys. In Chapter 4, I showed that boys, but not girls, who were small at birth had greater cortisol responses to stress coincident with the post-stress period.

An additional finding was that boys who were smaller at birth showed longer LVET. There is evidence from a study of men that LVET is inversely related to arterial stiffness, as measured by pulse-wave velocity. Therefore, one possible explanation of this finding is that arterial stiffness is greater in the boys who were smaller at birth. This is consistent with studies showing that poor arterial distensibility, assessed by the augmentation index, is associated with low birth weight in children and may be one of the ways in which low birth weight leads to hypertension in later life.

In further analysis, I examined the possibility that the associations between size at birth and cardiovascular or autonomic function in childhood might be explained by maternal factors such as body composition, smoking, or diet during pregnancy (Table 12).
Although this analysis revealed a number of interesting associations with prenatal maternal factors, they were independent of measures of size at birth and therefore do not further understanding of the primary findings of this study other than through exclusion of possible explanations. However, they reveal a number of additional potential mechanisms that are worthy of future study. In particular, the observation in boys that those with shorter, thinner mothers were more likely to have higher SVR and lower CO following stress and that this finding was independent of the children’s BSA, suggests that males with this maternal phenotype respond to stress with a predominantly vascular rather than myocardial activation. As discussed above, this pattern suggests that they might be at greater risk of developing hypertension. The finding that maternal smoking during pregnancy is associated with higher CO and a trend towards lower SVR following stress in the girls is difficult to interpret. As the finding is robust to adjustment for potential confounders including current smoking status of the parents, it may represent the effects of maternal smoking on cardiovascular development in utero. However, this finding should be viewed with caution given the binary nature of the variable and the weak associations and therefore requires confirmation in future studies.

Although the associations with maternal diet are also of interest, they should also be interpreted with caution given the potential limitations of recalled food frequency questionnaires for assessment of diet and the post-hoc nature of the analysis. In boys, low maternal protein consumption and folate consumption during pregnancy predicted lower PEP suggesting greater cardiac sympathetic drive post-stress. However, no such associations were present in the girls whilst higher maternal carbohydrate consumption in pregnancy was associated with higher MAP in girls but not boys. Given the sex-specific pattern of these relatively weak associations, confirmation is required from
studies which are better designed and better powered to address the effect of diet in pregnancy on cardiovascular function in boys and girls.

In contrast to the associations seen with maternal characteristics, the associations between cardiovascular function and markers of postnatal growth were stronger and similar in both sexes. Greater weight gain in infancy was associated with higher SV and CO, and lower MAP and SVRI in the post-stress period. In the first year of life, it is likely that this weight gain represents the predominant accumulation of non-adipose tissue (the maximum BMI of the children at this age was 22 which is likely to be normal although there are no reference data for this age). As this would include myocardial growth, it is tempting to speculate that the higher SV and CO may be a result of the development of larger, more powerful hearts which may account for the more beneficial phenotype of a greater reliance on CO and a lesser reliance on SVR to generate the blood pressure response to stress. This idea is supported by the lack of evidence of greater myocardial sympathetic stimulation (PEP) to account for the greater SV. However, such speculation is highly tentative and would require echocardiographic confirmation in future studies.

Of particular interest is that whilst post-infancy weight gain continued to be associated with higher SV and CO, the associations with MAP and SVRI reversed in direction. By the age of 8, nearly 12% of the children had a BMI above the 95\textsuperscript{th} percentile suggesting that post-infancy growth involves the accumulation of both adipose and lean tissue. Therefore, the development of obesity might account for greater SVRI and MAP seen in the boys with greater weight gain over this period. These findings on postnatal growth fit well with existing data. For example, a study of over 300 children aged 10 – 12 years in Kingston, Jamaica showed that both reduced birth weight and increased
weight gain in late childhood predicted higher blood pressure status in later childhood. Furthermore, this effect was greatest in those children whose growth in the first two years of life was poor. Such findings are not limited to risk factors for cardiovascular disease. A study of over 2000 men born in Helsinki, Finland in the decade following 1934 found that poor weight gain in the first year of life was strongly predictive of increased cardiovascular disease in adulthood and that this effect was stronger than, and independent of, an additional effect of birth weight. A subsequent study of both men and women from the same cohort confirmed that poor weight gain in infancy was predictive of cardiovascular disease in adulthood but also that individuals that grew poorly in infancy and then gained weight in later childhood had the greatest risk of cardiovascular disease. Therefore, my findings suggest a possible underlying mechanism for these epidemiological observations related to the relative emphasis of vascular over cardiac reactivity for generation of the blood pressure response to stress. This potential mechanism should be examined in more detail in prospective studies with more detailed postnatal growth data in the future.

In summary, I have shown for the first time that there are marked sex differences in the way that smaller size at birth is associated with alterations in autonomic and cardiovascular physiology in childhood. Although these associations differ between sexes, they suggest potential mechanisms by which smaller size at birth may lead to abnormal cardiovascular function in both sexes. Preliminary data on postnatal growth and maternal characteristics suggest that the developmental programming of cardiovascular function is complex with a multitude of potential mechanisms acting independently at different stages of early life.
Chapter 6. CONCLUSIONS

In this thesis, I have examined the hypothesis that prenatal development, assessed by measures of size at birth, affect later neuroendocrine control of cardiovascular function. This has been accomplished by examination of autonomic, cardiovascular and endocrine measures, not only at rest, but also when these systems are activated. I have presented the data from two clinical studies in which moderately sized groups (100–200 subjects) have undergone a psychologically stressful task or series of tasks. These tasks were designed to replicate the day-to-day stresses that we all experience in our lives and therefore allow greater understanding of how our neuroendocrine systems might behave when challenged. I believe the results of these studies demonstrate the value of careful and detailed study designs which merge the best ingredients of large-scale epidemiological investigations with traditionally small-scale detailed physiological examinations.

I have carried out studies which are unusual in their use of combinations of multiple technologies to examine developmental programming of stress systems by continuous and non-invasive collection of cardiovascular measures during psychological stress. Although many of the technologies that were used have been available for decades, few studies have combined them in the same way and fewer still use these technologies to generate continuous measures. The likely explanation for this is that the large amounts of data that result would exceed the ability of most research teams to process without sophisticated automated computer analysis. Robust automatic computer analysis is not generally available in commercial applications which may only work well with the clean, noiseless data that is rarely seen in ambulatory studies, leaving a residual burden on the user that often negates the utility of computer analysis in the
first place. To address this, I implemented a range of highly advanced signal processing algorithms which are not often used outside of the research laboratories in which they were developed. This dramatically cut down the degree of user intervention required for processing very large quantities of time series data in a highly reliable manner. As a result, the studies presented in this thesis have a significant advantage over many other studies because the continuous data allow more precise estimates (through averaging of multiple sequential measures) of cardiovascular and autonomic variables than would be possible with intermittent measurements.

Reliability is improved too, as peak responses to stress are often variable in their timing relative to the stressor and therefore studies using intermittent measures may be unable to collect appropriately timed measurements for all subjects, leading to sampling errors. For example, in addition to the continuous blood pressure measures that I collected in my study of children’s cardiovascular function (Chapter 5), I also collected single measures of blood pressure at rest and following the TSST-C, using a standard oscillometric device. The overwhelming majority of studies thus far have relied entirely upon such devices to study programming of blood pressure using similar protocols and yet, despite strong associations between measures of fetal growth and the continuous blood pressure measures in my study, there were no associations with the single oscillometric measures (data not shown). Thus, rather than addressing the high level of random variability seen in cardiovascular variables by examining very large populations, studies might address the same questions in smaller groups of subjects through the use of more precise techniques. By combining a range of outcome measures such as salivary cortisol, HR, blood pressure and markers of ANS function, the studies in this thesis also provide a more holistic picture of these intimately enmeshed systems. Indeed, I would suggest that the patterns of associations between
markers of size at birth and these multiple outcome variables have been more revealing, in terms of understanding underlying physiology, than any single result would have been.

There is strong evidence in this thesis that developmental programming of both cardiovascular and endocrine outcomes differs by sex. I carried out a sex-specific analysis because there is a large body of evidence from animal studies which supports the existence of such sex differences. However, I find it surprising that despite this evidence, many studies of developmental programming in animals and humans continue to be carried out in a single sex group or to make no distinction between the sexes. In some animals, there may be practical reasons why one sex or the other is under-studied. For example, where female animals are used for breeding or where male animals are difficult to control. Humans of both sexes are arguably equally amenable to study, however, and the results of this thesis suggest that greater consideration to this approach should be given in the future.

Taken together, the results in this thesis suggest a novel hypothesis with regard to sex-specific programming of hypertension. The findings in the girls and the young women in two different study groups support the idea that reduced fetal growth results in hyperactive SNS function from an early age that continues into adulthood. Whilst the girls did not demonstrate associations with blood pressure, these were present in the young women. In addition, the young women also showed reduced baroreflex sensitivity which resulted in greater blood pressure responses to stress. Such stress responsivity is increasingly being shown to influence risk of development of hypertension. Therefore, I would postulate that in early life, female humans who are small at birth have a hyperactive SNS, particularly during stress and possibly abnormal
baroreflex function as well (although I was not able to examine this in my study of children). As they age, the persistently abnormal SNS function leads to worsening blood pressure control, particularly during stress, perhaps by resetting of baroreflex function which may eventually lose its ability to buffer hypertensive episodes, leading to the eventual development of hypertension in late adulthood. Although the data from two time points and only two studies does not strongly support this hypothesis, it does suggest the need to study programming of autonomic function in a longitudinal and sex-specific manner. Thus, a sex-specific pathway for the developmental programming of hypertension in women is suggested.

In boys, a different pattern of associations was observed. Boys, but not girls, who were small at birth demonstrated greater HPAA responses to stress. This association was strong in comparison to the only similar published study which examined this association in adult male twins. Furthermore, a comparable but unpublished association was found by Ward et al. in the men, but not the women, who took part in the study presented in Chapter 3 but this did not reach traditional levels of significance \((P < .1)\). There are two possible explanations for these differences which suggest themselves:

First, it is possible (and I would argue most likely) that methodological differences account for the stronger findings in my study. In the study of adult twins, the baseline used for comparison with stress was taken at the beginning of the subjects' clinic visits. I have argued in section 2.2.1, that error is likely to be introduced into such measures of baseline HPAA function by the fact that some individuals respond strongly to the anticipation of the visit itself. As I used a baseline derived from salivary cortisol measures taken during a day when the children took part in no activities and rested at
home, it is likely that I was able to show a more consistent difference. Ward et al. obtained some data from subjects at home but response rates were very low and so they too compared measures at rest and during stress in the clinical environment. In the future, I would like to carry out a study in adults similar to my children's study where home baseline data is obtained.

The second possible explanation for my stronger findings is that I have studied pre-pubertal children whilst the other data comes from adults. It is possible that associations between prenatal growth and later HPAA function may be moderated by the activity of gonadal hormones. Additionally, it is possible that children respond in a more stereotypical fashion to the TSST-C than adults do. Whatever the explanation, it appears that HPAA responses to psychological stress may be programmed more strongly by prenatal life in male humans than in female humans. From this, I would draw a second tentative hypothesis regarding sex-specific programming of hypertension. In the study of cardiovascular function during stress in children, I found that small size at birth was associated with greater blood pressure due to increased SVR, particularly 20 minutes following the stress test, in boys but not in girls. This timing coincides with maximal stress-related secretion of cortisol which is a hormone known to accentuate the activity of the SNS. Therefore, I would postulate a separate pathway from small size at birth to hypertension in males which begins early in life and involves exaggerated HPAA responses that enhance the effect of the SNS on the peripheral vasculature during stress. This might lead to more prolonged blood pressure responses and, therefore, to the eventual development of hypertension. In summary, the sex differences presented in this thesis are provocative, if based on sparse data, and suggest that the well-known associations between reduced fetal growth and
hypertension in adulthood may exist due to separate, sex-specific programming mechanisms. Future studies should address this possibility.

Another interesting observation was that measures of cardiovascular or endocrine function in the low birth weight group of one sex were similar to the values of the other sex whilst those measures in the high birth weight group were attenuated. For example, in the study of adults, blood pressure profiles in response to stress were similar in men and low birth weight women whilst higher birth weight women showed attenuated blood pressure responses. In the study of children's HPAA function, cortisol profiles in response to stress were similar in girls and low birth weight boys whilst being relatively lower in the high birth weight boys. These observations may have no significance but they question the traditional interpretation that is given to associations with birth weight in programming studies. In general, the assumption is that reduced fetal growth is detrimental and leads to abnormal physiology which eventually leads to the development of disease. I would postulate that it may be the case that reduced fetal growth represents a situation of adversity that leads the developing fetus to adopt a physiology that is more similar to that of the opposite sex. Thus, it may be the physiology of the higher birth weight group of a given sex which differs from the remainder and this disparity may represent the development of beneficial physiology that is sacrificed in conditions of fetal adversity. Of course, this is speculative but may deserve consideration in future studies.

In section 1.1.4, I have discussed the relative advantages and disadvantages of using size at birth as an indirect measure of the effect of the maternal and fetal environments on fetal growth. Clearly, measures of size at birth are non-specific and non-sensitive markers of a variety of potential influences on fetal growth. Despite this, I would argue
that they remain a useful broad indicator of processes occurring in prenatal life. I have carried out preliminary analyses of additional prenatal measures which were available in my children's study. These produced evidence that both cardiovascular and endocrine outcomes may also be programmed by other factors such as maternal body composition, smoking habits and diet in pregnancy. However, it was particularly interesting that these findings were entirely independent of the associations with size at birth. In section 1.1.4, I discussed a study of sheep where a specific maternal dietary intervention was made in late gestation on the presumption that this would produce both a reduction in birth weight and alterations in outcome measures such as blood pressure and glucose tolerance in the adult offspring. Whilst a reduction in birth weight was observed to result from the dietary intervention, the authors expressed surprise that birth weight, independently of whether the offspring were from ewes who experienced restriction of their diet in pregnancy, was the strongest predictor of outcome measures.

There have been calls to abandon measures of size at birth entirely in studies of programming but the above example and my results argue for caution in this regard. Clearly there are many possible processes which may result in alterations of size at birth and I would suggest that understanding of these processes is not sufficiently advanced to replace size at birth entirely. Until there exists a comprehensive battery of measures that can be carried out throughout pregnancy that explain all of the associations between size at birth and the outcome variables, size at birth should continue to be examined. A picture might then emerge of which factors explain the well-known associations between birth weight and adult disease and which are describing additional processes. In the future, I intend to extend my preliminary analyses of my data to address the influence of maternal diet and body composition on
cardiovascular and endocrine outcomes. However, the size of my study may be a limiting factor and future studies are therefore likely to be needed.

The data on postnatal growth and cardiovascular function in boys was particularly interesting. This supports existing observations that poor weight gain in infant life and excess weight gain following infancy are associated with outcomes such as high blood pressure and cardiovascular disease. Although my postnatal growth data is sparse, my results suggest that differences in SV, CO and SVR may account for these observations. In the future, I would like to take this further by examining specific markers of endothelial function (such as pulse transit time), myocardial function and myocardial growth (using echocardiography or magnetic resonance imaging) in children to see how these differ with differing prenatal and postnatal growth trajectories.

The data presented in this thesis are novel and need confirmation. I have begun two major collaborations with colleagues in Finland and Holland to address this. I am working on data from over 700 adults born during World War II when mothers living in Holland endured a period of starvation. The adult offspring of these mothers took part in a repeat of the protocol described in Chapter 3 and therefore provide an opportunity to confirm the findings of my study in older adults. In Helsinki, colleagues are just beginning a repeat of my children's protocol in a similar age group (approximately 1 year younger) which closely follows the same methodology.
I have demonstrated that quite substantial differences may exist in the stress response physiology of apparently healthy, normal children according to their prenatal growth characteristics. This raises the question of whether this matters in terms of their future health. Existing cohort studies already suggest that exaggerated cardiovascular responses to stress are predictive of poor cardiovascular health in later life. In one of these studies, 'white coat' hypertension was strongly predictive of cardiovascular mortality. However, these studies only followed individuals from young adulthood. There is also evidence from a study of 12-year-old children that 'white coat' hypertension is associated with higher 24-hour urinary cortisol and endothelin concentrations, supporting the idea that children who are hyper-responsive to stress may also be susceptible to the development of hypertension. Interestingly, this finding was limited to boys and not girls which is compatible with my findings.

Ultimately, the data remains too sparse to definitively address the clinical relevance of my findings in children but future long-term cohort studies which follow people from birth to adulthood may do so. If they reveal, as the existing data suggest, that our cardiovascular health is substantively determined by processes occurring in childhood, this could have major implications for public health strategies and the delivery of healthcare. Healthcare providers might seek to optimise maternal and fetal health by providing evidence, for example, on the effects of maternal obesity, smoking and diet during pregnancy. The measures used in my studies of children might become important in clinical trials by helping to identify groups at greater risk of adult disease. The current medical mindset is limited to treatment of existing disease with a general reluctance to intervene in the health of apparently 'normal' individuals. This thesis suggests that cardiovascular disease in adulthood may come about, at least in part,
through the long-term detrimental effects of maladapted stress response systems beginning in early childhood.

The logical conclusion of this and future work may be a shift in the current medical mindset whereby physicians, and in particular, child health professionals seek to identify at risk individuals and perhaps even look to develop pharmacological interventions to reduce their risk. Physiological studies in higher mammals (primates) need to be carried out, together with further epidemiological investigations in humans, before this is a realistic proposition and the potential political and fiscal implications will present a major challenge. Eventually, however, the development of novel investigations and treatments for cardiovascular health in children might lead to a more preventative role for paediatric cardiologists, paediatricians, general practitioners, and other healthcare professionals who look after children.

Should medicine move towards this vision of future healthcare delivery? The growing incidence of cardiovascular disease is one of the primary global health concerns and there is an alarming trend towards onset of its risk factors, such as obesity, smoking, diabetes and high blood pressure even in childhood. It is becoming apparent that a reduction in this global trend towards ill-health may not be possible without a greater emphasis on the health of children. This will require more research in children, an awareness that children's health extends beyond childhood and the willingness to develop investigations and treatments for children. If we do not take these possibilities seriously, we may be doing future generations a great disservice.
REFERENCES


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(28) Benediktsson R, Lindsay RS, Noble J, Seckl JR, and Edwards CR. 


(119) Laghrissi-Thode F, Wagner WR, Pollock BG, Johnson PC, and Finkel MS. **Elevated Platelet Factor 4 and Beta-Thromboglobulin Plasma Levels in Depressed Patients With Ischemic Heart Disease.** *Biological Psychiatry.* 1997; 42: 290-5.


Jonas BS and Lando JF. **Negative Affect As a Prospective Risk Factor for Hypertension.** *Psychosomatic Medicine.* 2000; 62: 188-96.


(290) Altimiras J and Crossley DA, II. **Control of Blood Pressure Mediated by Baroreflex Changes of Heart Rate in the Chicken Embryo (Gallus gallus).** *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 2000; 278: R980-R986.


(336) Birch AA and Morris SL. Do the Finapres and Colin Radial Artery Tonometer Measure the Same Blood Pressure Changes Following Deflation of Thigh Cuffs? Physiological Measurement. 2003; 24: 653-60.


(342) von Haller A. Elementa Physiologicæ Corporis Humani. Lausanne, Switzerland: M. M. Bousquet et al.; 1760.


(461) Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, and Kirschbaum C. 
**HPA Axis Responses to Laboratory Psychosocial Stress in Healthy Elderly Adults, Younger Adults, and Children: Impact of Age and Gender.** 

(462) Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, and Kirschbaum C. 


APPENDIX A – ETHICAL APPROVAL

This appendix contains three letters from the Southampton & South West Hampshire Joint Local Research Ethics Committee. The original letter on the 24th of October, 2000 and the two following letters (which refer to protocol amendments) provide full ethical approval for the study of children that is detailed in this thesis.
Dear Professor Phillips

Submission No: 266/00 - Maternal nutrition, fetal growth and the programming of raised blood pressure in childhood: testing the stress hypothesis.

Following the conditional approval and in response to your letter dated 10th October 2000, I am pleased to confirm full approval having satisfied the Committees concerns on the "child behaviour checklist questionnaire.

This approval was granted by the Chairman Dr Audrey Kermode and will be brought to the attention of the Committee at their meeting on 22nd November 2000.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

Yours sincerely,

Clair Wright (Mrs)
Research Ethics Administrator
Submission No: 266/00: Maternal nutrition, fetal growth and the programming of raised blood pressure in childhood: testing the stress hypothesis

In response to your undated letter, I am pleased to confirm ethical approval for the Protocol amendment.

The following documents were reviewed:

- Amended Application form detailing changes to the plans for taking blood samples
- Amended Application form detailing changes to the timed collections of saliva
- Amended Application form detailing changes to replacing the Mirror-Tracing task and ‘challenging questions’ with the Trier Social Stress Test.
- Amended Application form detailing changes to blood pressure and pulse readings
- Finalised Questionnaire

This approval has been granted under Chairman’s action by the Vice-Chairman Dr Mervyn Griffiths and will be recorded at the Committee meeting in July.

Yours sincerely,

Mrs Clair Wright
Research Ethics Manager
Dear Professor Phillips,

Submission No. 266/00/t – Maternal nutrition, fetal growth and the programming of raised blood pressure in childhood: testing stress hypothesis.

In response to your letter dated 12th May 2003, I am pleased to confirm ethical approval for protocol amendment for the above study.

The following documents were reviewed:

- Letter 12th May 2003
- Correspondence to Jane Doe dated 12th May 2003
- Patient Information Sheet, dated May 2003
- General Health Questionnaire
- MRC Information Booklet (Titled Southampton’s Babies)

This approval has been granted under Chairman’s action by the Vice Chairman Mr Mervyn Griffiths and will be recorded at the committee meeting in June.

Yours sincerely,

Mrs Clair Wright
Research Ethics Manager
APPENDIX B – PARTICIPANT CONTACT

This appendix contains generic versions of the personalised letters which were sent to participants who took part in the study of children that is detailed in this thesis. The first two are the initial letter to the mother and an information sheet for the child. A more detailed information sheet was sent to those who expressed an interest in taking part, together with instructions on how to use an enclosed home saliva testing kit. Prior to our initial contact with potential participants, a letter was sent to their General Practitioner to ensure that our enquiries were appropriate.
Mrs Anne Onymous
0, No Where St.
No Where Land.

Dear Anne,

The Mothers, Children & Healthy Hearts Study

In 1995, you kindly took part in a survey of nutrition in pregnancy at the Princess Anne Hospital. With your help, we collected important information about your pregnancy with William. This gave us a much better understanding about how some foods eaten during pregnancy help to produce a healthy, well-grown baby. We enclose a booklet outlining our findings that we hope you will find interesting.

We are writing to invite you and William to help us with a new study. This is to find out how a mother’s diet during pregnancy influences her child’s responses to everyday tasks in later childhood. As these responses affect health and wellbeing, we hope to identify important new ways to help parents and their children.

If you are able to help us, we will ask you to come to our research centre at Southampton General Hospital. William will be asked to tell a short story and do some maths (similar to school work) for an audience of three people. To measure his body’s response to these tasks, we will check his pulse, blood pressure and hormone levels in his saliva. If you and William agree, we would also like to perform a scan to measure his bone size and strength. Absolutely NO blood tests, needles or painful procedures are involved!

William will receive a £10 Toys’R’Us voucher, a ‘picture’ of his skeleton if he has the bone scan, an educational workbook about the study and a toy as thanks for taking part. A free parking space will be provided and we will be happy to refund your travel costs.

If you think you might be able to help with the study and would like to find out more about it, please send us your completed reply card. Tracey Tudball, our research nurse, will phone you to describe the study and send a full information leaflet. Fifty children have now taken part and have enjoyed the project – some of their comments are printed overleaf.

Thank you very much for your help with this important research – it is most appreciated.

Yours Sincerely,

Professor David Phillips
Senior Clinical Scientist

Dr. Alex Jones
Research Paediatrician

Mrs. Tracey Tudball
Research Nurse

Local Research Ethics Committee Number: 266/90
These are comments from mothers and children who took part in our pilot study. There were no negative comments and the names of the children have been removed to protect their identity.

I really liked the playroom!

The team is excellent! They are helpful, kind, thoughtful and well organised.

It was an interesting insight into medical research and they had our child's best interests at heart.

It was a big adventure!

Our child felt very special. The whole day was very child-centred.

It was fun!

We were relaxed and made to feel at ease.

It was brilliant!
We are doctors and nurses at the hospital where you were born.

We are interested in how children grow and what makes them healthy. You and your mum helped us before when you were a baby.

We would like you to visit us at the hospital even though you are well. We would like you to tell a story and do some sums. We will do some tests that tell us how your body works. These tests do not hurt!

No you don’t. You can choose. You may want to talk to Mum or Dad about it.
Parents’ Information Sheet

The Mothers, Children & Healthy Hearts Study

Thank you for your interest in this study. This information sheet is intended to give you more information about why this research is being carried out and what taking part will involve.

What are the purposes of the study?

As we mentioned in our previous letter to you, we are interested in how the diet and health of mothers influence the growth and health of their children. Your participation in the study in 1995 helped us to understand what influences a child’s growth up until nine months of age. Now William is 8 years old, we would like to know more about how he has grown, what his health is like and how he responds to different tasks.

We feel that research into the health and development of children is important because it helps us to understand how people stay healthy, both as children and on into adult life.

What will we have to do if we take part?

If you have not already heard from us, we will contact you soon by telephone to explain the study in detail and answer any questions or concerns that you may have about taking part.

This will take up some time for you and William, so we would like to offer a £10 Toys‘R’Us voucher to show our gratitude.

You should also have received some clear plastic containers and instructions for collecting some saliva (spit) from William at home. This may seem like a strange thing to ask you to do but it will provide useful information. Saliva contains hormones that indicate how a person’s body is reacting to the world around them. The level of these hormones rises when a person meets a stranger or visits a new place. If we were there to show you how to collect the saliva, our presence would affect the result of the test. Therefore, we would like you and William to do this on your own. Please see the separate instruction sheet for details of how to do this.

You will be invited to attend the Wellcome Trust Clinical Research Facility at Southampton General Hospital (C Level, West Wing). If she has not already done so, Tracey, our research nurse, will phone you to arrange a time that is convenient for you.

If William is not well or something stressful has happened to him or your family in the week running up to your visit, please call and rearrange your visit for another week. If you are uncertain whether an event is likely to have affected William, please call and discuss it with us. Please call either Dr. Alex Jones on 023 8076 4097 or Tracey Tudball on 023 8076 4055.

Some skin creams, inhalers and medicines can cause problems with our saliva tests. Therefore, we would ask that you bring all medicines that William is using (particularly nasal sprays, inhalers and skin creams) with you to the clinic visit so that we may check their ingredients.
When you arrive, we will ask you some questions about you and William, his health, personality and major events that may have occurred in his life. On several occasions during his visit, we will check his temperature and ask him to provide a saliva sample. Meanwhile, the nurse will measure William. We will check his height, weight and how much fat is on his arms and body. We will attach some sticky pads to his skin to make a ‘heart tracing’ (you may know it as an ECG) and measure the sweatiness of his skin. He will then be asked to do some restful tasks such as watching a children’s video. We will also provide a workbook with some fun puzzles, drawing and colouring-in pages that relate to the study. He will then be given the beginning of a story and will be asked to spend five minutes thinking up an ending for this story. He will be asked to tell his story for a small audience of three people and do some simple mental arithmetic. To make this more exciting, there will be a microphone and video camera in the room but any recordings taken will be kept confidential and only accessible by researchers at the MRC. The ‘heart tracing’ and levels of hormones in his saliva will tell us how he has responded to this task. At the end of this task, he will get a toy of his choosing as a reward.

The nurse will ask William to blow into a tube to measure his breathing. Finally, if you agree, we will ask William to have a scan that will measure the density of his bones and the amount of fat on his body. Many parents and children find the pictures that the scanner takes interesting. If you and William would like a picture of his skeleton to take home, we will be happy to print one for you.

All the information that we collect will be kept in the strictest confidence.

**Are these tests safe?**

Yes. The bone scan involves William lying on a couch with a scanning arm moving two feet above him. The scan does not cause any pain and takes about six minutes to perform. It involves exposure to a tiny amount of X-rays (the equivalent of one day’s natural background X-rays).

The bone scan is widely used to measure bone density in people of all ages, including babies and children.

**Will it hurt?**

None of the procedures are painful. There will be NO blood tests or needles.

**Do we have to take part?**

No, taking part is voluntary. You do not have to give a reason for not taking part and if you agree to take part, you can change your mind at any time.

**What do we do now?**

Please read the instructions for collecting saliva from William at home. If you have not already been contacted, Tracey Tudball, our research nurse, will be contacting you soon by telephone to go through things with you.

Thank you very much for taking the time to read about this study.

We hope you will enjoy taking part and look forward to seeing you soon.

---

Professor David Phillips  
Senior Clinical Scientist

Dr. Alex Jones  
Research Paediatrician

Mrs. Tracey Tudball  
Research Nurse
Instructions for Taking Saliva Samples

**PLEASE NOTE:**
- William should NOT eat or drink anything for **one hour** before taking a sample.
- William should NOT eat or drink anything with **caffeine** in it (such as tea, coffee or cola drinks such as Coke and Pepsi) on the day when you are taking samples.
- If either of you have any questions, please call Alex on 023 80777624 / 07866 573685 or Tracey on 023 80764055. We are used to "strange questions" about this & will be happy to answer yours.

Here's what to do:

**Step 1.** Pick a day when William will be spending a quiet day at home at the weekend or on holiday. We would like you to collect five saliva samples on this day. The rest of this sheet explains how and when we would like you to do this.

**Step 2.** Check the time. This sheet (overleaf) has five coloured bars for each time of the day that you should take a sample. There are five containers in the package with matching coloured labels. There is also a spare container, gum and straw.

**Step 3.** Chew gum. William should chew one piece of gum until his mouth is watering then push the gum into his cheek with his tongue but NOT swallow his saliva. He may need to chew the gum several times to get enough saliva.

**Step 4.** Fill container. Choose the container with the coloured label that matches the coloured bar on the other side of this sheet. William should release his saliva down the straw into the container. Hold it firmly, as it can slip. Try to fill at least half of the container.

**Step 5.** Close container. Press the cap FIRMLY onto the container until it SNAPS shut.

**Step 6.** Fill in the sheet and the label. Record the time when you took the sample on the coloured bar on the other side of this sheet and the matching label.

**Step 7.** Return the samples. When all the samples are collected, please post them to us. They can be kept in their envelope in the fridge until they are all collected. The padded envelope has been addressed and stamped already.

**What to do if you can’t do this or something goes wrong:**

1. **If the timing isn’t spot on, the sample is still useful:** If the sample is up to an hour early or late that’s fine. Even if it’s more than that we will still get useful information from analysing it. All that matters is that you record the time that you took the sample accurately overleaf.

2. **If you miss a sample completely:** If you can, please try and collect any missing samples on a different day. If you do this, please remember to record the date as well as the time for that sample overleaf.

3. **Call us.** Please give us a call if you have any questions. We will be happy to help.

**FINALLY, Don’t forget to claim your reward for this when you visit us at the hospital!**
"WAKE UP" SAMPLE (AS CLOSE TO THE MOMENT OF WAKING UP AS POSSIBLE)

TIME WHEN YOU WOKE UP: _____________________________
DATE WHEN SAMPLE TAKEN: _____________________________
TIME WHEN SAMPLE TAKEN: _____________________________

30 MINUTES AFTER WAKING UP (BEFORE BRUSHING TEETH AND BEFORE BREAKFAST)

DATE WHEN SAMPLE TAKEN: _____________________________
TIME WHEN SAMPLE TAKEN: _____________________________

TWELVE THIRTY (12.30 PM) IN THE AFTERNOON (BEFORE LUNCH)

DATE WHEN SAMPLE TAKEN: _____________________________
TIME WHEN SAMPLE TAKEN: _____________________________

THREE THIRTY (3.30 PM) IN THE AFTERNOON (AT LEAST ONE HOUR AFTER LAST SNACK)

DATE WHEN SAMPLE TAKEN: _____________________________
TIME WHEN SAMPLE TAKEN: _____________________________

SIX THIRTY (6.30 PM) IN THE EVENING (JUST BEFORE OR ONE HOUR AFTER EVENING MEAL)

DATE WHEN SAMPLE TAKEN: _____________________________
TIME WHEN SAMPLE TAKEN: _____________________________
Dear Dr. xxxx

Re: The Mothers, Children & Healthy Hearts Study

The MRC Unit in Southampton has been studying the relationship between impaired fetal growth and high blood pressure in childhood and later life. The charity “Birthright” funded a prospective study of babies born in Southampton between 1994 and 1996. When these babies were neonates we carried out very careful measurements of their body size. In addition, we recorded a lot of information about the mother’s diet and health during pregnancy. We are now aiming to carry out a study of blood pressure in these children who are now aged 7-8 years. The purpose of this study is to characterise the early determinants of blood pressure in this sample of children. We are also looking at the factors that may lead to hypertension. We are particularly interested in their cardiovascular and salivary cortisol response to a mild psychological stressor (The Trier Social Stress Test for Children).

We are intending to invite parents and children (please see list below) to take part whom we believe to be your patients. If you think that it is inappropriate for us to contact any of these families, we should be grateful if you would let us know by annotating the list and returning it to us at the above address. If you would like more details about the study I should be pleased to supply them. Thank you for your help.

Yours sincerely,

Professor DIW Phillips PhD FRCP

Study participant —
APPENDIX C – INFORMED CONSENT

This appendix contains copies of the informed consent forms used in the study of children that is detailed in this thesis.

[Consent form image]

[Signature: [Name], Date: [Date], Signature: [Signature]]
Informed consent form: Parent and Child

Title of study: The Mothers, Children & Healthy Hearts Study

Child ID for this study: xxxxx

Please initial boxes

1. I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions. □

2. I understand that our participation is voluntary and that we are free to withdraw at any time, without giving any reason, and without our medical care or legal rights being affected. □

3. I agree that we (myself and my child) take part in the above study. □

Name of child

Name of parent Date Signature

Researcher Date Signature

1 copy for parent
1 copy to be kept with research notes
Informed assent form: William

Title of study: The Mothers, Children & Healthy Hearts Study

Child ID for this study: xxxx

I agree to take part in this study:

Please write your name or tick this box

Haven of parent

Date

Signature

Researcher

Date

Signature

1 copy for child
1 copy to be kept with research notes
Parent’s consent form: Liaison with outside agencies

Title of study: The Mothers, Children & Healthy Hearts Study

Child ID for this study: xxxx

Some of the families involved in this study may move house over the next few years. For those that do, we would like to ensure that we maintain contact with them, as the success of our research is dependent on information we collect about mothers and children over time.

If you agree now to be contacted about other studies, we would be grateful for your permission for other agencies (e.g. NHS Central Records, Local Health Authority and your GP) to give us your new contact details in the future.

I agree to the above.

Name of parent Date Signature

Researcher Date Signature

1 copy for parent
1 copy to be kept with research notes

Local Research Ethics Committee Number: 26/03