UNIVERSITY OF SOUTHAMPTON

Growth in utero, blood pressure and elasticity of the aorta and large conduit arteries.

by

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ABSTRACT

FACULTY OF MEDICINE, HEALTH AND BIOLOGICAL SCIENCES

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Doctor of Philosophy

GROWTH IN UTERO, BLOOD PRESSURE AND ELASTICITY OF THE AORTA AND LARGE CONDUIT ARTERIES

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Studies have shown that low birth weight is associated with raised blood pressure both in childhood and adult life. The mechanisms responsible for this relationship are unknown. The work in this thesis investigates the idea that diminished elastin synthesis during fetal and early postnatal life leads to modifications in arterial structure resulting in a reduction in the elastic properties of the arteries and a rise in blood pressure.

The work falls into two parts. The first concerns epidemiological studies in humans. The aim was to examine relationships between indicators of poor fetal growth and arterial compliance in three groups: elderly men and women, young adults and children. Detailed birth records were available for all.

Arterial compliance was estimated by measuring pulse wave velocity. The technique depends on the principle that the speed at which pulse waves travel through a liquid filled tube is inversely proportional to the square root of the compliance of the tube's wall.

The results of the studies in humans showed that people who were born with indicators of fetal growth restriction in mid to late pregnancy tended to have faster pulse wave velocities in the aorta to femoral, aorta to radial and aorta to foot arterial segments. Although the actual birth measurements that were related to pulse wave velocity were not the same across the studies, the likely timing of the growth restricting factors was consistent. For example, in elderly men and women, for each unit decrease in ponderal index ($oz/in^3 x\ 1000$), aorta to femoral pulse wave velocity increased by 16% ($p=0.05$). In elderly men and women, for each gram increase in placental weight, aorta to radial pulse wave velocity increased by 0.033 m/sec ($p=0.02$). For each day decrease in gestational age, aorta to foot pulse wave velocity increased by 0.5% ($p=0.01$) in young adults and by 0.013 m/sec ($p=0.04$) in children.

The second part of this thesis concerns experiments on animals. The aim was to quantify aortic scleroproteins in two established animal models of intrauterine growth restriction. In the first model, pregnant rats were fed either a low protein diet (9% casein) or a control diet (18% casein) throughout gestation. In the second model, pregnant rats were fed either a calorie restricted diet (70% of control diet) or a control diet throughout gestation. The offspring of nutritionally restricted mothers are born lighter in weight and have reliably higher blood pressure than control group offspring.

The animal experiments showed that alkali soluble + alkali insoluble elastin levels were 9.66% ($p=0.06$) lower in the thoracic aortas and 15.55% ($p=0.12$) lower in the abdominal aortas of the offspring of mothers who were fed a low protein diet compared to the offspring of mothers who were fed a control diet during pregnancy. Similarly the alkali soluble + alkali insoluble elastin levels were 7.49% ($p=0.04$) lower in the aortas of 200 day old rat pups whose mothers were fed a calorie restricted diet compared to rat pups born to mothers who were fed a control diet during pregnancy. Aortic collagen concentrations did not differ significantly between the offspring of nutritionally restricted or control diet fed mothers.

These studies provide evidence that indicators of fetal growth restriction are associated with permanent reductions in the elastic properties of the aorta. In the offspring of maternal dietary restricted rats, impaired fetal growth led to a deficit in aortic elastin. It is concluded that a reduction in synthesis of aortic elastin at a critical stage in early development is one mechanism by which subsequent risk of cardiovascular disease may be programmed.

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I would like to dedicate this thesis to my Dad, David Phillips, who was my source of inspiration and support.

Statement by the author

The author, Nirree Phillips, carried out the majority of the practical work and statistical analysis presented in this thesis. However, some assistance in carrying out the fieldwork and statistical analysis was received.

The pulse wave velocity measurements presented in the extension study detailed in Chapter 3 were made by one of two research nurses, Elizabeth Cooper and Sheila Walton after being trained to use the technique by Nirree Phillips. The Minnesota coding of the electrocardiogram was carried out by Nan Keen, and the variables ischaemic heart disease and electrocardiogram identification of ischaemic heart disease were calculated using a computer program written by Vanessa Cox, computer programmer at the MRC Environmental Epidemiology Unit, Southampton.

In chapter 4, the pulse wave velocity measurements of the young men and women were made by one of two research nurses, Paula Darroch and Paula Hickey who were trained to use the technique by Nirree Phillips.

Dr Simon Langley-Evans of the department of Biochemistry at the University of Southampton reared, measured blood pressures, killed and prepared the aorta samples of the rats that were used in the low protein animal model detailed in chapter 6. Dr Takashi Ozaki of the department of Obstetrics and Gynaecology, University College London was responsible for the rearing, blood pressure measurement, killing and aorta preparation of the rats that were used in the calorie restricted animal model. Aortic hydroxyproline concentrations were determined under the supervision of Caroline Clifford at the Institute of Orthopaedics, Stanmore, Middlesex. The multilevel models presented in chapter 6 were produced with the help of Alistair Sheill, statistician at the MRC Environmental Epidemiology Unit, Southampton.

Chapter 1

Introduction

1.1 Fetal origins of cardiovascular disease

Epidemiological studies have shown that individuals who were light or who had had a small body size at birth are at greater risk of cardiovascular diseases such as coronary heart disease and stroke in adult life.[1] In the earliest of these investigations, 5 654 men born in Hertfordshire, UK between 1911 and 1930 were studied. A two-fold increase in the risk of developing coronary heart disease was found in the men in the lowest of six birth weight categories compared to those in the highest.[1] Further studies throughout Europe,[2-6] India [7] and the USA [8] have reported similar associations between lighter birth weight and increased risk of coronary heart disease. Where it was also possible to study body dimensions at birth in relation to later cardiovascular disease, it was found that risk of coronary heart disease rose as head circumference, length and ponderal index at birth fell.[4; 7; 9] These findings suggest that restriction of growth during fetal life is associated with increased risk of cardiovascular disease and has led to the hypothesis that susceptibility to cardiovascular disease is influenced by events *in utero*.[10]

The question that follows is by what process could poor fetal growth lead to an increased risk of cardiovascular disease in adult life. The answer is still unclear although a number of possible mechanisms have been suggested. Theories for mechanisms have arisen from studies which show that associations between cardiovascular disease and small birth size are mirrored by similar trends with known independent cardiovascular risk factors. For example, low birth weight has been shown to be related to raised blood pressure,[11-13] non-insulin dependent diabetes mellitus,[14] high levels of low density lipoprotein cholesterol [15] and raised fibrinogen [16] and factor VII levels.[17]

1.2 Fetal origins of raised blood pressure

Repeatedly, epidemiological studies have shown that as blood pressure levels rise, the risk of cardiovascular diseases such as stroke and coronary heart disease also increases.[18] Raised blood pressure has therefore, been suggested as a possible link between the intrauterine environment and adult cardiovascular disease.[13] Indeed, of the mechanisms that might relate poor fetal growth to cardiovascular disease, raised blood pressure has been the most extensively examined. Many studies investigating the relationships between blood pressure and birth weight have been carried out in different countries across a wide range of age groups.[19] The recurrent finding of these studies has been that blood pressure levels fall progressively as weight at birth increases, although this relationship is not consistent in adolescent life.[19-22] Animal studies have shown the same inverse relationship between blood pressure and birth weight in for example, *guinea pigs* [23] *and rats.*[24]

In humans, abnormal body proportions at birth are also associated with raised blood pressure. For example, several studies have shown that babies born with a low ponderal index, [25-27] a short crown-heel length,[28] or a small head circumference [29-31] tend to have higher blood pressure in childhood and adult life. This evidence suggests that the tendency to develop raised blood pressure in later life is present at birth and originates in the uterine environment.

A fetal origin for raised blood pressure is further supported by studies which show that when individuals are ranked in order of their blood pressure level, they tend to maintain their rank on subsequent blood pressure recordings.[32-35] The magnitude of the correlation between successive blood pressure measurements rises from low and variable values in children (for example, r=0.17),[34] to high values in adults (for example, r=0.7).[36] The finding that hypertensive adults tend to have had higher blood pressure as children when compared to normotensive adults suggests that an individual's blood pressure pattern is already established in childhood. Such tracking of blood pressure has been demonstrated from as early as 4 to 6 days after birth.[34] Therefore, an individual's blood pressure pattern and their tendency to develop hypertension is already in existence in the days proceding birth and may have been established earlier, in fetal life.

1.3 Intrauterine growth

How could small size at birth predispose individuals to raised blood pressure in later life? To answer this question it is necessary to look at what happens to the fetus when normal intrauterine growth is constrained.

Three phases of normal fetal growth have been described.[37] The first phase takes place from conception to 16 weeks and involves a rapid increase in cell number (hyperplasia). Phase two occurs from 16 to 32 weeks gestation where cells both multiply in number and increase in size (hypertrophy). The third phase of fetal growth takes place from 32 weeks gestation to term and during this time cell size rapidly increases and the majority of glycogen and fat deposition occurs.

Intrauterine growth constraint leads to several different patterns of atypical growth. These altered growth patterns result in infants with either abnormally small body dimensions, marked disproportion between head size and either body length or abdominal circumference, or unusually small accumulations of soft tissue mass.[38] The occurrence of each of these different patterns of growth are thought to depend on the nature, the timing and the duration of the growth restricting factor (figure 1.1).

Growth restriction during the early phase of fetal growth impairs cellular hyperplasia and all fetal organs are proportionately reduced in size.[39] Fetal weight is low and there is a uniform reduction in all external body dimensions.[40] This type of proportionate growth restriction has been associated with factors such as maternal smoking and genetic abnormalities in the fetus, for example congenital malformations or chromosomal irregularities.[41]

Growth restricting factors acting in the latter stages of the second phase and in the third phase of fetal growth have a greater effect on cellular hypertrophy and fetal organ size is disproportionately reduced. The disproportionate pattern of growth is thought to occur because the fetus is able to redistribute its cardiac output in favour of the brain.[42] There is an unequal reduction in external fetal body dimensions with head growth being less affected than crown-heel length or abdominal growth.[38] This type of disproportionate growth restriction is thought to result from placental abnormalities or inadequate maternal oxygen or nutrient supplies. Growth restriction during the second phase of fetal growth leads to a mixture or intermediate pattern of atypical growth depending on whether the insult occurs during the earlier (proportionate) or latter half (disproportionate) of the second phase.

Growth restricting factors acting after the 34th week of gestation cause a reduction in fat deposition but have little effect on the growth of body dimensions such as crown-heel length.[40] The result will be an infant who is long and thin, a pattern which can be recognised through measurement of ponderal index (weight/length^3).

Figure 1.1 Summary of the effects of growth restricting factors on fetal body dimension according to the timing of the insult.

PHASE OF FETAL GROWTH	TYPE OF INTRA UTERINE GROWTH RESTRICTION	BODY DIMENSIONS
(1) 0 to 16 weeks (hyperplasia)	Proportionate	↓ birth weight ↓ head circumference ↓ abdominal circumference ↓ length normal ponderal index
(2) 16 to 32 weeks (hyperplasia and hypertrophy)	Proportionate	mixture depending on early or late exposure
	Disproportionate	↑ or ↓ placental size
(3) 32 weeks to term (hypertrophy)	Disproportionate	↑ head : ↓ abdominal circumference ↑ head : ↓ length
34 weeks +	Low Ponderal Index	↑ length ↓ birth weight

(↓ = smaller ↑ = larger / longer)

The placenta provides nutrition and respiratory support to sustain growth and development during fetal life.[39] From the 13th day of gestation to the end of the 4th month the placenta undergoes active proliferation. Thereafter, there is a period of slow placental enlargement which spans from the fifth month of gestation to term.[43] Animal and human studies have provided evidence that alterations in the growth rate of the placenta occur as an adaptive response to lower maternal nutrient or oxygen supplies. For example, maternal undernutrition during mid pregnancy led to a reduction in placental weight in two animal studies,[44; 45] and an increase in placental weight in

another two studies.[46; 47] Further investigations have shown that the placenta enlarges in response to hypoxia in rats [48] or to anaemia or low haemoglobin levels in humans.[49; 50]

1.4 Programming of raised blood pressure

The question that follows is how is it possible that reduced fetal or placental development could predispose individuals to have high blood pressure in later life. One suggestion is that poor intra-uterine growth could lead to alterations in the structure of blood vessels leading to changes in their ability to function efficiently within the cardiovascular system.

There is some evidence that vascular structure is altered by poor fetal growth. Animal studies have shown that factors which restrict fetal growth may permanently alter the structure and physiology of a range of the body's organs and systems such as the pancreas, liver, kidney and indeed the blood vessels.[51; 52] This is thought to occur because in fetal life the tissues and organs of the body develop in periods of rapid cell division during which maturation must be achieved.[53] The timing of these periods of growth is dependent on the particular organ or tissue. A growth restricting stimulus may slow cell division and in doing so permanently alters the structure and function (DNA content and cell size) of the particular organ or tissue undergoing rapid development at that time.[37]

Blood vessels grow and develop rapidly in fetal life and may therefore be particularly susceptible to growth restricting stimuli. As a result, growth restricted babies may develop blood vessels with an altered structure. This could be deleterious because the physical properties of the arterial walls in particular have a major impact on circulatory function and efficiency.

1.5 Elastic requirements of the aorta

The aorta and the large arteries carry out two main functions within the circulatory system. One of these functions is to act as conduit vessels supplying blood from the left ventricle to the organs and tissues of the body with minimum loss of perfusion pressure. The other is a cushioning function to smooth the pulsations from the heart.[54]

During systole, the left ventricular blood is ejected into the thoracic aorta. To accommodate the left ventricular load, the aorta stretches and as much as two thirds of the stroke volume is stored.[55] As the left ventricle relaxes, elastic recoil of the aorta causes forward propulsion of the blood against a closed aortic valve and pressure within the aorta falls. The next ventricular contraction occurs before pressure has declined by about a third of the peak systolic pressure. In this way a pressure head is established which is maintained through the arterial system. The pressure head drives blood through the sites of controlled resistance to the capillary networks.[55]

As the arteries stretch and accommodate the volume changes of the heartbeat, this allows storage of the stroke volume during systole and minimises the rise in systolic pressure. Elastic recoil of

the arterial wall enables continued blood flow during diastole. In this way the pulsations from the heart become smoothed. Dampened pulsatile blood flow is present in the small arteries, arterioles and capillaries, but near continuous flow is achieved at the venous level.[54]

1.6 Importance of the elastic properties of the arterial system

An elastic arterial system is necessary to minimise systolic pressure and dampen pulsatile flow. The adverse effects of a non-compliant system can be demonstrated when the elasticity of the aorta is decreased. Systolic blood pressure rises and diastolic blood pressure falls. Therefore, pulse pressure is increased and the heart must work harder to maintain the same level of blood flow to the periphery.[56] Coronary blood flow occurs mainly during diastole. The elastic recoil of the aorta produces the energy necessary for the flow of blood through the coronary arteries. Reduced arterial elasticity can therefore, also lead to a fall in coronary driving pressure.

1.7 Arterial compliance

The term arterial compliance is used to describe the elastic properties of an artery.[56] Estimates of arterial compliance can be made by determining the pressure strain elastic modulus (Peterson's modulus). The pressure strain elastic modulus describes the relationship between the pressure applied to the arterial wall (stress) and the distension (strain) that this pressure achieves.[57] Strain is measured in the circumferential direction as the relative increase in vessel diameter (D) for a given change in pressure (P). Therefore, the pressure strain elastic modulus is calculated by $\Delta P \times D / \Delta D$ (detailed in chapter 2).

1.8 Measurement of arterial compliance

Direct *in vivo* measurements of arterial compliance are not easy in humans. They require a system in which changes in pressure, wall thickness and luminal volume can be made along the length of the artery.[58] Volume change measurements are possible using methods such as pulsed ultrasound or magnetic resonance imaging.[207,208] However, aortic pressure can only be measured accurately by catheterisation.[57]

Indirect *in vivo* measurements of arterial compliance can be made more easily. The methods used are based on the fact that the speed at which a pulse wave travels through a liquid filled tube is inversely proportional to the square root of the compliance of the tube's wall. Applied to the circulatory system, ejection of blood into the aorta following contraction of the left ventricle creates a pulse wave that is propagated along the arterial tree at a speed that varies according to the stiffness of the artery (detailed in chapter 2). As arterial elasticity decreases, the speed at which the pulse wave travels increases proportionally.[59] The elastic modulus of an artery can thus be calculated from measuring pulse wave velocity and applying the following equation: - $E = 2\rho (c + u)^2$ where E is the pressure strain elastic modulus, ρ is the density of blood, c is the pulse wave velocity and u is the mean velocity of blood flow[60] (detailed in chapter 2).

Measurement of pulse wave velocity has been used since the beginning of the last century to estimate the elasticity of the arterial system.[57] The pressure wave generated by contraction of the left ventricle gives rise to a diameter and a flow wave, both of which can be detected along the path of an artery.[58] The diameter wave can be measured using photoplethysmographic techniques and the flow wave using Doppler ultrasound methods. Pulse wave velocity is estimated by dividing the distance between the two consecutive sensors that are used to detect the pulse wave by the time taken for the pulse wave to travel between these two points (detailed in chapter 2).

1.9 Arterial compliance is a risk factor for cardiovascular disease

Recently, improvements in non-invasive methods for measuring pulse wave velocity have lead to renewed interest in reduced arterial compliance as a risk factor for cardiovascular disease.[61] Arterial compliance is an important determinant of systolic blood pressure, left ventricular load and coronary blood flow (section 1.6) so its measurement provides an indication of how efficiently the cardiovascular system is functioning.

Several studies have shown that reduced arterial compliance is associated with cardiovascular diseases such as atherosclerosis, cerebrovascular disease and coronary heart disease.[61; 62] For example, one study showed a strong relationship between arterial stiffness measured 60 to 370 days before death and the severity of atherosclerosis assessed at necropsy.[63] Other studies have found that patients with cerebrovascular disease, coronary heart disease and myocardial infarction had lower arterial compliance measurements compared to healthy age and sex matched controls.[64-69] A number of cardiovascular risk factors are also associated with increased arterial stiffness. These include, age, male gender, lipid status, [70; 71] non-insulin dependant diabetes mellitus [71; 72] and raised blood pressure.[73] This evidence has led to the recognition that reduced arterial compliance may serve as a marker for cardiovascular disease.[74]

1.10 Arterial compliance and hypertension

As far back as 1880 it was demonstrated that arterial compliance decreases as blood pressure levels increase.[75] Whether reduced arterial compliance plays a role in the aetiology of hypertension is less clear. Animal studies have shown that experimentally reducing arterial compliance increases systolic blood pressure levels.[76] In humans, reduced arterial compliance has been demonstrated in hypertensive people compared to normotensive individuals, at different stages of the disease and in a wide range of age groups.[56; 77; 78] However, increasing blood pressure causes arteries to stretch which in turn lowers their compliance. Reduced arterial compliance, may therefore be a mechanical consequence of blood pressure levels rather than the cause of raised blood pressure through structural alterations in the vessel wall.[78; 79]

Several forms of evidence suggest that raised blood pressure can not solely account for the decrease in arterial compliance seen with hypertension. For example, one study found that

arterial compliance was abnormal in hypertensive individuals before significant elevations in their blood pressure.[80] This same study showed that in patients with borderline hypertension the observed decrease in arterial compliance could not be accounted for by the marginal increase in blood pressure.[80] In another study, forearm arterial compliance measurements were similar in people with established hypertension irrespective of how high their blood pressure had risen above the diagnostic criteria for identifying hypertension (≥160mmHg for systolic pressure and ≥ 90mmHg for diastolic pressure).[81] Other studies, which compared arterial compliance at isobaric pressure by mathematically modelling arterial compliance as a function of blood pressure, have found reduced arterial compliance in hypertensive patients compared to normotensive individuals.[78; 82] It has also been shown that antihypertensive drugs exert different effects on arterial compliance despite similar reductions in blood pressure. For example, for the same blood pressure reducing effect, angiotensin converting enzyme inhibitors, nitrates and calcium channel blockers increase brachial artery compliance, but beta-blockers and dihydralazine and its derivatives do not.[83]

Two important lines of evidence emerge from these studies. Firstly, the fact that arterial compliance remains lower in hypertensive people compared to normotensive individuals when blood pressure levels are reduced suggests that raised blood pressure alone is not wholly responsible for the decrease in arterial elasticity seen in hypertension. Other structural and functional changes that lower arterial compliance must therefore, be involved.[80] The second important point is that reduced arterial compliance is already detectable in people with borderline hypertension. This indicates that the structural changes in the arteries are already present before blood pressure levels are elevated. This raises the question of when such structural changes might take place. Is it possible that alterations in the development of blood vessels *in utero* following fetal growth restriction could provide the starting point? In order to investigate this idea further it is necessary to recognise how the structure of the arterial wall enables the vessel to stretch and recoil and how these properties develop during fetal growth.

1.11 Elastic properties of vertebrate mammalian tissues are due to elastin

Biological elasticity is required so that tissues are able to stretch, allowing movement and other specialised functions to take place. Several natural elastomers have evolved. These include octopus elastomer from octopus aorta, resilin in the cuticle and hinges of insect wings, abductin in the hinges of molluscs and elastin from different tissues in vertebrates.[84; 85] Indeed it is elastin in the walls of the arteries that is primarily responsible for the elasticity of the human arterial system.

Elastin is a scleroprotein that is found in all vertebrates with the exception of hagfish and lampreys. It first arose in cartilaginous fish in the Devonian era 400 million years ago just after the divergence of the cyclostome (jawless fish) and gnathastome (vertebrates with distinct jaws) lines.[86] The appearance of elastin coincided with the achievement of a fully closed circulatory system. [87;88]

The elastic properties of elastin result from its molecular structure. Each elastin molecule is made up of covalent cross-links and is rich in hydrophobic amino acids, such as glycine (30%), valine (15%), and proline (11%), but poor in hydrophilic amino acids.[84] The ability of elastin to stretch and recoil results from this combination of hydrophobicity and the high degree of cross-linking.[89] When the elastin molecule is stretched, water is forced to associate with its non-polar side chains and this disrupts the hydrophobic interactions. Aggregation of the non-polar groups causes the water to be expelled and this gives rise to elastic recoil.[89]

Positive selection for increased hydrophobicity of elastin molecules appears to have taken place during the evolution of more complex circulatory systems.[85] This corresponds to comparative changes in systolic blood pressure, which has risen from 30 mmHg in fish to 120 to 150 mmHg in mammals.[86] Whatever the selective pressures were for increasing the hydrophobicity of elastin molecules, the result is that elastin has become more elastic with evolutionary time. The advantage of greater elasticity within circulatory systems has been the maintenance of higher blood pressure levels.

1.12 Structure of the arterial wall

The elastic behaviour of the arterial wall at normal physiological pressure is almost entirely determined by the structure of the tunica media. The tunica media forms the largest part of the vessel wall and its elastic properties depend on the amount and relative proportion of the elastin and collagen that it contains.[58] Elastin and collagen account for 50-80% of the dry weight of the aorta and are essential for maintaining tensile strength and allowing elastic recoil. Elastin is characterised by a high extensibility and a low elastic modulus.[90] Indeed, elastin can be extended to 100-150% of its original length before breaking. Collagen fibres are much stiffer; they have an elastic modulus 100 to 1000 times greater than elastin and break at about 5-8% extension.[89]

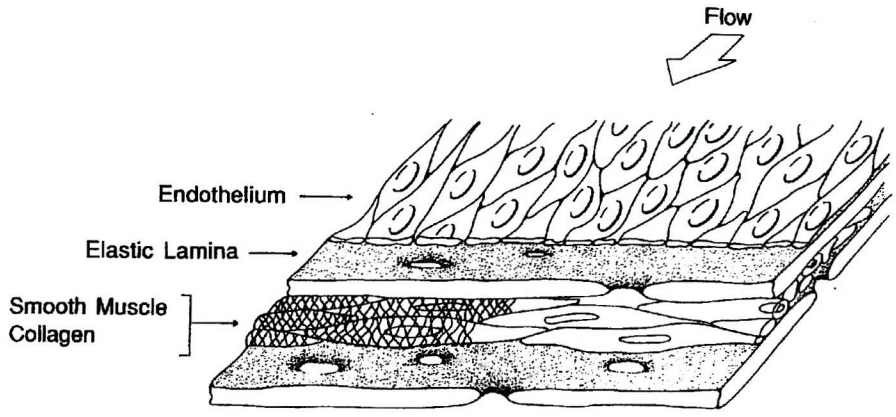
Elastin and collagen function together in a biphasic manner.[58] At normal physiological blood pressures (systolic blood pressure less than 200 mmHg) elastin mediates an elastic response to increased blood pressure. As blood pressure rises further, the stress is borne more and more by the stiffer collagen component of the arterial wall. Finally all the stress is transferred to the collagen fibres which become wholly responsible for resistance to stretch.[59] As blood pressure increases arteries therefore become functionally stiffer. This is an important relationship because it enables the arteries to remain stable over a wide range of pressures and helps prevent vessel rupture.

1.13 Elastin distribution and function

The structural arrangement of elastin within the arterial wall is central to its function. Elastin makes up the major amorphous component (90%) of elastic fibres and microfibrillar proteins, present as small fibrils, constitute the rest (10%).[90] Elastic fibres are arranged in laminae in the intima media of the vessel wall concentric to the blood vessel lumen. Interspersed with each of the elastic

laminae are collagen fibres and smooth muscle cells which are arranged circumferentially and make up the lamellar unit (figure 1.2). At normal physiological pressures the elastic fibres, collagen and smooth muscle cells are precisely aligned and form distinct layers.[59]

Figure 1.2 Illustration of the lamellar unit. [91]



The nature of the relationship between arterial structure and elasticity is not clearly understood. Various models have been proposed to explain why an artery becomes stiffer as it is stretched. For example, one theory suggests that when the artery is unstretched the elastin and collagen fibres within the lamellar units are arranged in parallel with the elastin fibres largely straight and the collagen fibres kinked and bent.[92] At low pressures stress is borne by the elastin fibres which do not strongly resist stretch. As pressure increases the arterial wall stretches further and collagen fibres begin to reach their normal resting lengths. With further pressure the collagen fibres resist further stress strongly and the load becomes transferred to collagen. From this model however, a rapid transition would be expected from the low elastic modulus that is characteristic of elastin to the high elastic modulus of collagen as the collagen fibres become taut. This is not what happens *in vivo*, the elastic modulus of an artery increases gradually as it is distended. Other models have suggested that elastin and collagen may exist in a combination of parallel elements that are connected in series (Greenwald, personal communication). However, no connections between elastin and collagen have yet been demonstrated.[59]

Collagen and elastin are differentially distributed along the arterial system in accordance with distance from the heart. Elastin predominates in the thoracic aorta and decreases in concentration along the arterial tree. Collagen increases in concentration distally from the heart and prevails in the abdominal aorta.[93] This distribution gradient is reflected in the spatial arrangement of elastic laminae.[94] The laminae are most clearly defined in the large vessels near the heart, whereas in the muscular arteries they are fewer and more fragmented. A comparative study of 10 mammalian species found that the number of lamellar units at any section in the thoracic aorta was proportional to the thickness of the vessel wall and closely correlated to transmural pressure.[95]

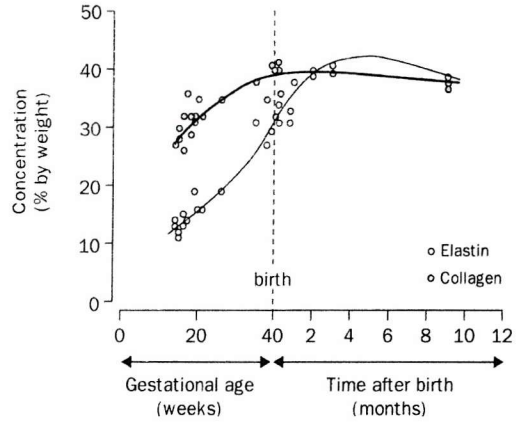
This evidence suggests that the lamellar unit is the structural element responsible for the elastic function of the arterial wall.

The question that follows in how and when during growth and development are these elastic laminae laid down.

1.14 Elastin synthesis

The synthesis of elastin occurs during fetal and early postnatal life. Elastic fibres first appear in the extracellular space as bundles of microfibrils arranged parallel to one another. Elastin is synthesised as a soluble precursor called tropoelastin, which is deposited onto the extracellular microfibril scaffold. The enzyme lysyl oxidase mediates cross-linking of the tropoelastin molecules through oxidation of selective lysine residues. The cross-linking results in the production of mature elastin which has a highly stable structure.[96] Fragments of mature elastin form in this way and merge together to form continuous elastic membranes. In human fetuses, elastic fragments have been identified from as early as 8 weeks gestation. By the third gestational month continuous elastic membranes are detectable.[94] Throughout the remainder of fetal growth these elastic membranes continue to increase in thickness and number.[97] Elastin production increases rapidly in the days just before birth and synthesis continues during early postnatal life (figure 1.3).[98]

Figure 1.3 Scleroprotein content of the human aorta during fetal and early postnatal life.[99]



After the postnatal period of growth is completed elastin production ceases in healthy vessels. In the porcine aorta, elastin synthesis is negligible 3 months after birth. Similarly, elastin synthesis ceases 12 weeks after hatching in chickens and 4 weeks after birth in rats.[93; 100] *In vivo* studies and various cell models have shown that tropoelastin mRNA levels are undetectable in adult blood vessels. The loss of ability to synthesise elastin in early postnatal life and the suppression of elastin production in adult tissue appears to be controlled by a post-transcriptional mechanism. This precise mechanism involved is unclear, although it is mediated at least in part by selective destabilisation of elastin specific mRNA.[100; 101]

1.15 Regulation of elastin synthesis

Synthesis of arterial elastin is closely regulated during development by distinct mechanisms that act at different stages of growth. Elastin production begins with transcription of the tropoelastin gene, which appears to be controlled at both the transcriptional and post-transcriptional level.[101; 102] Cell culture experiments have shown that several modulators are able to up-regulate expression of the tropoelastin gene. Modulators include for example, Insulin-like Growth Factor-1, interluekin-1β and glucocorticoids.[102] However, only Insulin-like Growth Factor-1 has so far been shown to increase tropoelastin mRNA transcription *in vivo*. Another modulator, Transforming Growth Factor-β has also been found to increase tropoelastin production, which it achieves through stabilisation of tropoelastin mRNA.[103]

1.16 Longevity of elastin

Once elastin has been laid down it is remarkably stable. Indeed, elastin is one of the most long-lived proteins in the body along with lens crystallins and tooth dentine and enamel.[104] Turnover rates of proteins range from minutes to years.[105] Animal studies have shown that the half life of metabolically labelled elastin is measurable in periods of years, for example, 27 years in the mouse aorta and 40 years in the rat aorta.[106]

In humans, elastin longevity has been studied by measuring racemisation of aspartic acid where the prevalence of d-aspartate correlates with the time elapsed since protein synthesis. Studies have shown that d-aspartate levels in the human lung and aorta increase linearly with age, which indicates that the age of the elastin correlates with the age of the subject.[105] This is consistent with elastin being laid down during fetal and early postnatal life with little or no new elastin synthesis thereafter. Further evidence of elastin longevity in humans comes from a study that measured nuclear weapons related ^{14}C deposition in lung tissue. The study showed data consistent with elastin being metabolically stable over the human life span.[104]

1.17 Elastin formation is critical

In summary, the majority of elastin is deposited in the aorta within a short period of its overall growth time. Synthesis is regulated during fetal and early postnatal life after which production ceases. The elastin laid down in the aorta is metabolically stable over the human lifetime and there is no appreciable elastin synthesis in the healthy adult aorta. Any imperfections in elastin deposition during early development therefore, can not be rectified in later life. The elastic properties of arteries are dependent on the structure, arrangement and proportion of elastin in their walls. Arterial elasticity is necessary to smooth the pulsations from the heart into near continuous blood flow to the periphery and to minimise the systolic pressure rise and reduce cardiac load. The circulatory system therefore relies on the elastin accumulated during early development to remain intact and function throughout life. The correct formation of elastin during fetal and early postnatal life is of critical importance to the efficient functioning of the circulatory system.

The question that follows is whether it is possible that poor fetal growth leads to a reduction in elastin synthesis in the artery walls and causes a decrease in arterial compliance that can not be rectified later.

1.18 Evidence for altered elastin synthesis in utero

One animal study has provided evidence that elastin synthesis is affected by factors acting *in utero*. The study showed that the DNA content of rat aortas was altered by growth restriction during fetal development. Inhibition of DNA synthesis through maternal administration of methotrexate during pregnancy led to chemical alterations in the cellular characteristics of the aortic wall in the rat pups. The number of lamellar units remained the same, but there was an increase in elastic tissue intersects and a transient increase in interlamellar elastin deposits.[107]

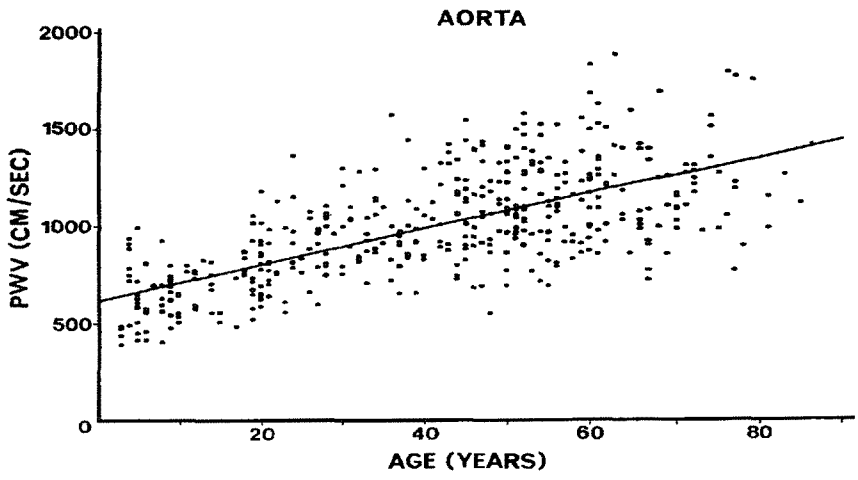
There is also evidence that fetal elastin synthesis may become impaired in humans. This evidence comes from studies of children born with a single umbilical artery. In these children blood flow to the placenta is transported by the iliac vessels on only one side of the body so the intrauterine blood flow pattern is altered. In a group of these children aged 5 to 8 years old, the common iliac artery was found to be much less compliant on the side of the missing umbilical artery.[108] Histological examination showed that the iliac vessels on the side of the absent umbilical artery were small and muscular compared to the iliac arteries of the opposite side which were large and elastic.[109]

If the amount of arterial elastin is reduced by adverse factors during fetal development as suggested by these studies, this could be translated into pathology in adult life through acceleration of the normal ageing process.

1.19 Elastin and ageing

During the normal ageing process elastin fibres undergo progressive characteristic changes. Over the human lifetime the elastic laminae lose their orderly arrangement, become thinner, split, fray and fragment [59; 110] and there is an associated increase in collagen and ground substance.[111] As the elastic laminae fracture, stress is transferred from elastin to the collagen fibres which are about 100 times stiffer than elastin and therefore, arterial compliance decreases. Several studies have shown that pulse wave velocity of the aorta and large arteries increases progressively with age indicating that the arteries become stiffer over time.[112-116] This relationship has been demonstrated both within populations (figure 1.4) and within individuals.[117]

Figure 1.4 Aortic pulse wave velocity in a population ranging in age from 3 to 89 years.[118]



The characteristic changes in the elastic laminae associated with age become histologically apparent in the second decade of human life. However, it has been suggested that degeneration begins in childhood [113] and may start at birth.[111] Indeed figure 1.4 suggests that pulse wave velocity increases progressively from birth onwards indicating a reduction in arterial elasticity and deterioration of arterial function from this point forward throughout life.

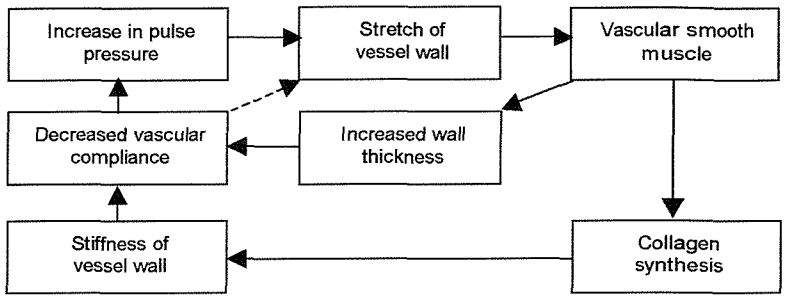
1.20 Elastin and hypertension

Animal studies have shown that in response to raised blood pressure restructuring of the arterial wall takes place. The restructuring process involves hyperplasia and hypertrophy of the smooth muscle cells, thickening of the lamellar units and accumulation of elastin, collagen and other matrix proteins.[119; 120] As a result vessel wall geometry changes, but the absolute amounts of elastin and collagen increase in relative proportion to one another and so reflect the normal composition at any site in the arterial tree.[120] The additional elastin that accumulates does not result in the thickening of the elastic lamellae nor in the formation of new ones, instead it is deposited as irregular islands in the interlamellar tracts.[121] Therefore, the elastic properties of the arterial wall are reduced despite the unchanged proportions of elastin and collagen.[122] Several of the changes that take place in the arterial wall in response to raised blood pressure are similar to those that occur during the ageing process. Hypertension may therefore, be caused by an accelerated form of ageing.[123]

Thickening of the arterial wall during hypertension protects the vessel wall from the elevated blood pressure. Protection is achieved because vascular stress is inversely proportional to wall thickness according to Lamés equation (chapter 2). However, elastin is unusually stable and its

accumulation over a period of hypertension is not reversible. Therefore, the elastin persists long after blood pressure levels have been lowered.[120; 122] Arterial compliance is reduced as a result of the increased wall thickness and this causes pulse pressure to rise. The increase in pulse pressure causes the vessel wall to stretch and this stimulates synthesis of matrix proteins and further vessel wall hypertrophy. However, as vessel wall thickness increases and arterial compliance decreases, the degree of stretch for a particular pulse pressure falls. Even so, the process becomes self-perpetuating and a feedback mechanism is set up which helps to maintain the hypertensive state (figure 1.5).[99]

Figure 1.5 Inter-relationships between arterial compliance, collagen synthesis and pulse pressure.[99]



If the elasticity of the arterial system is reduced at birth because of poor elastin synthesis *in utero,* an acceleration of the normal degenerative effects of ageing could lead to raised blood pressure in later life. Through a feedback mechanism, the raised blood pressure levels then become self-perpetuating.

1.21 Hypothesis

People who were light or small at birth tend to have higher blood pressure later in life. The mechanisms that mediate this relationship are unknown. The hypothesis is that persistent structural changes in blood vessels are involved. Formation of elastin, the scleroprotein primarily responsible for the elastic properties of the arteries, is adversely influenced by poor intrauterine conditions. A reduction in arterial elastin content leads to persistently stiffer arteries and the genesis of high blood pressure through acceleration of the normal ageing process.

Elastin is produced maximally in late gestation and early postnatal life. Therefore, the growth restricting factors that reduce elastin synthesis will be those acting in the latter stages of fetal growth. It is predicted that people who were either disproportionately growth restricted or thin at birth will have lower arterial compliance.

1.22 Evidence

There is already some evidence to support this hypothesis although it comes from only one study. Arterial compliance was recorded in 194 men and women aged between 50 and 53 years who had been measured in detail at birth.[28] The aorto-iliac and femoro-popliteal-tibial segments were investigated. On average, aorto-iliac pulse wave velocity was faster in men and women who were light, short or who had had a smaller abdominal circumference at birth (figure 1.6). However, these relationships were weak. Consistent and stronger relationships were found in the femoro-popliteal-tibial segment. Femoro-popliteal-tibial pulse wave velocity tended to be faster in men and women who were light at birth, or who were shorter in length, or who had had a smaller head or abdominal circumference at birth. No relationships were found between pulse wave velocity and ponderal index or placental weight in either of the arterial segments studied.

Figure 1.6 Results of the previous published study.[28]

	Mean pulse wave velocity (m/s)					
	Aorto-iliac segment	p Value	n	Femoro-Popliteal-tibial segment	p Value	n
Birthweight (lb)						
≤5.5	9.1		11	12.8		10
-6.5	8.5		37	12.4		45
-7.5	8.6		73	11.5		79
-8.5	8.3		58	10.7		60
>8.5	8.6	0.5	15	11.3	0.05	19
Length at birth (in)						
<20	8.6		65	11.6		74
20	8.6		70	11.8		76
>20	8.3	0.5	59	10.8	0.4	63
Abdominal circumference (in)						
≤11.5	9.3		44	13.1		48
- 12.25	8.3		42	10.8		46
- 13	8.4		62	11.1		73
>13	8.0	0.01	45	10.8	0.03	44
Occipitofrontal circumference (in)						
≤13	8.2		56	12.8		56
- 13.8	9.1		68	11.4		77
>13.8	8.2	0.2	70	10.7	0.03	80

1.23 Aims and objectives

The aims of the present studies are twofold:

1. To explore whether measures of fetal growth influence the elastic properties of arteries by measuring pulse wave velocity in three populations at different ages. The first population was a group of elderly people in whom raised blood pressure and its consequences are likely to be high. The second population was a group of young adults. The third population was a group of children in whom the degenerative effects of age are likely to be minimal.

   Pulse wave velocity was measured in up to four different arterial segments in these populations. These were the aorta to femoral segment (elastic artery), the aorta to radial segment (muscular artery with a high elastin content), the femoral to foot segment (muscular

artery with a low elastin content) and the aorta to foot segment (includes elastic and muscular arteries).

2. To explore whether the scleroprotein content of the aorta is altered in two different animal models of intrauterine growth restriction.

Chapter 2

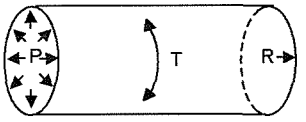
Arterial compliance measurement method

2.1 Background

Elasticity describes the relationship between the force applied (stress) to a material and its consequent deformation (strain).[59] The relationship between stress and strain is expressed as the elastic modulus. For most non-biological elastic materials applying a given force causes a proportional increase in length, this is known as Hookes law. The force divided by the extension in the longitudinal direction is called Young's modulus.

For a cylindrical tube, the applied force will be the circumferential tension (T) in the wall due to the lengthening caused by pressure (figure 2.1). Circumferential tension can be calculated from Laplace's law where tension in the wall is the product of pressure (P) and the radius (R).

Figure 2.1 Law of Laplace applied to a cylindrical tube such as a blood vessel.[124]



$$T = P\,R$$

Where the wall of the cylinder has a thickness h, the circumferential tension is calculated by PR/h (Lamés equation).[59]

When a material is stretched in a longitudinal direction, as it extends it will also get thinner in the transverse direction. The ratio of transverse to longitudinal strain is called the Poisson ratio (σ). This ratio is a characteristic property of the material and for small strains is constant. For arteries the Poisson ratio is approximately 0.5.[59]

2.2 Structural stiffness

Arteries have non-linear elastic properties and do not obey Hooke's law because they become stiffer the more they are stretched. In order to quantify arterial stiffness, the distension of the radius (ΔR) over small increases in pressure (ΔP) is calculated. This is called the incremental elastic modulus (E_{inc}), and it is calculated using the following equation, where σ is the Poisson ratio for arteries, $\overline{R}$ is the mean radius over the pressure increment and h is the wall thickness.

$$\text{Incremental elastic modulus } (E_{inc}) = (1-\sigma^2)\,\frac{\Delta P \overline{R}^2}{\Delta R\, h}$$

2.3 Functional stiffness

The incremental elastic modulus provides a measure of stiffness of the material within the arterial wall. However, it does not give a measure of functional stiffness, that is the actual vessel distension that would occur in response to a known pressure change.[58] Functional stiffness can be quantified using the pressure strain elastic modulus (E_p), which is calculated using the following equation.

$$\text{Pressure strain elastic modulus } (E_p) = \frac{\Delta P \overline{R}}{\Delta R}$$

The pressure strain elastic modulus is related to the incremental elastic modulus by the expression $E_p = E_{inc}h/(1-\sigma^2)R$. Therefore, functional stiffness depends not only on the structural stiffness (E_{inc}) but also the vessel geometry (ratio of wall thickness to radius).

2.4 Pulse wave velocity

The pressure strain elastic modulus can be estimated by measuring the velocity of the pulse wave that is generated through contraction of the left ventricle. During systole the left ventricle expels blood at high pressure into the aorta. The aorta expands to accommodate much of the stroke volume. The expansion stretches the walls at the root of the aorta and the wall tension of this section increases. This pulls on the adjacent section of arterial wall so that a wave of pressure is created that propagates throughout the arterial system.[55] Waves of diameter and flow are also created and the speed at which these pulse waves travel along the arteries increases in proportion to their wall stiffness.[57]

The Moens Korteweg equation describes the relationship between the pulse wave velocity and the pressure strain elastic modulus assuming that the artery is perfectly elastic, that it has a thin wall and that it is filled with an incompressible fluid.[59]

$$\text{Moens Korteweg equation} \qquad c = \frac{\sqrt{E_p}}{2\rho} + u$$

Where c is the pulse wave velocity, ρ the density of blood and u the velocity of blood.

2.5 Validity of Moens Korteweg equation

The Moens Korteweg equation was derived from theoretical work investigating the velocity of a pressure wave travelling through water in a rubber tube.[59] When applied experimentally in animal studies to the speed of the pulse wave propagating along the arterial wall, the equation has been shown to work well. For example, in anaesthetised dogs, values of the elastic modulus measured directly from the pressure diameter relationship are comparable to those calculated from the

Moens Korteweg equation following simultaneous intra-arterial measurements of the pressure and flow wave velocities.[125; 126]

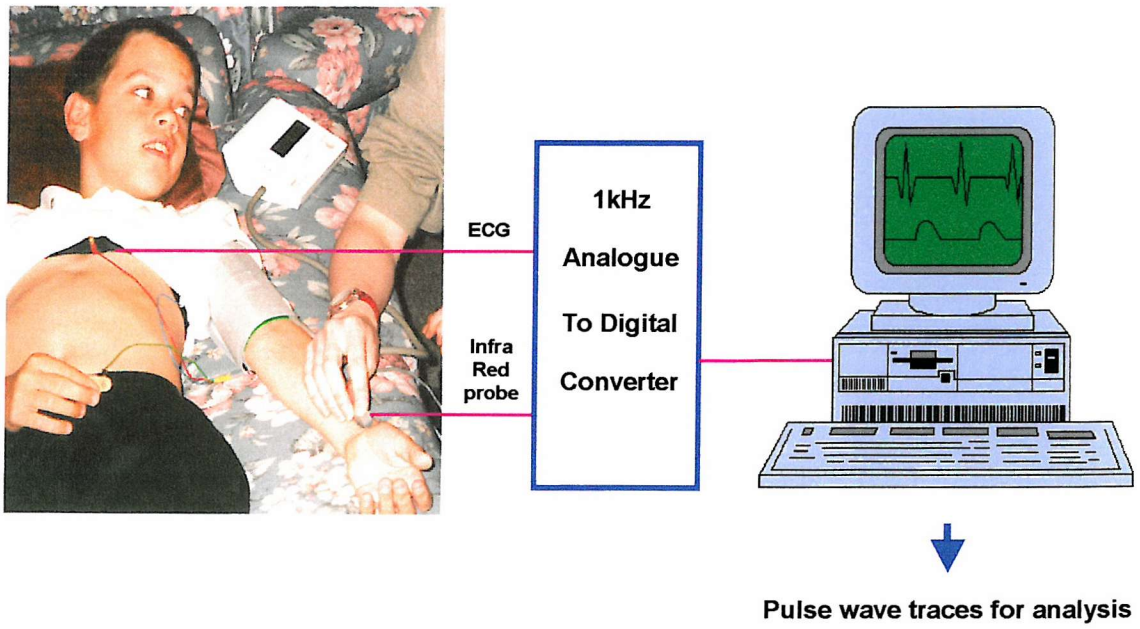
2.6 Measurement of pulse wave velocity

Pulse wave velocity is calculated by measuring the time delay between detection of the flow, pressure or diameter wave as it travels past two sites a known distance apart. Previous studies have used Doppler ultrasound methods to determine the flow wave velocity [127] or sphygmographic methods to determine the pressure wave propagation speed.[57] In the present studies an optical technique based on the principle of photoplethysmography was used to detect the diameter wave.[60]

2.7 Apparatus

A two-lead electrocardiogram was used to provide the first signal. A probe consisting of an infra red emitter and detector was positioned on the skin above a large artery, such as the radial artery (figure 2.2) to provide the second signal. Infra red radiation from the probe's emitter passes into the skin, vascular tissue and blood where it is absorbed and scattered.[128] As the arterial wave of dilatation passes the probe, the volume of blood in the artery increases. Blood absorbs infra red radiation more strongly than dermal and vascular tissue so the magnitude of the detected signal decreases in proportion to the increase in vessel diameter.[60] This is the basis of the technique of photoplethysmography. Variations in the probe detector signal pass through an integral amplifier, an analogue to digital converter sampling at a rate of 1kHz and a variable gain amplifier, which are interfaced to a computer. The computer runs software that allows the signals from the detectors to be captured, displayed on screen and then saved for later analysis.[129]

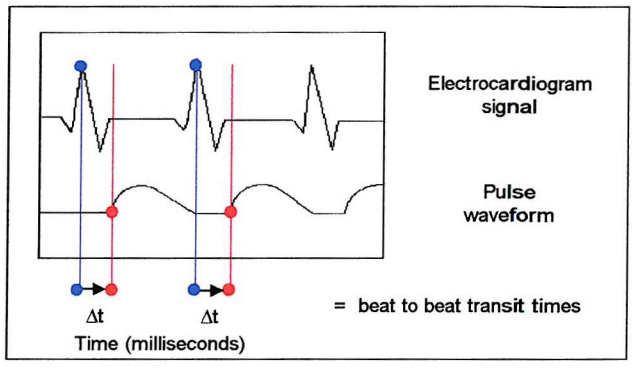
Figure 2.2 Apparatus for measuring pulse wave velocity.



2.8 Trace Analysis

The pulse wave traces are analysed using a customised computer program that plots the electrocardiogram and diameter wave signals on screen. The data are smoothed using an unweighted moving average technique. The program then automatically identifies the peak of the R wave (●) on the electrocardiogram signal and the position of the foot, the geometric minimum value, of the pulse waveform (●). It does this by using an algorithm described by Kontis, which identifies the point before the fall of the R wave or the upswing of the pulse waveform.[130] The time delay (horizontal distance (Δt) in milliseconds) between these two points is then measured, beat by beat, for the duration of the trace (figure 2.3). From these points a mean average transit time is calculated.

Figure 2.3 Method for beat by beat determination of the pulse wave transit time.



Pulse wave velocity can then be calculated from the following equation: -

$$\text{Pulse wave velocity} = \frac{\text{skin surface approximation of the distance between electrocardiogram and probe}}{\text{mean transit time}}$$

2.9 Use of the foot of the pulse waveform

When the pulse propagation velocity is measured from the phase difference between corresponding Fourrier components, regular fluctuations have been shown to occur at low frequencies.[131] These fluctuations are due to reflected waves.[129] At higher frequencies the attenuation of reflected waves is large so their net effect is minimal and measured velocities approximate the true propagation speed.[131] The high frequency components of the wave are represented by the part of the wave at which the rate of change in the gradient is greatest. This corresponds to the foot of the main peak of the pulse wave, therefore this point is used to determine pulse wave velocity.

2.10 Quality of measurement procedure

The quality of the optical measurement technique has been evaluated through assessment of its validity and reproducibility.

2.11 Validation of the measurement technique

Several studies have been carried out to verify that the signals from the infra red probe do indeed measure changes in the arterial diameter wave, and that the diameter wave reproduces the characteristics of the pressure wave. For example, comparisons with high precision echo tracking pulsed ultrasound measurements have confirmed that the infra red signal reproduces changes in arterial diameter.[60] Also signals from the infra red probe measured transcutaneously in humans and over an exposed artery in sheep have been found to be similar in shape and timing to simultaneous intra arterial measurements of the pressure wave.[60; 129]

2.12 Reproducibility of the measurement technique

Two studies have been carried out to assess the reproducibility of pulse wave velocities measured using the optical measurement technique in the short-term.[129] In these studies no significant differences were found between repeat measurements recorded either beat by beat or after 3 hourly intervals. No long term reproducibility studies have been carried out, therefore repeat pulse wave velocity measurements were made on a group of men and women (n=30) after a period of 2 to 14 months from first measurement.

2.13 Subjects and method

Repeat pulse wave velocity recordings were made using the optical measurement technique on 23 men and 7 women ranging in age from 69 to 72 years. First measurements were made between the months of July 1996 and February 1997 during clinics at the Northern General Hospital, Sheffield. Subjects were asked to lie supine whilst an electrocardiograph was attached via a chest strap. An infra red probe was then placed over the radial artery and the computer was activated to capture the output of the electrocardiograph and the pulse waveform over a 20 second measurement period. Systolic and diastolic blood pressures were recorded at the brachial artery directly after completion of the capture period using an automated Omron HEM 711 sphygmomanometer. Heart rate was also recorded. The distance from the sternal notch to the central position of the probe was then measured using a tape measure to follow the approximate path of the artery over the skin. This measurement procedure was then repeated with the infra red probe placed over the femoral artery, and then over the dorsalis pedis or posterior tibial artery. Repeat measurements were made using the same procedure during a second clinic attendance that took place between the months of April and September 1997.

2.14 Results

Table 2.1 shows the mean values and standard deviations of the repeat pulse wave velocity, blood pressure and heart rate measurements.

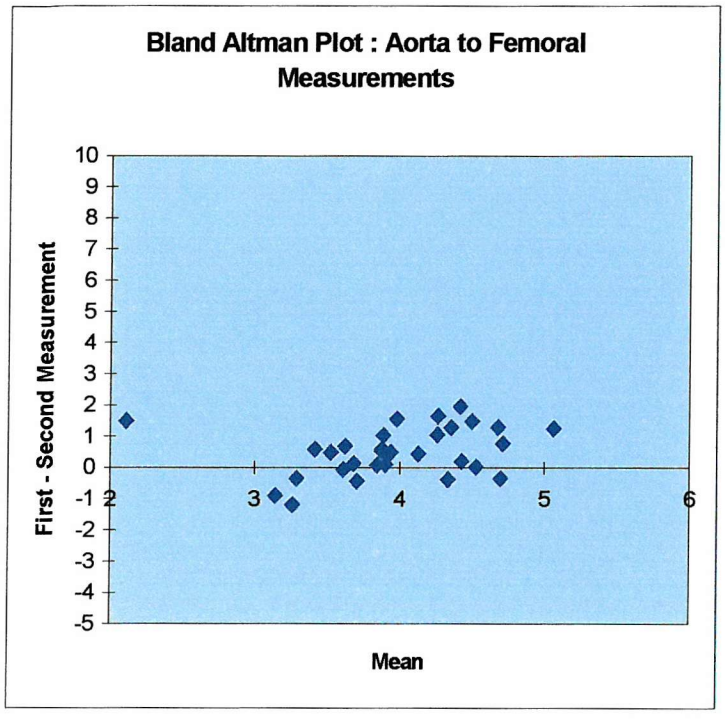
Table 2.1 Mean and standard deviation (SD) of pulse wave velocity (m/sec), blood pressure (mmHg) and heart rate (beats per minute) at first and second measurement (n=30).

Segment	Pulse wave velocity (m/sec)		Systolic Pressure (mmHg)		Diastolic Pressure (mmHg)		Heart rate (bpm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aorta to Femoral								
Measurement 1	4.2	0.7	147.3	19.4	81.3	11.5	64.6	13.1
Measurement 2	3.7	0.5	141.8	8.3	80.8	10.6	66.2	10.9
Aorta to Radial								
Measurement 1	4.4	0.6	147.8	6.5	82.6	13.9	66.6	12.3
Measurement 2	4.1	0.6	147.6	24.4	84.8	11.9	66.9	10.8
Aorta to Foot								
Measurement 1	6.2	0.8	144.2	23.1	79.6	12.0	66.3	13.6
Measurement 2	5.9	0.7	141.9	17.8	80.8	9.3	65.0	11.2

The mean pulse wave velocity of the second measurement was 0.5 m/sec slower in the aorta to femoral segment and 0.3 m/sec slower in the aorta to radial and aorta to foot segments when compared to the first measurement. Mean systolic blood pressure tended to be lower on the second measurement compared to the first.

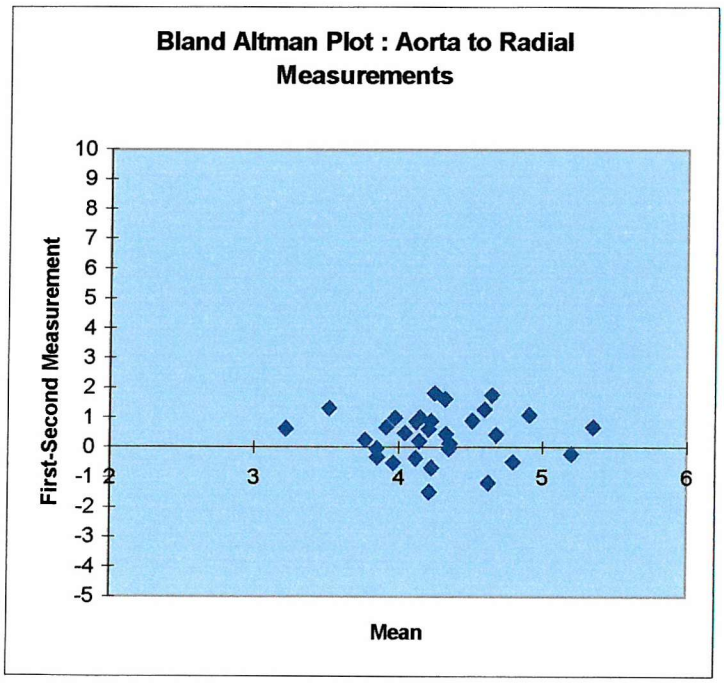
Bland-Altman plots were produced for each arterial segment (figures 2.4 to 2.6) where the differences between the first and second pulse wave velocity measurements were plotted against the mean of the two measurements. Correlation coefficients were not used because when repeat measurements are plotted against each other they will only be perfectly reproducible if the points lie along the line of equality, but perfect correlation will occur if the data points lie across any straight line on the graph.[132] Similarly rank correlations were not used because rank order can be preserved but still give a poor measurement of reproducibility.[132]

Figure 2.4 Bland-Altman plot of aorta to femoral pulse wave velocity measurements.



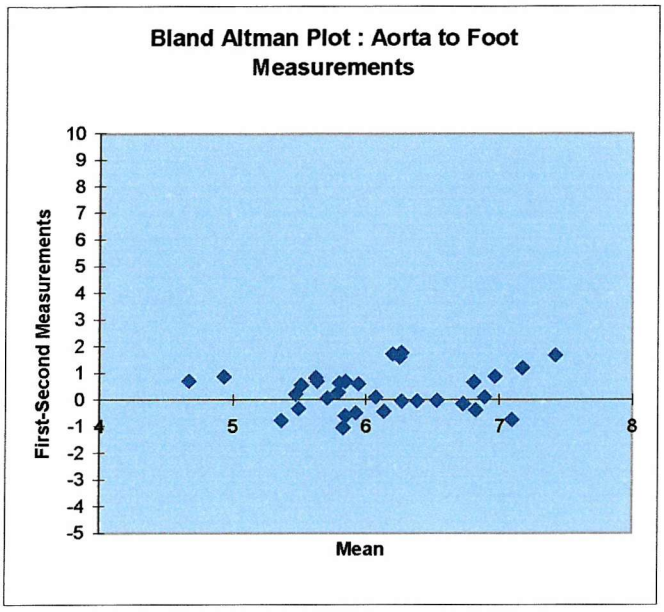
mean difference =	1.30
standard error =	0.14
t statistic =	9.25
p value of t statistic =	< 0.01

Figure 2.5 Bland-Altman plot of aorta to radial pulse wave velocity measurements.



mean difference =	0.41
standard error =	0.15
t statistic =	1.42
p value of t statistic =	0.17

Figure 2.6 Bland-Altman plot of aorta to foot pulse wave velocity measurements.



mean difference =	0.33
standard error =	0.13
t statistic =	2.56
p value of t statistic =	0.02

The Bland-Altman plots show that the first pulse wave velocity measurements tended to be faster than the second in all the arterial segments and that these differences, although small, were statistically significant in the aorta to femoral (p= <0.01) and aorta to foot (p= 0.02) segments.

Coefficients of variation (standard deviation ÷ mean) between the first and second pulse wave velocity measurements were calculated with systolic and diastolic blood pressure included as covariates. Table 2.2 shows the results.

Table 2.2 Coefficients of variation for pulse wave velocities adjusting for the effects of systolic and diastolic blood pressure.

Arterial segment	Coefficient of variation
Aorta to femoral	16.0%
Aorta to radial	15.0%
Aorta to foot	9.6%

After taking into account the effects of systolic and diastolic blood pressure, aorta to femoral pulse wave velocity varied by 16% between first and second measurements. Similarly, aorta to radial pulse wave velocity varied by 15% and aorta to foot pulse wave velocity varied by 9.6% between first and second measurements.

2.15 Discussion

Bland-Altman graphs of the repeat pulse wave velocity measurements were plotted in order to assess the reproducibility of the measurement technique. The mean difference between the first and second measurements should be zero if the measurement technique is perfectly reproducible.[132] However, a statistically significant difference from zero was found between the repeat measurements made in the aorta to femoral and aorta to foot segments where the second measurement was systematically lower than the first. There are two main sources of measurement error that may provide an explanation for these differences. They are observer variation and subject variation.

2.16 Observer Variation

Observer variation can occur either between or within each observer's measurements. In the present studies pulse wave velocities were measured by the same observer therefore, only within-observer variation was relevant.

Within observer variations, if present, are likely to be random and therefore would not be expected to influence the mean pulse wave velocity values.[133] However, possible sources of within-observer variation were examined by investigating the differences between the first and second pulse wave velocity measurements according to (a) the month (over an 8 month period) that the first measurement was made and (b) the period of time between the first and second measurements. This was carried out in order to investigate whether there had been any learning effects as the observer became more experienced with the measurement technique. No statistically significant differences were found.

2.17 Subject variation

A variety of biological factors could be responsible for the variations in the pulse wave velocity that were found between repeat measurements. These might include for example, differences in blood pressure level, heart rate, peripheral resistance or smooth muscle activity.[130] All of these factors can influence the propagation speed of the pulse wave and may vary daily.

In the present study repeat data was collected on systolic and diastolic blood pressure and heart rate, so it was possible to investigate whether variation in these biological parameters could account for the differences between the repeat pulse wave velocity measurements. No statistically significant differences in either diastolic blood pressure or heart rate were found between first and second measurements. However, after producing Bland-Altman plots for repeat systolic blood pressure readings, a systematic difference was revealed between the recordings made after the aorta to femoral and the aorta to foot pulse wave velocity measurements. The systolic blood pressure recordings that were made following the aorta to femoral pulse wave velocity measurements tended to be 5.5 mmHg higher on the first recording compared to the second and this difference was statistically significant ($p=0.04$). Similarly, systolic blood pressure recorded

after the first aorta to foot pulse wave velocity measurement also tended to be higher than the second, although the difference was smaller (2.3 mmHg) and was not statistically significant (p=0.38). Systolic blood pressure may have been lower in the subjects on their second pulse wave velocity recording because they were more familiar with the measurement techniques.

The variation in systolic blood pressure reading between the first and second measurements might explain why the pulse wave velocities also differed. This is possible because the velocity of the pulse wave is related to blood pressure levels.[134] The stress-strain relationship exhibited by arteries is curvilinear, so as blood pressure levels rise the arteries become less extensible and the velocity of the pulse wave increases.[58] In accordance with this relationship in the present study, mean systolic blood pressure was higher and pulse wave velocity was faster at the first measurement compared to the second. Therefore, the subsequent mean decrease in systolic blood pressure level on the second measurement could account for the mean reduction in pulse wave velocity that was also found.

Coefficients of variation adjusted for systolic and diastolic pressure were calculated to explore the data further. These showed that even after the effects of blood pressure had been taken into account a variation in pulse wave velocity of between 9.6 to 16% still existed between first and second measurements (Table 2.2).

Adjusting for blood pressure level during statistical analysis assumes that the increase in pulse wave velocity with rising blood pressure levels is the same for everybody within the population, which might not be the case (figure 5.9). Therefore, mathematical adjustment may not be an adequate means of controlling for the relationship between blood pressure and pulse wave velocity. However, in the present study the blood pressure adjusted coefficients of variation for repeat pulse wave velocity measurements are consistent with those of a previous study. In the previous study, aortic pulse wave velocity measured by Doppler ultrasonography was found to vary by factors of up to 18% over the course of a day.[130] Therefore, the differences in repeat measurements in the present study appear to be within the normal variations for pulse wave velocity that might be expected due to biological variations alone.

2.18 Changes in pulse wave velocity over time

Studies in humans have shown that the pulse wave velocity of the aorta and large arteries increases progressively with age both within populations [118] and within individuals.[117] Therefore, pulse wave velocity might have been expected to increase over the time period (ranging from 2 and 14 months) between the first and second measurements. However, no statistically significant difference in pulse wave velocity was found with time between measurements whether the time difference was explored either as a continuous variable or when categorised into < 6 months, 6-12 months and >12 month periods.

2.19 Conclusions

Estimates of pulse wave velocity in 30 subjects were found to be systematically lower on second measurement after a period of 2 to 14 months. However, the differences between measurements, although statistically significant, were small, and can probably be accounted for by variations in systolic blood pressure. This was indicated by a similar systematic difference between systolic blood pressure measurements. However, pulse wave velocity still varied by a factor of up to 16% after systolic and diastolic blood pressure had been taken into account in the statistical analysis, although the adequacy of statistical adjustment for blood pressure level is uncertain. The method appears to be reproducible in long term, but the importance of the effects of systolic blood pressure on pulse wave velocity readings should be emphasised.

Chapter 3

Arterial compliance in elderly men and women

This chapter describes a study of a group elderly men and women who were born in the Jessop hospital for Women, Sheffield between 1922 and 1930 where they were measured in detail at birth and in whom recent arterial compliance measurements were made.

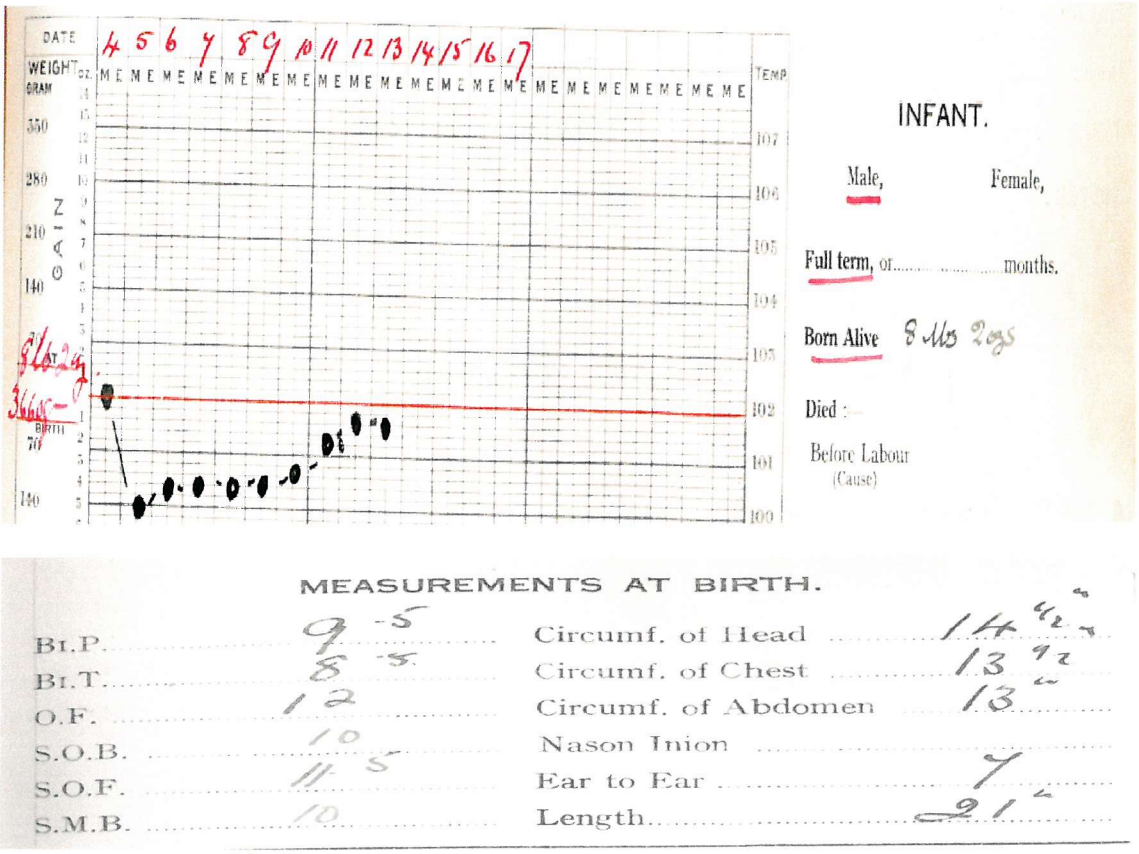
3.1 Subjects

The study population consists of men and women who took part in a follow up study in 1996 and also subjects who participated in an extension study, which began in 1997.

3.1.1. Follow up study population

There are 2 232 complete birth records of singletons that were born to married mothers in the Jessop Hospital for Women in Sheffield, UK between the years of 1922 and 1926. Each record consists of a standard form that was used to enter details of each mother and child. Information on the form included the date of the mother's last menstrual period, the child's birth weight, length, chest, head and abdominal circumference (figure 3.1) and the weight of the placenta.

Figure 3.1 Excerpts from the Jessop Hospital Labour Ward Records showing details of infant weight and size at birth.



From each birth record, the subject's name and their date of birth were sent to the Office for National Statistics who performed a search of the UK National Health Service Central Register in order to try and trace each individual. It was found that 829 of the 2 232 people, for whom Jessop

Hospital birth records were available were currently registered with a General Practitioner in the UK and 442 of these people were registered with a General Practitioner in the Sheffield area.

For 47 of the 442 people who still lived in Sheffield, either General Practitioner permission to contact them was denied, they were no longer living in the Sheffield area or they were deceased. The remaining 395 subjects were invited to participate in a study of cardiovascular risk factors and 322 (82%) agreed to take part. These 322 men and women formed the study population for the present follow up study.

After ethical approval had been gained from the Northern General Hospital Ethics Committee, a letter was sent to each of the 322 subject's General Practitioners to verify the subjects address and to request permission to approach their patient. 295 of the subjects were still registered with a General Practitioner in the Sheffield area. General Practitioner permission was denied for 5 of these people leaving a total of 290 men and women.

3.1.2 Extension study population

The extension study population consisted of 1480 singleton men and women who were born to married mothers in the Jessop Hospital for Women between the years of 1922 to 1930 and who were thought to be registered with a General Practitioner in Sheffield in January 1997. This group of people also included all the men and women from the study population detailed in section 3.1.1, although those who had refused to take part in either the original study or the follow up study were excluded, leaving a total of 1289 people. The 1289 people were divided into 5 categories according to their weight at birth (<5.5 lb, 5.5 to 6.5 lb, 6.5 to 7.5 lb, 7.5 to 8.5 lb and >8.5 lb). The study sample was then selected by including all of the subjects in the highest (n= 159) and lowest (n=77) birth weight groups, and randomly selecting 85 males and 85 females from the remaining 3 groups. The total number of subjects in the group was 746 and included 100 men and women who had taken part in the original study of cardiovascular risk factors (detailed in section 3.1.1).

The names, and NHS numbers of the 746 men and women were sent to Sheffield Family Health Services Authority who were asked to search their records to identify each subject's General Practitioner. 711 of the men and women were still registered with a General Practitioner in Sheffield.

After ethical approval had been gained from the Northern General Hospital Ethics committee, letters were sent to each General Practitioner requesting permission to contact their patient. General Practitioner approval was granted for 660 of the subjects. For the other 51 subjects, either General Practitioner permission was denied, the subject was no longer living in the Sheffield area or the subject was deceased.

3.2 Methods

Once General Practitioner approval had been given, a letter was sent to the subject inviting them to participate in the study. A nurse then contacted those who had agreed to take part and arrangements were made to visit each subject in their own home.

During the home visit a questionnaire was administered which enquired about recent illnesses, symptoms of coronary heart disease (Rose chest pain questionnaire), stroke and peripheral vascular disease and current medication. At the end of the interview the subjects were invited to the Northern General Hospital, Sheffield to attend a clinic for further investigations.

At the clinic, height was measured using a portable stadiometer and weight using a portable digital Seca scale. Resting 12 lead electrocardiograms were recorded and arterial compliance and blood pressure measurements were made using the methods detailed in chapter 2.

Pulse waveforms were recorded with the infra red probe placed firstly over the radial artery of the left wrist, then over the femoral artery just below the inguinal ligament on the left hand side and finally over the posterior tibial artery immediately posterior to the medial malleolus or the dorsalis pedis artery of the left foot. This enabled pulse wave velocity to be estimated in the aorto-iliac, aorto-brachial and aorto-femoro-popliteal-tibial arterial segments. Systolic and diastolic blood pressure measurements were recorded at the brachial artery using an automated Omron HEM 711 sphygmomanometer directly after each pulse waveform measurement period.

3.2.1 *Derivations from the data*

The presence of ischaemic heart disease was determined from a resting 12 lead electrocardiogram and answers to the Rose chest pain questionnaire. The variable 'Rose chest pain questionnaire identification of ischaemic heart disease' was defined according to the Rose chest pain questionnaires diagnostic criteria for angina pectoris.[135] The electrocardiograms were coded according to the Minnesota protocol by a trained coder.[136] The variable 'electrocardiogram identification of ischaemic heart disease' was defined as follows (table 3.1).

Table 3.1 Minnesota code definitions for the variable 'electrocardiogram identification of ischaemic heart disease.

Ischaemic heart disease	Minnesota codes
Definite	1-1, 1-2, 7-1
possible	1-3, 4-1, 4-2, 4-3, 5-1, 5-2, 5-3
absent	all other codes

The variable 'ischaemic heart disease' was determined as presence of one or more of the following: - angina pectoris according to the Rose chest pain questionnaire; Minnesota codes as outlined in table 3.1; self reported history of stroke, heart attack, coronary artery angioplasty, coronary artery bypass grafting or carotid endarterectomy.

An estimate of pulse wave velocity in the femoro-popliteal-posterior tibial segment, although not measured directly, was calculated from the values obtained in the aorta to femoral and aorta to foot segments using the following equation: -

$$\text{femoral to foot pulse wave velocity} = \frac{\left[\text{probe separation in the aorta to foot segment} - \text{probe separation in the aorta to femoral segment}\right]}{\left[\text{transit time in the aorta to foot segment} - \text{transit time in the aorta to femoral segment}\right]}$$

3.3 Analysis

Tabulation of means, univariate and multivariate linear regression analysis was used to examine the relationships between measurements of body size at birth, adult blood pressure and pulse wave velocity. Where the frequency distribution of pulse wave velocities were skewed, log transformations were used. Scatterplots were produced to display the data.

3.4 Results

3.4.1 Follow up study

Of the 290 men and women who were invited to take part in this study, 211 agreed to participate and were visited at home by a nurse. 146 of these people then attended a clinic where blood pressure and arterial compliance measurements were made.

3.4.2 Extension study

419 of the 660 subjects who were invited to participate in this study agreed to take part and home visits were carried out. 393 men and women also agreed to attend a clinic. Of the 393 people, 58 had already taken part in the follow up study. Arterial compliance and blood pressure was not re-measured in these 58 men and women, leaving a total of 335 subjects.

The data from the follow up study (n=146) and the extension study (n=335) was then combined resulting in a total study sample of 481 men and women. The distribution of birth size, blood

pressure and pulse wave velocities of the elderly men and women are presented as histograms in appendix A.

Mean current body size and birth measurements of the elderly men and women are summarised in table 3.2

Table 3.2 Mean current body size and birth measurements in the 65 to 75 year old men and women.

	Men (n=276)	**SD**	**Women** (n=205)	**SD**	**p value of difference**	**All** (n=481)
Current measurements						
Height (cm)	170.1	6.77	157.2	6.01	<0.001	164.6
Weight (kg)	77.6	12.4	67.6	12.6	<0.001	73.4
Body Mass Index (kg/m^2)	26.8	3.90	27.4	4.98	0.129	27.0
Age at clinic attendance (years)	69.0	1.6	69.7	2.0	<0.001	69.3
Birth measurements						
Birth weight (lb)	7.47	1.26	7.15	1.19	0.004	7.34
Head circumference (in)	13.7	0.734	13.5	0.660	<0.001	13.6
Chest circumference (in)	13.1	0.863	12.9	0.803	0.002	13.0
Abdominal circumference (in)	12.4	0.998	12.2	0.927	0.010	12.3
Length (in)	20.2	1.12	20.0	1.06	0.062	20.1
Head:abdominal circumference	1.11	0.064	1.11	0.070	0.865	1.11
Head:length	0.679	0.039	0.673	0.032	0.063	0.676
Ponderal index (oz/in^3 x 1000)	144.7	20.8	142.4	18.8	0.239	143.8
Placental weight (oz)	22.4	4.64	21.8	4.93	0.236	22.2
Placenta:birth weight	0.189	0.034	0.195	0.036	0.119	0.192
Gestational age (days)	280	15	278	16	0.244	279

1 lb = 0.454 kg; 1 in = 2.54 cm SD = standard deviation

The men who participated in this study were on average 12.9 cm taller, 10 kg heavier and 8.4 months younger than the women and these differences were statistically significant. Women tended to have a larger body mass index than the men, although the difference was not statistically significant.

Men tended to be heavier, larger babies at birth. For example, men tended to be 0.32 lb heavier, with head, chest and abdominal circumferences that were 0.2 in larger at birth than those of the women and all these differences were statistically significant.

3.5 Blood pressure

The mean blood pressure measurements of the 481 men and women who took part in the study are summarised in table 3.3

Table 3.3 Mean systolic and diastolic pressure (mmHg) in the 65 to 75 year olds.

	Men (n=276)	SD	Women (n=205)	SD	p value of difference	All (n=481)
Systolic blood pressure	146.0	20.7	141.0	21.7	0.010	143.9
Diastolic blood pressure	81.6	10.1	78.4	9.65	0.001	80.2

Men had systolic blood pressure that was on average 5.0 mmHg higher and diastolic blood pressure that was 3.2 mmHg higher than the women. Both these differences were statistically significant.

Linear regression analysis was used to investigate the relationships between blood pressure and current and birth size measurements. The results are summarised in table 3.4.

Table 3.4 Univariate analysis of blood pressure measurements with current and birth size measurements.

	Systolic Pressure Regression coefficient (p value)	Diastolic Pressure Regression Coefficient (p value)
Current measurements		
Height (cm)	0.026 (0.809)	0.108 (0.035)
Weight (kg)	0.049 (0.501)	0.952 (0.006)
Body Mass Index (kg/m^2)	0.183 (0.414)	0.187 (0.076)
Age (years)	0.478 (0.387)	-0.403 (0.119)
Birth measurements		
Birth weight (lb)	-0.108 (0.891)	0.086 (0.817)
Head circumference (in)	0.268 (0.845)	1.319 (0.042)
Chest circumference (in)	0.967 (0.416)	1.033 (0.068)
Abdominal circumference (in)	1.003 (0.333)	0.919 (0.062)
Length (in)	0.652 (0.484)	0.369 (0.402)
Head:abdominal circumference	-12.044 (0.458)	-4.695 (0.516)
Head:length	0.193 (0.995)	19.881 (0.136)
Ponderal index (oz/in^3 x 1000)	-5.175 (0.482)	-2.444 (0.481)
Placental weight (oz)	-0.051 (0.826)	-0.005 (0.963)
Placenta:birth weight	-19.743 (0.527)	-7.730 (0.599)
Gestational age (days)	0.104 (0.155)	0.078 (0.027)

In the 65 to 75 year old men and women, systolic blood pressure tended to be higher in people with a larger current body size, although none of the relationships were statistically significant. There were no relationships between systolic blood pressure and birth measurements. Diastolic blood pressure tended to be higher in people who were taller or heavier as adults. Diastolic blood pressure also tended to be higher in men and women who had had a larger head circumference at birth or who were born after a longer length of gestation. All of these relationships were statistically significant.

Multiple linear regression models were produced to investigate the relationships between systolic and diastolic blood pressure and birth size measurements after adjusting for the potential confounding effects of age, sex and body mass index. The results are summarised in tables 3.5 and 3.6.

3.5.1 Systolic blood pressure

Table 3.5 Multivariate analysis of systolic blood pressure and birth measurements adjusted for : -

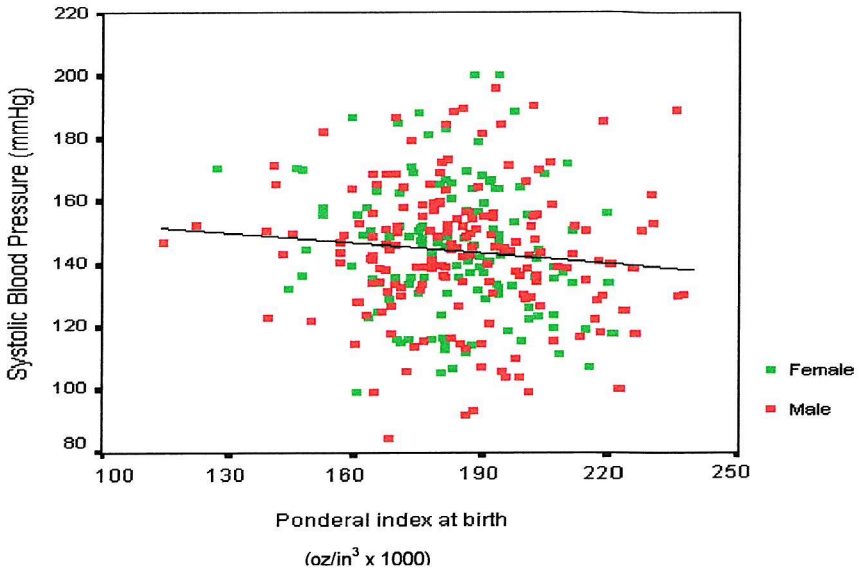
i age, sex and body mass index.

ii age, sex, body mass index and gestational age.

Birth measurements	**i**	**ii**
	Regression coefficient (p value)	Regression coefficient (p value)
Birth weight (lb)	-0.836 (0.305)	-1.069 (0.303)
Head circumference (in)	-0.721 (0.616)	-1.580 (0.374)
Chest circumference (in)	0.354 (0.774)	0.017 (0.991)
Abdominal circumference (in)	0.420 (0.697)	-0.244 (0.851)
Length (in)	0.241 (0.825)	0.885 (0.438)
Head:abdominal circumference	-9.573 (0.549)	0.493 (0.979)
Head:length	-5.549 (0.850)	-39.046 (0.250)
Ponderal index (oz/in^3 x 1000)	-9.828 (0.203)	-16.395 (0.076)
Placental weight (oz)	-0.150 (0.523)	-0.159 (0.561)
Placenta:birth weight	-13.092 (0.682)	-36.148 (0.335)
Gestational age (days)	0.099 (0.189)	

Systolic blood pressure was not strongly related to any of the birth measurements after adjusting for age, sex and body mass index. After gestational age had been added into model (ii), a relationship between raised systolic blood pressure and a smaller ponderal index at birth became apparent (figure 3.2). However, it was not statistically significant at conventional levels (p=0.05).

Figure 3.2 Scatterplot of systolic blood pressure against ponderal index at birth after adjusting for age, sex, body mass index and gestational age.



3.5.2 Diastolic blood pressure

Table 3.6 Multivariate analysis of diastolic blood pressure and birth measurements adjusted for : -

i age, sex and body mass index.

ii age, sex, body mass index and gestational age.

Birth measurements	**i** Regression coefficient (p value)	**ii** Regression coefficient (p value)
Birth weight (lb)	-0.309 (0.417)	0.002 (0.997)
Head circumference (in)	0.662 (0.321)	1.048 (0.217)
Chest circumference (in)	0.573 (0.320)	0.992 (0.201)
Abdominal circumference (in)	0.545 (0.280)	0.678 (0.277)
Length (in)	-0.059 (0.896)	0.341 (0.531)
Head:abdominal circumference	-3.955 (0.597)	-1.619 (0.856)
Head:length	20.234 (0.137)	12.797 (0.430)
Ponderal index (oz/in^3 x 1000)	-3.160 (0.378)	-2.973 (0.502)
Placental weight (oz)	-0.057 (0.620)	-0.016 (0.901)
Placenta:birth weight	-3.172 (0.830)	-14.346 (0.424)
Gestational age (days)	0.058 (0.108)	

There were no relationships between birth size measurements and diastolic blood pressure in the 65 to 75 year old men and women in either of the multivariate models.

3.6 Pulse wave velocity

The mean pulse wave velocities of the men and women that took part in the study are summarised in table 3.7.

Table 3.7 Mean pulse wave velocity (m/sec) in the 65 to 75 year old men and women.

Arterial segment	Men (n=276)	SD	Women (n=205)	SD	p value of difference	All (n=481)
Aorta to femoral	5.31*	1.24	4.83*	0.99	< 0.001	5.11
Aorta to radial	4.99	1.07	4.58	1.03	< 0.001	4.81
Aorta to foot	6.98	1.07	6.56	1.08	< 0.001	6.80
Femoral to foot	10.20*	3.24	11.08*	5.70	0.034	10.57

* geometric mean

Pulse wave velocity tended to be faster in men than women in all of the arterial segments except for the femoral to foot segment. On average, aorta to femoral pulse wave velocity was 0.48 m/sec faster, aorta to radial pulse wave velocity was 0.41 m/sec faster, aorta to posterior tibial artery was 0.42 m/sec faster and femoral to posterior tibial artery pulse wave velocity was 0.88 m/sec slower in men compared to women. All these differences were statistically significant.

The frequency distribution of pulse wave velocities in the aorta to femoral and femoral to foot segments were skewed. Transformation using logarithms produced approximately normal distributions. Linear regression analysis was then used to investigate the relationship between pulse wave velocity and current and birth size measurements and blood pressure levels (table 3.8).

Table 3.8 *Univariate analysis of pulse wave velocity with current body size measurements, blood pressure and birth measurements adjusted for: -*

i(Logged) Aorta to femoral pulse wave velocity.

ii Aorta to radial pulse wave velocity.

iii Aorta to foot pulse wave velocity.

iv (Logged) Femoral to foot pulse wave velocity.

	i Regression coefficient (p value)	ii Regression coefficient (p value)	iii Regression coefficient (p value)	iv Regression coefficient (p value)
Current measurements				
Height (cm)	0.006 (<0.001)	0.018 (0.001)	0.021 (<0.001)	-0.003 (0.101)
Weight (kg)	0.000 (0.629)	0.002 (0.581)	0.001 (0.697)	0.000 (0.752)
Body Mass Index (kg/m^2)	-0.007 (0.005)	-0.018 (0.116)	-0.025 (0.025)	0.003 (0.401)
Age (years)	0.020 (<0.001)	0.049 (0.078)	0.051 (0.067)	-0.001 (0.987)
Blood pressure				
Systolic pressure	0.002 (<0.001)	0.017 (<0.001)	0.018 (<0.001)	0.003 (<0.001)
Diastolic pressure	0.004 (<0.001)	0.024 (<0.001)	0.034 (<0.001)	0.006 (<0.001)
Heart rate	0.004 (<0.001)	0.020 (<0.001)	0.023 (<0.001)	0.002 (0.157)
Birth measurements				
Birth weight (lb)	0.006 (0.486)	0.047 (0.234)	0.025 (0.530)	-0.003 (0.804)
Head circumference (in)	0.018 (0.233)	0.043 (0.532)	0.081 (0.254)	-0.007 (0.737)
Chest circumference (in)	0.033 (0.009)	0.102 (0.093)	0.155 (0.011)	0.010 (0.588)
Abdominal circumference (in)	0.036 (0.001)	0.105 (0.046)	0.152 (0.004)	0.007 (0.677)
Length (in)	0.015 (0.151)	0.111 (0.020)	0.078 (0.109)	-0.002 (0.897)
Head:abdominal circumference	-0.550 (0.001)	-1.621 (0.035)	-2.160 (0.005)	-0.201 (0.406)
Head:length	-0.096 (0.755)	-2.193 (0.130)	-0.371 (0.800)	0.054 (0.903)
Ponderal index (oz/in^3 x 1000)	-0.081 (0.310)	-0.429 (0.253)	-0.393 (0.302)	0.023 (0.838)
Placental weight (oz)	0.002 (0.521)	0.026 (0.020)	-0.000 (0.984)	-0.005 (0.171)
Placenta:birth weight	0.026 (0.934)	2.686 (0.071)	-0.833 (0.580)	-0.547 (0.264)
Gestational age (days)	0.000 (0.969)	0.005 (0.169)	0.005 (0.198)	0.003 (0.025)

Aorta to femoral, aorta to radial and aorta to foot pulse wave velocities tended to be higher in men and women who were taller, or who had a smaller body mass index or who were older. However, not all of these relationships were statistically significant at conventional levels (p=0.05). Femoral to foot pulse wave velocity was not related to any of the current body size measurements.

In each arterial segment, pulse wave velocity tended to be faster in men and women with higher systolic or diastolic blood pressure, or a faster heart rate. These relationships were all statistically significant except for that between femoral to foot pulse wave velocity and heart rate.

Aorta to femoral and aorta to foot pulse wave velocity tended to be faster in people who had had a larger chest circumference at birth. Aorta to femoral, aorta to radial and aorta to foot pulse wave

velocities tended to be faster in people who had had a larger abdominal circumference and a smaller head to abdominal circumference ratio at birth. Aorta to radial pulse wave velocity tended to be faster in men and women who were born longer in length, or who had had a heavier placenta. Femoral to foot pulse velocity was not related to any birth size measurement. However, femoral to foot pulse wave velocity did tend to be faster in men and women who were born after a longer period of gestation, and this relationship was statistically significant.

The relationship between pulse wave velocity and evidence of cardiovascular disease or taking of antihypertensive medication was investigated using tabulation of means. The results are displayed in table 3.9.

Table 3.9 Tabulation of means for pulse wave velocity (m/sec) according to presence of ischaemic heart disease and taking of antihypertensive medication.

		Aorta to femoral pulse wave velocity*		Aorta to radial pulse wave velocity		Aorta to foot pulse wave velocity		Femoral to foot pulse wave velocity*	
Ischaemic heart disease									
Absent	*n=277*	5.21		4.94		6.97		9.97	
Possible	*n=84*	4.95		4.83		6.81		9.97	
Definite	*n=118*	4.57	(<0.001)	4.52	(0.003)	6.41	(<0.001)	9.78	(0.954)
Rose chest pain questionnaire identification of ischaemic heart disease									
Absent	*n=312*	5.05		4.89		6.93		10.18	
Present	*n=150*	4.66	(<0.001)	4.53	(0.002)	6.32	(<0.001)	9.21	(0.011)
Electrocardiogram identification of ischaemic heart disease									
Absent	*n=328*	5.16		4.93		6.96		9.97	
Possible	*n=101*	4.85		4.73		6.72		9.87	
Definite	*n=49*	4.14	(<0.001)	4.30	(<0.001)	6.02	(<0.001)	9.39	(0.506)
Antihypertensive medication									
Not taking	*n=287*	5.10		4.85		6.89		9.97	
Taking	*n=194*	4.81	(0.082)	4.76	(0.391)	6.66	(0.094)	9.87	(0.890)

* geometric mean values in parentheses are the p values of the difference in the mean

In all arterial segments, pulse wave velocity tended to be slower in men and women with possible or definite ischaemic heart disease compared to those without. This was found to be the case whether ischaemic heart disease was identified through the Rose chest pain questionnaire, or through Minnesota coding of the electrocardiogram. Each of the differences were statistically significant, except in the femoral to foot segment where only the change in mean pulse wave velocity according to Rose chest pain questionnaire identification of ischaemic heart disease was statistically significant. Mean pulse wave velocity was slower in men and women who were taking antihypertensive medication, although these differences were not statistically significant.

Multiple linear regression analysis was used to investigate the simultaneous relationships between pulse wave velocity and birth size measurements after adjusting for the potential confounding effects of age, sex, height, electrocardiogram identification of ischaemic heart disease (referred to as electrocardiogram finding), systolic blood pressure and gestational age. The results are summarised in tables 3.10 to 3.13.

3.6.1 Elastic arteries : aorta to femoral segment

Table 3.10 Multivariate analysis for logged aorta to femoral pulse wave velocity with birth size measurements adjusted for: -

i age, sex and height.

ii age, sex, height and electrocardiogram finding.

iii age, sex, height, electrocardiogram finding and systolic blood pressure.

iv age, sex, height, electrocardiogram finding and gestational age.

Birth measurement	**i** Regression coefficient (p value)	**ii** Regression coefficient (p value)	**iii** Regression coefficient (p value)	**iv** Regression coefficient (p value)
Birth weight (lb)	-0.007 (0.403)	-0.012 (0.146)	-0.014 (0.089)	-0.018 (0.094)
Head circumference (in)	-0.004 (0.978)	-0.005 (0.742)	-0.006 (0.668)	-0.008 (0.629)
Chest circumference (in)	0.019 (0.152)	0.007 (0.543)	0.003 (0.825)	0.001 (0.949)
Abdominal circumference (in)	0.026 (0.024)	0.015 (0.165)	0.010 (0.353)	0.016 (0.204)
Length (in)	0.004 (0.650)	0.006 (0.550)	0.002 (0.842)	-0.002 (0.886)
Head:abdominal circumference	-0.494 (0.002)	-0.354 (0.024)	-0.276 (0.067)	-0.382 (0.028)
Head:length	-0.126 (0.681)	-0.233 (0.420)	-0.189 (0.498)	-0.145 (0.660)
Ponderal index (oz/in^3 x 1000)	-0.120 (0.128)	-0.188 (0.014)	-0.175 (0.017)	-0.173 (0.050)
Placental weight (oz)	-0.003 (0.884)	-0.001 (0.605)	-0.002 (0.438)	0.000 (0.924)
Placenta:birth weight	0.102 (0.747)	0.134 (0.656)	0.126 (0.662)	0.357 (0.295)
Gestational age (days)	-0.002 (0.756)	0.000 (0.624)	0.000 (0.323)	/

After adjusting for the effects of age, sex and height, aorta to femoral pulse wave velocity tended to be faster in men and women who had had a larger abdominal circumference or a larger abdominal circumference in relation to their head circumference at birth. Adding electrocardiogram identification of ischaemic heart disease into the model weakened these relationships. After electrocardiogram identification of ischaemic heart disease had been added into the model, aorta to femoral pulse wave velocity tended to be faster in men and women who had had a larger abdominal circumference in relation to their head circumference (figure 3.3) or a smaller ponderal index at birth (figure 3.4). Adding systolic blood pressure into the model slightly weakened these relationships. Adding gestational age into the model strengthened the relationship between a faster aorta to femoral pulse wave velocity and a larger abdominal

circumference in relation to head circumference at birth. However, the relationship between a faster aorta to femoral pulse wave velocity and a smaller ponderal index at birth was changed little in model iv.

Figure 3.3 Scatterplot of logged aorta to femoral pulse wave velocity against head to abdominal circumference ratio after adjusting for age, sex , current height and electrocardiogram finding.

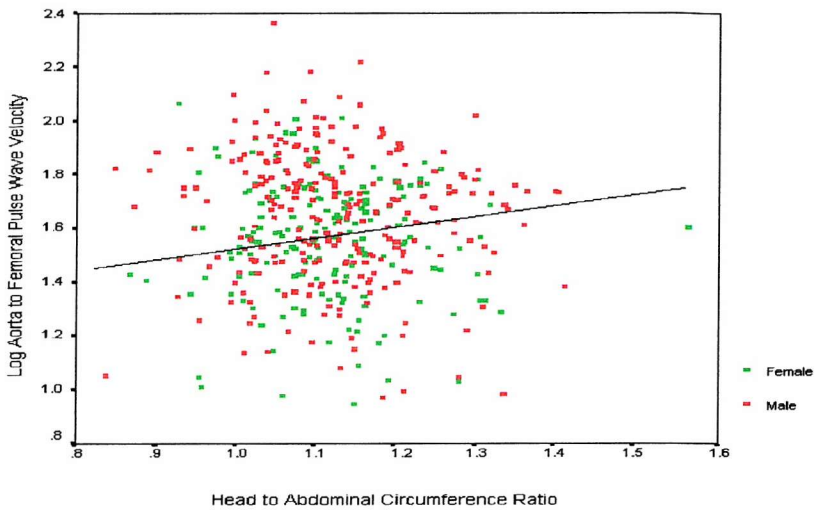
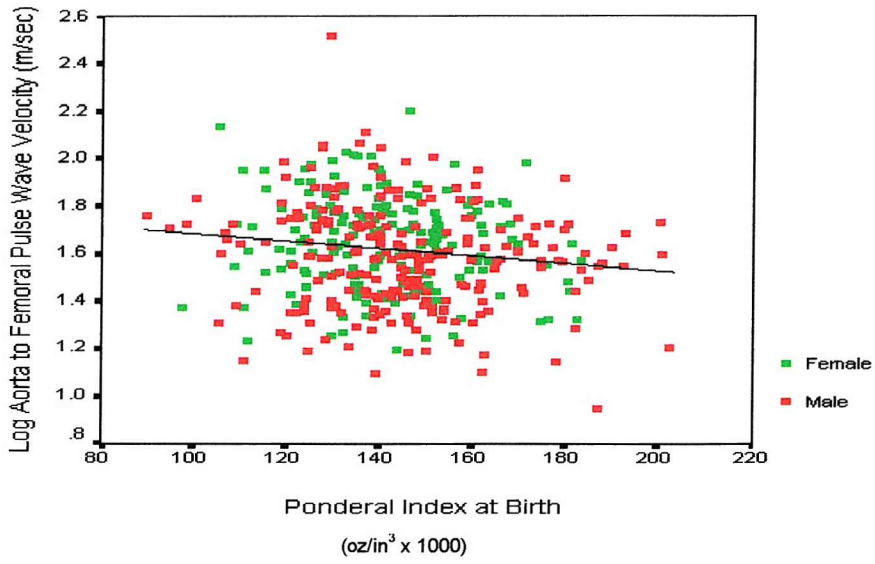


Figure 3.4 Scatterplot of logged aorta to femoral pulse wave velocity against ponderal index at birth after adjusting for age, sex, current height and electrocardiogram finding.



3.6.2 Muscular arteries with high elastin content : aorta to radial segment

Table 3.11 Multivariate analysis for aorta to radial pulse wave velocity with birth size measurements adjusted for: -

i age, sex and height.

ii age, sex, height and electrocardiogram finding.

iii age, sex, height, electrocardiogram finding and systolic blood pressure.

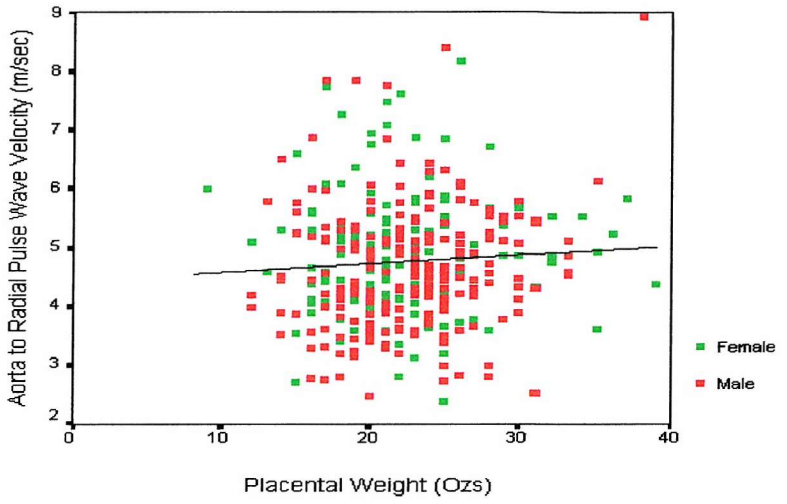
iv age, sex, height, electrocardiogram finding and gestational age.

Birth measurement	**i** Regression coefficient (p value)	**ii** Regression coefficient (p value)	**iii** Regression coefficient (p value)	**iv** Regression coefficient (p value)
Birth weight (lb)	0.024 (0.566)	0.013 (0.764)	0.022 (0.591)	0.008 (0.889)
Head circumference (in)	-0.021 (0.774)	-0.035 (0.631)	-0.029 (0.664)	-0.146 (0.103)
Chest circumference (in)	0.052 (0.415)	0.030 (0.641)	0.014 (0.810)	-0.049 (0.538)
Abdominal circumference (in)	0.077 (0.170)	0.054 (0.332)	0.036 (0.496)	0.015 (0.823)
Length (in)	0.092 (0.068)	0.094 (0.059)	0.085 (0.070)	0.072 (0.223)
Head:abdominal circumference	-1.580 (0.051)	-1.326 (0.103)	-0.996 (0.191)	-1.419 (0.126)
Head:length	-2.584 (0.081)	-2.918 (0.046)	-2.827 (0.040)	-4.111 (0.015)
Ponderal index (oz/in^3 x 1000)	-0.448 (0.250)	-0.617 (0.115)	-0.466 (0.207)	-0.450 (0.335)
Placental weight (oz)	0.027 (0.020)	0.025 (0.028)	0.029 (0.008)	0.033 (0.015)
Placenta:birth weight	3.515 (0.020)	0.549 (0.018)	3.956 (0.005)	4.072 (0.021)
Gestational age (days)	0.005 (0.209)	0.004 (0.251)	0.002 (0.505)	

After adjusting for the effects of age, sex and height, aorta to radial pulse wave velocity tended to be faster in men and women who had had a larger abdominal circumference in relation to their head circumference, a heavier placenta (figure 3.5) or a lighter birth weight in relation to their placental weight at birth. When electrocardiogram identification of ischaemic heart disease was added into the model (ii), these relationships were weakened. However, the relationships between a faster aorta to radial pulse wave velocity and a heavier placenta or a lighter birth weight in relation to placental weight at birth remained statistically significant. A relationship between faster aorta to radial pulse wave velocity and longer length in relation to head circumference also became apparent. When head circumference and length at birth were added into model ii together, the relationship between aorta to radial pulse wave velocity and longer length at birth was stronger (regression coefficient =0.118, p=0.003) than that between aorta to radial pulse wave velocity and a small head circumference at birth (regression coefficient = 0.079, p=0.21). Similarly, after adding placental weight and birth weight into model ii together, the relationship between aorta to radial pulse wave velocity and placental weight (regression coefficient = 0.040, p=0.007) was stronger than that between aorta to radial pulse wave velocity and birth weight (regression coefficient = -0.008, p=0.115). Adding systolic pressure into model iii had little effect on the

relationships between aorta to radial pulse wave velocity and size at birth, except for the relationship with placenta to birth weight ratio, which was strengthened. Adding gestational age into the regression models also strengthened the relationships between a faster aorta to radial pulse wave velocity and a smaller head to length ratio, a heavier placenta and a larger placenta to birth weight ratio.

Figure 3.5 Scatterplot of aorta to radial pulse wave velocity against placental weight at birth after adjusting for age, sex, current height and electrocardiogram finding.



3.6.3 Elastic and muscular arteries : aorta to foot segment

Table 3.12 Multivariate analysis for aorta to foot pulse wave velocity with birth size measurements adjusted for: -

i age, sex and height.

ii age, sex, height and electrocardiogram finding.

iii age, sex, height, electrocardiogram finding and systolic blood pressure.

iv age, sex, height, electrocardiogram finding and gestational age.

Birth measurement	**i** Regression coefficient (p value)	**ii** Regression coefficient (p value)	**iii** Regression coefficient (p value)	**iv** Regression coefficient (p value)
Birth weight (lb)	-0.020 (0.644)	-0.041(0.337)	-0.039 (0.420)	-0.046 (0.421)
Head circumference (in)	0.011 (0.886)	-0.010 (0.887)	-0.018 (0.972)	-0.059 (0.531)
Chest circumference (in)	0.099 (0.124)	0.062 (0.330)	-0.041 (0.506)	0.055 (0.498)
Abdominal circumference (in)	0.104 (0.065)	0.068 (0.221)	0.053 (0.392)	0.070(0.317)
Length (in)	0.034 (0.506)	0.037 (0.461)	-0.019 (0.459)	-0.051 (0.400)
Head:abdominal circumference	-1.695 (0.037)	-1.281 (0.110)	-1.153 (0.284)	-1.534 (0.107)
Head:length	-0.237 (0.873)	-0.707 (0.625)	-0.550 (0.617)	-1.554 (0.371)
Ponderal index (oz/in^3 x 1000)	-0.407 (0.297)	-0.679 (0.078)	-0.522 (0.102)	-0.748 (0.116)
Placental weight (oz)	-0.006 (0.576)	-0.010 (0.389)	-0.010 (0.616)	-0.001 (0.959)
Placenta:birth weight	-0.422 (0.780)	-0.362 (0.807)	-0.075 (0.851)	0.358 (0.841)
Gestational age (days)	0.004 (0.261)	0.004 (0.308)	0.001 (0.700)	

After adjusting for the effects of age, sex and height, aorta to foot pulse wave velocity tended to be faster in men and women who had had a large abdominal circumference in relation to their head circumference at birth. When electrocardiogram identification of ischaemic heart disease was added into the model, this relationship was weakened and was no longer statistically significant. There were no relationships between aorta to foot pulse wave velocity and birth measurements in the other models.

3.6.4 Muscular artery with low elastin content: femoral to foot segment

Table 3.13 Multivariate analysis for logged femoral to foot pulse wave velocity with birth size measurements adjusted for: -

i age, sex and height.

ii age, sex, height and electrocardiogram finding.

iii age, sex, height, electrocardiogram finding and systolic blood pressure.

iv age, sex, height, electrocardiogram finding and gestational age.

Birth measurement	**i** Regression coefficient (p value)	**ii** Regression coefficient (p value)	**iii** Regression coefficient (p value)	**iv** Regression coefficient (p value)
Birth weight (lb)	0.006 (0.655)	0.005 (0.720)	0.007 (0.633)	0.003 (0.876)
Head circumference (in)	0.005 (0.830)	0.004 (0.869)	0.007 (0.758)	0.010 (0.728)
Chest circumference (in)	0.025 (0.224)	0.023 (0.270)	0.017 (0.398)	0.024 (0.357)
Abdominal circumference (in)	0.016 (0.366)	0.014 (0.433)	0.009 (0.627)	0.092 (0.676)
Length (in)	0.005 (0.770)	0.005 (0.759)	0.005 (0.719)	0.017 (0.366)
Head:abdominal circumference	-0.223 (0.392)	-0.227 (0.447)	-0.080 (0.753)	-0.222 (0.460)
Head:length	0.125 (0.787)	0.121 (0.828)	0.097 (0.827)	-0.332 (0.534)
Ponderal index (oz/in^3 x 1000)	0.704 (0.543)	0.706 (0.621)	0.067 (0.572)	-0.023 (0.874)
Placental weight (oz)	-0.004 (0.332)	-0.004 (0.315)	-0.030 (0.426)	-0.004 (0.390)
Placenta:birth weight	-0.568 (0.259)	-0.564 (0.266)	-0.424 (0.383)	-0.451 (0.445)
Gestational age (days)	0.003 (0.033)	0.003 (0.034)	0.002 (0.071)	

Femoral to foot pulse wave velocity was not related to any of the birth measurements. However, after adjusting for age, sex and height, femoral to foot pulse wave velocity tended to be faster in men and women who were born after a longer period of gestation. However, this relationship was weakened and was not statistically significant after systolic blood pressure had been added into the regression model.

3.7 Discussion

On average systolic blood pressure was higher in people with a larger current body size although none of the relationships were statistically significant. Diastolic blood pressure tended to be higher in men and women who were taller or heavier, and these relationships were statistically significant. These results are consistent with other studies which have reported that people with a larger current body size tend to have raised blood pressure.[21; 137]

In order to investigate the effects of potential confounding variables, information about known factors that may confuse the relationships between birth size and raised blood pressure was collected during the study. Lifestyle factors such as social class, alcohol consumption and smoking were examined and were found to be unrelated to blood pressure level.

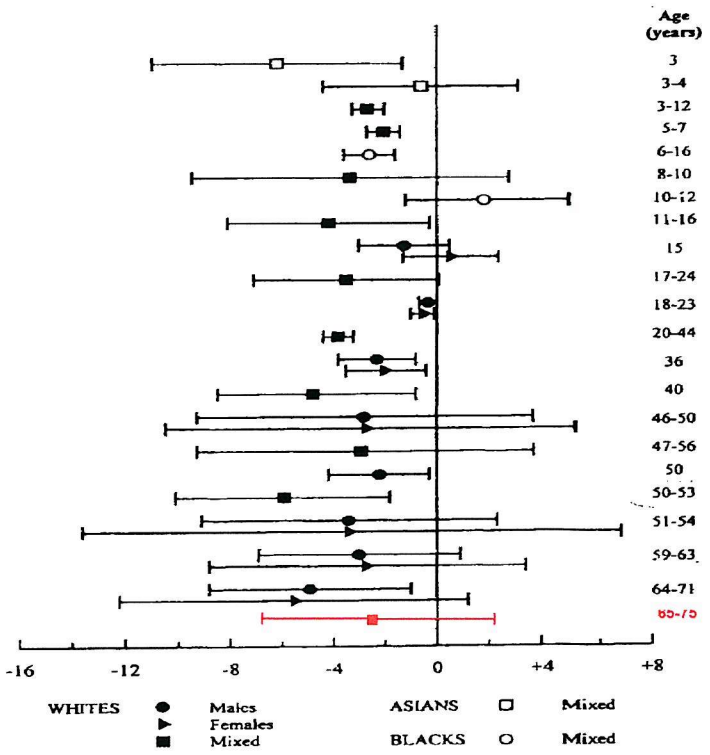
3.8 Blood pressure and birth weight

After adjusting for the effects of age, sex, current weight and gestational age, systolic blood pressure tended to be higher in people who were lighter at birth. Multiple linear regression analysis showed that for each pound increase in birth weight, systolic blood pressure tended to fall by 1.1 mmHg (95% C.I. -3.13 to 0.93), although the relationship was not statistically significant.

A relationship between blood pressure and birth weight may have been obscured in subjects who were taking antihypertensive medication. If men and women with a lower birth weight were more likely to be taking antihypertensive medication, the blood pressure lowering effect of the medication could obscure any relationship with low birth weight. The mean birth weight of people who were taking antihypertensive medication was indeed lower (7.28 lb) than those who were not (7.41 lb), but the difference was not statistically significant (p=0.2). Including a term for antihypertensive medication in multiple linear regression analysis had no statistically significant effect on the relationship between systolic blood pressure and birth weight.

A systematic review which included 34 studies that describe the relationship between fetal growth and blood pressure in more than 66 000 children, adolescents and adults, showed that systolic blood pressure tended to be higher in individuals who had been light as babies.[19] The review also suggested that the magnitude of the relationship between weight at birth and raised blood pressure tended to increase with age (figure 3.6). Therefore, the results of the present analysis were compared with studies that investigated similar elderly age groups. Only one such study was found and this showed that in 64 to 71 year old men and women, systolic blood pressure tended to increase by 5.2 mmHg (95% C.I. -1.8 to -8.6) with each kilogram decrease in birth weight.[11] Conversion of the birth weight values from pounds to kilograms (1 lb = 0.454 kg) in the present study revealed that systolic blood pressure tended to increase by 2.4 mmHg (95% C.I. -6.9 to 2.1) for each kilogram decrease in birth weight.

Figure 3.6 Difference in systolic blood pressure per kilogram increase in birth weight.[19]



The results of the present study have been superimposed onto figure 3.6 (**shown in red**) in order to compare them with those of the previous studies. The regression coefficient of the present study is within the 95% confidence intervals of all of the studies of both middle aged and elderly adults. Therefore, the results of the present study although not statistically significant are consistent with previously published findings.

3.9 Blood pressure and birth size

Birth weight is a summary measure of fetal growth, which includes for example head size, length and fatness. Birth weight alone does not distinguish between babies who were born proportionately small, disproportionate or thin. Therefore, investigating birth size measurements provides more information about fetal growth.[138] In the present study, after taking into account the effects of age, sex and current body mass index, systolic pressure tended to be higher in men and women who had had a lower ponderal index at birth. However the relationship was not statistically significant (table 3.5). People who were born after a shorter length of gestation may have had smaller body dimensions at birth because they were delivered prematurely rather than because they were growth restricted in utero. However, adding of gestational age into the model in table 3.5 strengthened the relationship between raised systolic blood pressure and a low ponderal index. This finding suggests that men and women tended to have raised systolic blood

pressure because they had had a smaller ponderal index at birth, not because they had been delivered prematurely.

Ponderal index describes soft tissue mass in relation to skeletal growth. A low ponderal index indicates thinness. In the present study systolic blood pressure tended to be higher in men and women who had been longer in length at birth. If these people were long in length but had a normal birth weight their ponderal index would be low and this may explain the relationship between raised systolic blood pressure and a low ponderal index rather than actual thinness at birth. To investigate this, weight and length at birth were added into the regression model together (model i, table 3.5). The strength of the relationship between systolic blood pressure and longer length at birth (regression coefficient = 2.66, p=0.07) was similar to that between systolic blood pressure and a lighter birth weight (regression coefficient = -2.64, p=0.06). This suggests that thinness, not longer length at birth is responsible for the relationship between systolic blood pressure and ponderal index.

The results of the present study are consistent with several other studies that have investigated blood pressure in relation to fetal size. For example, a study of 4 year old children in Salisbury and an investigation of 45 year old men and women in China both found that systolic and diastolic blood pressure fell progressively as ponderal index at birth increased.[10; 25] Similarly a study of 46 to 54 year olds in Preston showed a strong relationship between thinness at birth and raised blood pressure after taking into account placental weight.[139] However, these findings are not universal. For example, two studies of 8 to 11 year old children have shown no relationship between raised systolic blood pressure and ponderal index at birth.[29; 31]

The process that links a low ponderal index to raised systolic blood pressure in later life is not known. However, it has been suggested the impaired blood vessel growth resulting in reduced vessel compliance could be responsible. In the present study, arterial compliance data was collected through measurement of pulse wave velocity to investigate arterial stiffness as a possible mechanism linking poor fetal growth to raised blood pressure.

3.10 Pulse wave velocity

The average pulse wave velocities in the 65 to 75 year old men and women in the present study were 5.1 m/sec in the aorta to femoral segment, 4.8 m/sec in the aorta to radial segment, 6.8 m/sec in the aorta to foot segment and 10.6 m/sec in the femoral to foot segment.

Pulse wave velocity has been shown to increase with age.[113] Therefore, the pulse wave velocity values found in the present study were compared with other estimates of pulse wave velocity in similar age groups. Two previous studies, which report pulse wave velocity in elderly subjects were identified and the results are summarised in table 3.14. In the first of the studies [140] an

optical technique similar to that of the present study was used, the other study used Doppler methodology.[118]

Table 3.14 Mean pulse wave velocities measured in elderly populations.

1st Author and year of publication	Age (years)	Arterial Segment	Pulse wave velocity (m/sec)
Eliakim 1971	> 60	Heart to dorsalis pedis	7.2
Present study	**65 to 75**	**Aorta to foot**	**6.8**
Avolio 1985	70	Aorta to femoral	12.5
Avolio 1985	70	Aorta to femoral	8.7
Present study	**65 to 75**	**Aorta to femoral**	**5.1**

The study by Eliakim et al. showed that in men aged over 60 years, mean heart to dorsalis pedis pulse wave velocity was 7.2 m/sec.[140] This finding is comparable with the results of the present study where mean pulse wave velocity of the aorta to foot segment was 6.8 m/sec. The study by Avolio et al. reported aorta to femoral pulse wave velocities of 8.7 m/sec in inhabitants of rural Guangzhou at age 70 years, and of 12.5 m/sec in residents of urban Beijing also at 70 years old.[118] In the present study the mean aorta to femoral pulse wave velocity was 5.1 m/sec, which is much slower than both these findings. A difference between the optical and the Doppler methods used in each of the studies can explain much of the variation between these pulse wave velocity estimates. Each of the studies calculated pulse wave velocity by measuring the transit time of the pulse waveform detected between two sites a known distance apart. However, the position of the first of the two sites that were used differed between studies. In the current study the R wave of the electrocardiogram was used as the first site, whereas in the study by Avolio et al. the aortic arch provided the first site. The transit time in the current study therefore included left ventricular contraction time whereas the transit times measured by Avolio et al. did not. An estimate of average left ventricular contraction time (from aortic catheterisation traces of elderly subjects) was subtracted from the aorta to femoral transit times in the present study. The resulting aorta to femoral pulse wave velocity was 11.9 m/sec, which is similar to the estimates measured by Avolio et al. for urban Beijing (12.5 m/sec).[118]

The results of the present study are consistent with previous studies which have shown that pulse wave velocity is associated with several known cardiovascular risk factors including male sex, current height, a faster heart rate and raised systolic and diastolic blood pressure.[70; 141-143] For example, studies have shown that pulse wave velocity tends to be faster in males ranging from the ages of 4 to 70 years compared to females of the same age.[114; 127; 141] This apparent gender effect is thought to be due either to a protective effect of oestrogens in females,[127] atherosclerotic involvement, or differences in vessel structure.[114] Recently, a study has reported a relationship between shorter stature and faster pulse wave velocity.[143] The findings of the present study were not consistent with this because pulse wave velocity tended to be faster in taller subjects. This

relationship with taller height was not explained by the fact that males, who were on average taller than females, also tended to have faster pulse wave velocities. When sex and height were added into a regression model with aorta to femoral pulse wave velocity for example, the effect of height (regression coefficient 0.43, p=0.007) was stronger than that of sex (regression coefficient -0.03, p=0.255). Another recent study found that a faster aortic pulse wave velocity was related to a faster heart rate.[142] Similar associations were shown in the present study. A faster heart rate may be associated with faster pulse wave velocity through poorer physical fitness.[144] The findings that a faster pulse wave velocity was related to higher systolic and diastolic blood pressure is also consistent with other studies which have shown that pulse wave velocities tend to be higher in hypertensive and borderline hypertensive individuals compared to normotensives, although the findings are not universal.[80; 81]

The finding that pulse wave velocity tends to be faster in people with both raised systolic and diastolic blood pressure suggests that an association with antihypertensive medication and pulse wave velocity should have been found. Pulse wave velocity did tend to be slower in people taking antihypertensive medication in all arterial segments, however, none of the relationships were statistically significant (table 3.9). This may be explained by the fact that the various classes of antihypertensive drugs exert different effects on arterial compliance. For example, calcium channel blockers and ACE inhibitors have been shown to decrease pulse wave velocity for an equivalent fall in blood pressure. However, dihydralazine-like drugs, propranolol and diuretics have no effect on pulse wave velocity as blood pressure is lowered.[83; 145]

3.11 Pulse wave velocity and ischaemic heart disease

In the present study, pulse wave velocity was slower in men and women with evidence of ischaemic heart disease. Several previous studies have also reported an association between pulse wave velocity and ischaemic heart disease. For example, one study showed that pulse wave velocity was faster in people with ischaemic heart disease compared to those without.[66] Another study reported no difference in pulse wave velocity between people with ischaemic heart disease and healthy age and sex matched controls.[146] A further study reported that pulse wave velocity was slower in people with ischaemic heart disease, compared to control subjects, although the relationship was not statistically significant.[140]

The finding that pulse wave velocity was slower in people with ischaemic heart disease was unexpected because low arterial compliance has been suggested as a risk factor for cardiovascular disease (detailed in section 1.9). One explanation for these results might be a lowering of blood pressure following myocardial infarction, which has been reported in several epidemiological studies.[147; 148] Due to the curvilinear relationship between arterial compliance and blood pressure, a fall in blood pressure following myocardial infarction would also cause pulse wave velocity to fall. However, systolic blood pressure tended to be higher in people with possible (148 mmHg) or definite (146 mmHg) ischaemic heart disease in the present study compared to

men and women with absent (143 mmHg) ischaemic heart disease, although the difference was not statistically significant at conventional levels (p=0.07). There was no difference in diastolic blood pressure with presence or absence of ischaemic heart disease.

Another possible explanation for the finding that pulse wave velocity was slower in people with ischaemic heart disease lies within the methodology. The pulse wave velocity measurement method used in the present study measured the length of time between the peak of the R wave of the electrocardiogram and the arrival of the wave of dilatation generated from contraction of the left ventricle at a probe placed on a peripheral artery. If the aortic ejection time is longer in people with ischaemic heart disease this would increase the transit time of the pulse wave between the detection sites and result in a slower calculated pulse wave velocity. There is some evidence to suggest that aortic ejection time is indeed longer in people with ischaemic heart disease. For example, male coronary patients aged 60 years and over had a longer mean aortic ejection time (0.127 seconds) measured from the earliest onset of the QRS complex to the foot of the pulse wave form measured at the aortic origin, compared to healthy age matched male controls (0.109 seconds). The difference in mean aortic ejection times was statistically significant (p=<0.01).[66] In order to overcome this methodological problem in the present study, electrocardiogram identification of ischaemic heart disease was added into multivariate models (tables 3.10 to 3.13). Adding electrocardiogram identification of ischaemic heart disease into the models affected the regression coefficients in all the segments, except for the femoral to foot segment where aortic ejection time was not included in the transit time.

3.12 Pulse wave velocity and birth size

3.12.1. Proportionately small

In all of the regression models (tables 3.10 to 3.13), pulse wave velocity in the aorta to femoral, aorta to radial and aorta to foot segments tended to be faster in men and women who had been light at birth although none of these relationships were statistically significant. Aorta to femoral, aorta to radial and aorta to foot pulse wave velocities also tended to be faster in people who had had smaller head circumferences, or larger chest or abdominal circumferences or who were longer in length at birth. Again none of these relationships were statistically significant. In the femoral to foot segment, pulse wave velocity tended to be faster in men and women who were heavier at birth, or in people who had had a larger head, chest or abdominal circumference, or who were longer in length at birth. However, none of these relationships were statistically significant. Therefore, there was little evidence to suggest that pulse wave velocity was related to proportionate growth restriction in any of the arterial segments studied.

3.12.2. Disproportionately small abdominal circumference or length in relation to head size

There were no relationships between aorta to femoral, aorta to foot or femoral to foot pulse wave velocities and head to length ratio at birth in any of the multivariate models. Aorta to radial pulse

wave velocity tended be faster in men and women who were long in relation to their head circumference at birth after adjusting for the effects of age, sex, height, and electrocardiogram identification of ischaemic heart disease and this relationship was statistically significant. However, this relationship was explained by a tendency for men and women to be longer in length at birth rather than being disproportionate in body size (detailed in section 3.6.2). Aorta to femoral pulse wave velocity tended to be faster in men and women whose abdominal circumference was large in relation to their head circumference at birth. However, this relationship was no longer statistically significant when systolic blood pressure was added into the regression model (model iii, table 3.10). Neither aorta to radial, aorta to foot or femoral to foot pulse wave velocities were related to head to abdominal circumference ratio at birth. Pulse wave velocity in the segments studied was therefore, not related to disproportionate fetal growth restriction as identified through head to abdominal or head to length ratios at birth.

3.12.3 Low ponderal index

After adjusting for the effects of age, sex, height and electrocardiogram identification of ischaemic heart disease, aorta to femoral pulse wave velocity tended to be faster in men and women who had had a low ponderal index at birth. Adding gestational age into the regression models had little effect on this relationship. Aorta to radial, aorta to foot and femoral to foot pulse wave velocities were not related to ponderal index at birth. This provides evidence to suggest that pulse wave velocity in the elastic arterial segment was related to a lower ponderal index at birth.

3.12.4 Placental size

There were no relationships between pulse wave velocity in the aorta to femoral, aorta to foot or femoral to foot segments with either placental weight or birth weight in relation to placental weight. In all of the multivariate models (table 3.11) aorta to radial pulse wave velocity tended to be faster in people who had had a heavier placenta and in men and women who were born light in relation to their placental weight. Both of these relationships were statistically significant although the latter finding was explained by a tendency for a heavier placenta rather than disproportion between birth weight and placental weight (detailed in section 3.6.2).

3.12.5 Gestational age

Pulse wave velocity was not related to gestational age in any of the segments, except for the femoral to foot segment. After adjusting for age, sex and height, femoral to foot pulse wave velocity tended to be faster in men and women who were born after a longer period of gestation. The relationship was no longer statistically significant after systolic blood pressure had been added into the model (table 3.13).

3.13 Birth size in relation to 'type' of artery

The results of the present study showed that faster pulse wave velocities in an elastic arterial segment (aorta to femoral) and a muscular arterial segment with high elastin content (aorta to

radial) were related to a low ponderal index (aorta to femoral segment) and a heavier placenta (aorta to radial segment) at birth. Both these birth size measurements indicate fetal growth restriction in the mid to late stages of pregnancy (detailed in section 1.3). Pulse wave velocity in the muscular arterial segment with low elastin content (femoral to foot) was unrelated to any birth size measurements.

These results are consistent with the hypothesis that intrauterine growth restriction in mid to late pregnancy leads to a permanent reduction in arterial elasticity through a reduction in the content of elastin in the arteries. The elastin content of the aortic wall increases linearly through the latter half of pregnancy (figure 1.3), therefore, growth restricting factors would have the greatest effect on elastin production during this time. In the femoral to foot segment, intrauterine growth restriction might not lead to a reduction in pulse wave velocity because the elastin content is too low to be affected. Alternatively the majority of elastin in this segment may have been laid down after the period of intrauterine growth restriction. Indeed elastin synthesis in the femoral to foot segment may have taken place in early postnatal life. Studies on the aortas of pigs have suggested that a temporal gradient of elastin synthesis occurs with distance from the heart.[149] In the days surrounding birth elastin synthesis was found to be maximal in the thoracic aorta and this maximal level of synthesis became progressively distal in the days following birth.[93]

How could being thin or having a heavier placenta at birth reduce arterial compliance? One possibility could be altered activity of trophins or mitogens causing changes in the vessel wall constituents.

3.14 Trophins and mitogens

During fetal growth restriction metabolic adaptations take place that result in changes in the concentration of fetal and placental hormones and growth factors.[150; 151] These include Insulin like Growth Factors, fibroblast growth factor and cortisol. For example, Insulin like Growth Factor 1 levels in blood taken at delivery from the umbilical cords of growth restricted babies who were born at term are lower than those of non-growth restricted infants. Among growth restricted babies, those with a low ponderal index at birth have also been shown to have lower Insulin like Growth Factor 1 levels at delivery.[152; 153] Another study showed that cord serum fibroblast growth factor-2 concentrations at delivery decreased as the birth weights of babies who were born to mothers with diabetes mellitus fell.[154] The change in cortisol in intrauterine growth restricted infants is less clear. For example, an animal study reported lower levels of cortisol in guinea pigs who were growth restricted following unilateral uterine artery ligation.[155] A study in humans found slightly raised levels of cortisol in umbilical cord plasma samples in intrauterine growth restricted babies at delivery compared to controls.[156] Another study found that cord blood plasma cortisol levels were higher in growth restricted babies at delivery compared to controls. These metabolic changes that occur in intrauterine growth restriction may provide the basis for altered fetal programming and

could lead to alterations in the structure of the vasculature through interference with the biosynthesis of vessel wall structural proteins, such as elastin.

Vascular elastin content could be reduced if the hormones whose concentrations are altered during fetal growth restriction are also involved in elastin biosynthesis. For example, developmental regulation of elastin gene expression occurs at the transcriptional level and several modulators have been proposed to control its activation. These include Insulin-like Growth Factor 1, and cortisol.[102;103;157] Animal experiments have shown that in rat neonatal aortic smooth muscle cells, increasing the concentration of Insulin-like Growth Factor 1 results in higher levels of both tropoelastin mRNA and soluble elastin.[158] Similarly, cortisol and glucocorticoids have also been shown to stimulate elastin synthesis in fetal abdominal aortas.[159] Elastin synthesis could also be modified through alterations in the expression of the enzyme lysyl oxidase, which is involved in elastin crosslinking. Effectors such as Fibroblast Growth Factor [160] upregulate expression of this enzyme.

Vascular elastin content could therefore be reduced if the levels of any of these modulators are decreased during periods of fetal growth restriction because elastin biosynthesis would slow. The reduced vascular elastin levels may persist and lead to permanently stiffer arteries. During early postnatal life elastin synthesis ceases so any deficit in elastin content would not be rectifiable.

3.15 Comparison with other studies

There has been one published study that investigated the effects of reduced fetal growth on arterial compliance.[28] A group of 50 year old men and women living in Sheffield, UK were studied. Pulse wave velocity in the aorto-iliac segment tended to be faster in people who had been light or short or who had had a smaller abdominal circumference at birth.[28] The reported trends were weak and not statistically significant. Consistent and stronger relationships were found in the femoro-popliteal-tibial arterial segment (figure 1.6).

The results of the present study are not consistent with those of the previous study. In the present study, although aorta to femoral pulse wave velocity tended to faster in men and women who were lighter at birth, the relationship was not statistically significant. Aorta to femoral pulse wave velocity was not related to shorter length or smaller abdominal circumference at birth in the elderly men and women. No relationships were found between femoral to foot pulse wave velocity and birth measurements in the present study.

3.16 Conclusions

In the present study, after adjusting for sex and current weight, it was found that babies who were thin at birth tended to have higher systolic blood pressure at the age of 65 to 75 years old, although the relationship was of borderline statistical significance. Pulse wave velocity was measured to investigate whether reduced arterial compliance could be a mechanism linking poor

fetal growth and raised blood pressure in adult life. The results showed that men and women who were thin at birth tended to have faster pulse wave velocities in the aorta to femoral segment, and people with heavier placentas at birth tended to have faster pulse wave velocities in their aorta to radial segments. This suggests that reduced arterial compliance could indeed be a mechanism linking reduced fetal growth in mid to late pregnancy to raised blood pressure in adult life.

Chapter 4

Arterial compliance in young adults

This chapter describes a study of young adults who were born in the Farnborough hospital, Kent between 1975 and 1977 where they were measured in detail at birth and in whom recent arterial compliance measurements were made.

4.1 Subjects

The study population consists of a group of men and women who took part in the Brompton study, which was established to investigate the development of children's blood pressure.[161] 1895 babies from 2088 consecutive births at the Farnborough hospital Kent between April 1975 and May 1977 entered the study. All of the infants were born after 37 completed weeks of gestation. Systolic blood pressure was measured in these infants at 4 days and then again at 6 weeks, 6 months, 1 year and then annually until the age of 10 years. Information about each infant's size at birth (birth weight, length and head circumference) was abstracted from the routine obstetric records.

It was not possible to make a full set of blood pressure measurements in each of the 1895 children. The present study is a follow up of those subjects whose blood pressure was measured at 4 days, 6 weeks, 6 months and at least once between 1 and 6 years and at least once between 6 and 10 years of age. 1 188 subjects fulfilled these criteria.

4.2 Methods

Approval was gained from the South and West Multi-centre Research Ethics Committee and the ethics committees of Surrey, Hampshire, Sussex and Kent (detailed in appendix B). The name and date of birth of each of the subjects was then sent to the Office for National Statistics who performed a search of the National Health Service Central Register to trace each individual still living in the defined geographical area (Bromley, Kent, Hampshire, Surrey, East and West Sussex). 765 subjects were identified. The name and NHS number of each subject was sent to the local Family Health Services Authority who were asked to search their records and identify each subjects General Practitioner. Each General Practitioner was then written to and asked to send a letter to their patient inviting them to take part in the study.

731 letters were sent out to General Practitioners to forward to their patients. 21 letters were returned either because the patients were no longer registered with the General Practitioner or because the subject had moved from the address on the General Practitioners records. 402 subjects replied to their letters by returning a slip detailing their address and a contact telephone number. Of the 402 subjects who replied, 360 agreed to participate in the study. 33 of the 42 people who declined gave no reason for their decision, 6 were abroad, 2 were unable to spare

time due to work commitments and 1 subject was pregnant. A field worker then contacted each of the subjects who had agreed to participate and arranged to visit them at home. During the home visit a questionnaire was administered which enquired about factors that may affect blood pressure. These factors included for example, alcohol consumption, taking of therapeutic or recreational drugs and smoking habits. Height was measured with a portable stadiometer and weight was measured using a digital Seca scale. Arterial compliance and blood pressure measurements were made using the methods detailed in chapter 2.

Pulse waveforms were recorded with the infra red probe placed firstly over the radial artery of the left wrist, and then over the posterior tibial artery immediately posterior to the medial malleolus or on the dorsalis pedis of the left foot. This enabled pulse wave velocity to be estimated in the aorto-brachial and aorto-femoro-popliteal-tibial arterial segments. Systolic and diastolic blood pressure was measured using an Omron HEM711 automated sphygmomanometer before the first pulse wave measurement and then directly after each pulse wave recording. Pulse waveforms were not recorded in the aorta to femoral segment in this study because it was felt that measurement at the femoral artery in young adults in their own homes could pose a risk to the personal safety of the fieldworker.

4.3 Analysis

Tabulation of means, univariate and multiple linear regression analysis were used to examine the relationships between measurements of body size at birth, blood pressure and pulse wave velocity. Where frequency distributions of the variables were skewed logarithmic transformations were used.

4.4 Results

347 of the subjects were visited at home by a fieldworker. It was not possible to visit 13 of the 360 subjects who had agreed to participate because 10 were unable to find suitable times for a home visit, 1 had moved away after having agreed to participate and 2 subjects returned their consent forms after the study had finished.

The distribution of birth size measurements, blood pressure and pulse wave velocities of the young adults are presented as histograms in appendix C.

The mean birth measurements and current body size of the 347 men and women who took part in the study are summarised in table 4.1.

Table 4.1 Mean birth measurements and current body size of the young adults.

	Males (n=162)	SD	Females (n=185)	SD	p value of difference	All (n=347)
Current measurements						
Age (years)	22.4	0.67	22.4	0.72	0.836	22.4
Height (cm)	177.09	6.64	164.54	6.46	<0.001	170.41
Weight (kg)	75.02	11.76	62.60	10.93	<0.001	68.40
Body mass index (kg/m^2)	24.10	4.07	23.08	3.84	0.013	23.56
Birth measurements						
Birth weight (kg)	3.43	0.46	3.31	0.42	0.011	3.36
Head circumference (cm)	35.04	1.25	34.57	1.33	0.002	34.80
Length (cm)	50.90	2.93	50.39	2.54	0.089	50.63
Head circumference:length	0.69	0.04	0.69	0.04	0.618	0.69
Ponderal index (kg/m^3)	26.27	4.26	26.04	3.37	0.662	26.15
Placental weight (g)	628.31	132.35	621.32	116.06	0.441	624.63
Placental:birth weight	0.18	0.03	0.19	0.03	0.190	0.19
Gestational age (days)	278	7	279	8	0.111	279

There was no difference in the average age of the men and women who took part in the present study. Men were on average 12.55 cm taller, 12.95 kg heavier and had a body mass index that was 1.02 kg/m^2 greater. All these differences were statistically significant.

On average the men in the present study were larger as babies compared to the women. In men, mean birth weight was 0.12 kg heavier and mean head circumference at birth was 0.47 cm larger than in the women and these differences were statistically significant. On average men were longer in length at birth and had heavier placentas, although neither of these differences were statistically significant.

4.5 Blood pressure

The mean systolic and diastolic blood pressure measurements of the 347 men and women are summarised in table 4.2.

Table 4.2 Mean systolic and diastolic blood pressure measurements (mmHg).

Blood pressure	Males (n=162)	SD	Females (n=185)	SD	P value of difference	All (n=347)
Systolic blood pressure	125.52	10.75	110.26	8.74	<0.001	117.38
Diastolic blood pressure	72.98	7.18	70.25	6.11	<0.001	71.52

On average, men had systolic blood pressure that was 15.26 mmHg higher and diastolic blood pressure that was 2.73 mmHg higher than the women. Both these differences were statistically significant.

Univariate analysis of systolic and diastolic blood pressure with current body and birth size measurements is summarised in table 4.3

Table 4.3 Univariate analysis of systolic and diastolic blood pressure (mmHg) with current body size and birth measurements.

	Systolic pressure		**Diastolic pressure**	
	Regression coefficient	*(p value)*	*Regression coefficient*	*(p value)*
Current body size				
Age (years)	0.969	(0.311)	1.535	(0.003)
Height (cm)	0.595	(<0.001)	0.098	(0.015)
Weight (kg)	0.468	(<0.001)	0.123	(<0.001)
Body mass index (kg/m^2)	0.912	(<0.001)	0.297	(0.002)
Birth measurements				
Birth weight (kg)	0.103	(0.945)	-1.142	(0.166)
Head circumference (cm)	0.038	(0.945)	-0.233	(0.456)
Length (cm)	0.093	(0.721)	-0.084	(0.559)
Head circumference:length	-5.103	(0.781)	-0.469	(0.963)
Ponderal index (kg/m^3)	-1.086	(0.945)	0.346	(0.973)
Placental weight (g)	-0.100	(0.713)	0.022	(0.877)
Placental:birth weight	-0.011	(0.604)	0.012	(0.285)
Gestational age (days)	-0.267	(0.002)	-0.060	(0.210)

Systolic blood pressure tended to be higher in men and in women who were older, or taller, or heavier or who had a larger body mass index. The relationships between systolic blood pressure and current height, weight and body mass index were statistically significant. Systolic blood pressure was not related to birth size measurements in univariate analysis. However, systolic pressure did tend to be higher in men and women who were born after a shorter length of gestation and this relationship was statistically significant.

Diastolic blood pressure tended to be higher in men and women who were older, or taller, or heavier or who had a larger body mass index. All of these relationships were statistically significant. Diastolic blood pressure was not related to birth size measurements in univariate analysis.

Systolic and diastolic blood pressures were related to age, sex and current body mass index, therefore, these variables were adjusted for in multivariate analysis.

Mean blood pressures of men and women who were taking antihypertensive medication compared to those who were not are summarised in table 4.4. The mean blood pressure of women who were taking the contraceptive pill compared to those who were not is also displayed in this table.

Table 4.4 Tabulation of means for blood pressure (mmHg) according to antihypertensive medication and taking of the contraceptive pill.

		Systolic blood pressure		Diastolic blood pressure	
Antihypertensive medication					
Taking medication	n=3	117.45		68.33	
Not taking medication	n=344	109.67	(0.278)	71.55	(0.412)
Contraceptive pill					
Taking the pill	n=115	112.48		71.05	
Not taking the pill	n=70	106.61	(<0.001)	68.95	(0.023)

(values in parentheses show p value of the difference of the mean)

Only 3 men and women were taking antihypertensive medication. Although mean systolic blood pressure was higher in people who were taking antihypertensive medication compared to those who were not, the difference was not statistically significant. Women who were taking the contraceptive pill tended to have systolic blood pressure that was 5.9 mmHg higher and diastolic blood pressure that was 2.1 mmHg higher than women who were not taking the contraceptive pill and both these differences were statistically significant. Therefore, taking of the contraceptive pill was also adjusted for in multivariate analysis.

4.5.1 Systolic blood pressure

The results of the multivariate analysis for systolic pressure are summarised in table 4.5.

Table 4.5 Multivariate analysis of systolic blood pressure (mmHg) with birth size adjusted for: -

i age, sex, current body mass index and taking of the contraceptive pill.

ii age, sex, current body mass index , taking of the contraceptive pill and gestational age.

Birth measurement	**i**		**ii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-2.750	(0.016)	-2.003	(0.092)
Head circumference (cm)	-1.168	(0.004)	-0.948	(0.020)
Length (cm)	-0.242	(0.216)	-0.087	(0.664)
Head circumference:length	-11.190	(0.409)	-15.913	(0.235)
Ponderal index (kg/m^3)	-2.289	(0.596)	-6.881	(0.611)
Placental weight (g)	-0.241	(0.214)	-0.218	(0.256)
Placenta:birth weight	0.002	(0.893)	-0.006	(0.699)
Gestational age (days)	-0.178	(0.007)		

After adjusting for the effects of age, sex, current body mass index and taking of the contraceptive pill, systolic blood pressure tended to be higher in men and women who were light (figure 4.1) or who had had a smaller head circumference at birth (figure 4.2) or who were born after a shorter length of gestation (figure 4.3). Each of these relationships was statistically significant. These relationships were weakened after adding gestational age into the model (ii) and only the relationship between raised systolic blood pressure and a smaller head circumference at birth remained statistically significant.

Figure 4.1 Scatterplot of systolic blood pressure adjusted for age, sex, current body mass index and taking the contraceptive pill against weight at birth.

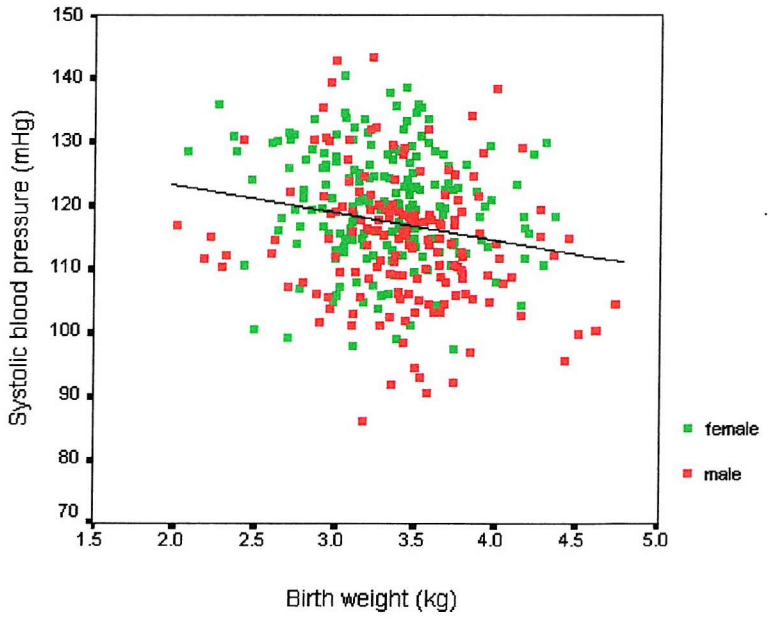


Figure 4.2 Scatterplot of systolic blood pressure adjusted for age, sex, current body mass index and taking the contraceptive pill against head circumference at birth.

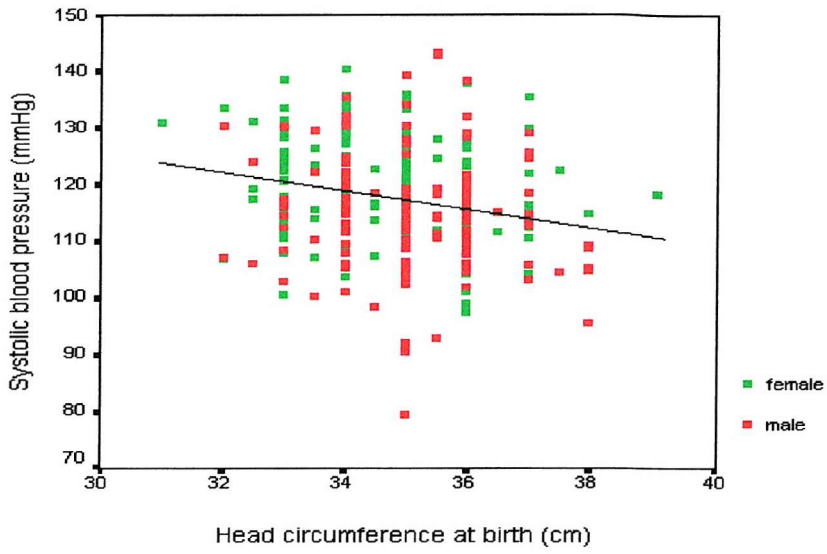
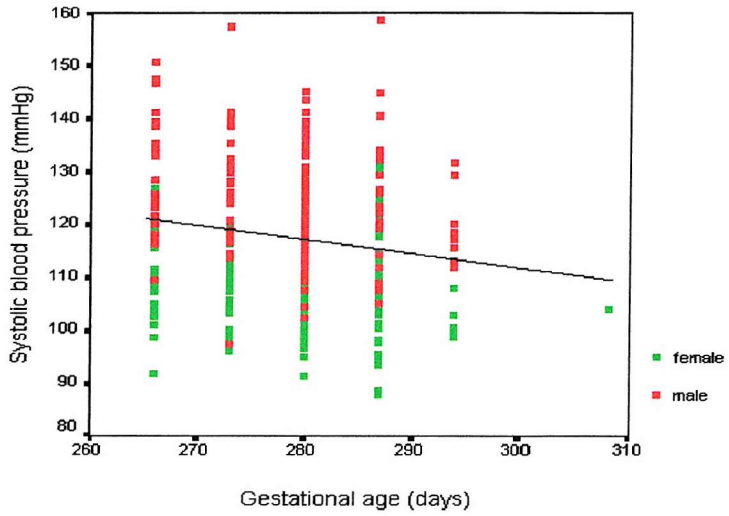


Figure 4.3 Scatterplot of systolic blood pressure adjust for sex, current body mass index and taking the contraceptive pill against gestational age at birth.



4.5.2 Diastolic blood pressure

The results of the multivariate analysis for diastolic blood pressure are summarised in table 4.6.

Table 4.6 Multivariate analysis of diastolic blood pressure (mmHg) with birth size adjusted for: -

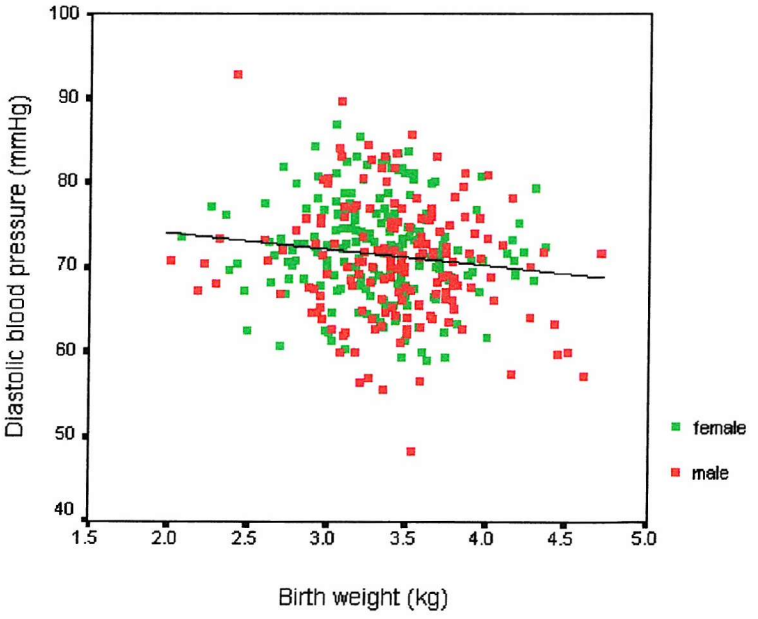
i age, sex, current body mass index and taking of the contraceptive pill.

ii age, sex, current body mass index, taking of the contraceptive pill and gestational age.

Birth measurement	**i**		**ii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-1.643	(0.040)	-1.604	(0.057)
Head circumference (cm)	-0.476	(0.099)	-0.424	(0.150)
Length (cm)	-0.144	(0.300)	-0.109	(0.451)
Head circumference:length	-1.474	(0.879)	-2.717	(0.780)
Ponderal index (kg/m^3)	-0.963	(0.922)	-0.854	(0.931)
Placental weight (g)	-0.041	(0.763)	-0.036	(0.792)
Placenta:birth weight	0.011	(0.310)	0.010	(0.383)
Gestational age (days)	-0.036	(0.443)		

After adjusting for the effects of age, sex, current body mass index and taking of the contraceptive pill, diastolic blood pressure tended to be higher in men and women who were lighter at birth and this relationship was statistically significant (figure 4.4). Adding gestational age into the model (ii) weakened this relationship, which was of borderline statistical significance.

Figure 4.4 Scatterplot of diastolic blood pressure adjusted for age, sex, current body mass index and taking the contraceptive pill against weight at birth.



4.6 Pulse wave velocity

Mean pulse wave velocities in the aorta to radial and aorta to foot segments of the 347 men and women are summarised in table 4.7.

Table 4.7 Mean pulse wave velocities (m/sec) in young adults.

Arterial segment	Males (n=162)	SD	Females (n=185)	SD	P value of difference	All (n=347)
Aorta to radial	4.88	0.68	4.49	0.59	<0.001	4.67
Aorta to foot	5.66*	0.68	5.36*	0.69	<0.001	5.50

* geometric mean

On average, pulse wave velocity was 0.39 m/sec faster in the aorta to radial segment and 0.30 m/sec faster in the aorta to foot segment in the men compared to the women. Both of these differences were statistically significant.

Univariate analysis of pulse wave velocity with current body size, current blood pressure and size at birth are summarised in table 4.8.

Table 4.8 Univariate analysis of pulse wave velocity (m/sec) with current body size and birth measurements.

	Aorta to radial		(Logged) Aorta to foot	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Current body size				
Age (years)	-0.077	(0.129)	-0.026	(0.007)
Height (cm)	0.019	(<0.001)	0.002	(0.004)
Weight (kg)	0.008	(0.004)	0.009	(0.002)
Body mass index (kg/m^2)	-0.002	(0.813)	0.013	(0.171)
Blood pressure				
Systolic pressure (mmHg)	0.019	(0.003)	0.004	(<0.001)
Diastolic pressure (mmHg)	0.024	(<0.001)	0.005	(<0.001)
Heart rate (beats per minute)	0.017	(<0.001)	0.002	(0.007)
Birth measurements				
Birth weight (kg)	0.071	(0.376)	-0.006	(0.695)
Head circumference (cm)	0.049	(0.086)	0.004	(0.484)
Length (cm)	0.005	(0.734)	-0.002	(0.439)
Head circumference:length	0.830	(0.388)	0.230	(0.202)
Ponderal index (kg/m^3)	0.631	(0.519)	0.112	(0.542)
Placental weight (g)	0.015	(0.304)	0.001	(0.780)
Placenta:birth weight	0.001	(0.593)	0.001	(0.670)
Gestational age (days)	-0.008	(0.069)	-0.002	(0.014)

Pulse wave velocity in the aorta to radial segment tended to be faster in men and women who were younger, or taller, or heavier or who had a smaller body mass index. Only the relationships with height and weight were statistically significant. Pulse wave velocity in the aorta to radial segment tended to be faster in people with higher systolic and diastolic blood pressure and in men and women with a faster heart rate. All of these relationships were statistically significant. Pulse wave velocity in the aorta to radial segment also tended to be faster in men and women who had had a larger head circumference at birth and in people who were born after a shorter length of gestation although neither of these relationships were statistically significant at conventional levels.

Pulse wave velocity in the aorta to foot segment tended to faster in people who were younger, or taller or heavier and these relationships were statistically significant. On average, pulse wave velocity in the aorta to foot segment was also faster in men and women with higher systolic and diastolic pressure and in people with a faster heart rate. These relationships were all statistically significant. Aorta to foot pulse wave velocity was not related to any of the birth size measurements. However, aorta to foot pulse wave velocity tended to be faster in people who were born after a shorter length of gestation and this relationship was statistically significant.

Mean pulse wave velocities of men and women who were taking antihypertensive medication or women who were taking the contraceptive pill compared to people who were not are summarised in table 4.9.

Table 4.9 Tabulation of means for pulse wave velocity (m/sec) according to antihypertensive medication and taking of the contraceptive pill.

		Aorta to radial Pulse wave velocity (m/sec)		Aorta to foot pulse wave velocity* (m/sec)	
Antihypertensive medication					
Taking medication	n=3	4.84		5.43	
Not taking medication	n=344	4.67	(0.656)	5.50	(0.910)
Contraceptive pill					
Taking the pill	n=115	4.58		5.68	
Not taking the pill	n=70	4.35	(0.030)	5.65	(0.093)

* geometric mean values in parentheses show p value of the difference of the mean

There were no statistically significant differences in pulse wave velocity between men and women taking antihypertensive medication and those who were no, although the statistical power was insufficient for comparison. On average pulse wave velocity was faster in both the aorta to radial and aorta to foot segments of women who were taking the contraceptive pill, however, the difference was small.

Aorta to radial and aorta to foot pulse wave velocities were related to age, sex, height and taking the contraceptive pill. Therefore, these variables were adjusted for in multivariate analysis.

4.6.1 Muscular arteries with high elastin content : aorta to radial segment

Multivariate analysis of aorta to radial pulse wave velocity and birth size is summarised in table 4.10.

Table 4.10 Multivariate analysis of aorta to radial pulse wave velocity (m/sec) and birth measurements adjusted for the following variables:

i age, sex, height and taking the contraceptive pill.

ii age, sex, height, taking the contraceptive pill and systolic blood pressure.

iii age, sex, height, taking the contraceptive pill and gestational age.

Birth measurements	**i**	**ii**	**iii**
	Regression coefficient (p value)	Regression coefficient (p value)	Regression coefficient (p value)
Birth weight (kg)	-0.012 (0.878)	0.023 (0.764)	0.056 (0.489)
Head circumference (cm)	0.020 (0.456)	0.037 (0.169)	0.046 (0.089)
Length (cm)	-0.002 (0.875)	-0.003 (0.981)	0.006 (0.680)
Head circumference:length	0.581 (0.525)	0.833 (0.351)	0.671 (0.455)
Ponderal index (kg/m^3)	0.338 (0.714)	0.497 (0.580)	0.497 (0.580)
Placenta:birth weight	0.011 (0.419)	0.014 (0.275)	0.015 (0.255)
Placental weight (g)	0.001 (0.356)	0.001 (0.327)	0.001 (0.423)
Gestational age (days)	-0.007 (0.097)	-0.005 (0.250)	

After adjusting for the effects of age, sex, current height and taking the contraceptive pill, aorta to radial pulse wave velocity tended to be faster in men and women who were born after a shorter period of gestation, although this relationship was not statistically significant. Adding systolic blood pressure into the model (ii) weakened this relationship. When gestational age was added into the model (iii) a relationship between a faster aorta to radial pulse wave velocity and a larger head circumference became apparent, although this was not statistically significant at conventional levels (p=0.05).

4.6.2 Elastic and muscular arteries : aorta to foot segment

The frequency distribution of aorta to foot pulse wave velocity was skewed. Transformation using logarithms produced approximately normal distributions.

Multivariate analysis of logged aorta to foot pulse wave velocity and birth size measurements are summarised in table 4.11

Table 4.11 Multivariate analysis of logged pulse wave velocity (m/sec) in the aorta to foot segment and birth measurements adjusted for the following variables :

i age, sex, height and taking the contraceptive pill.

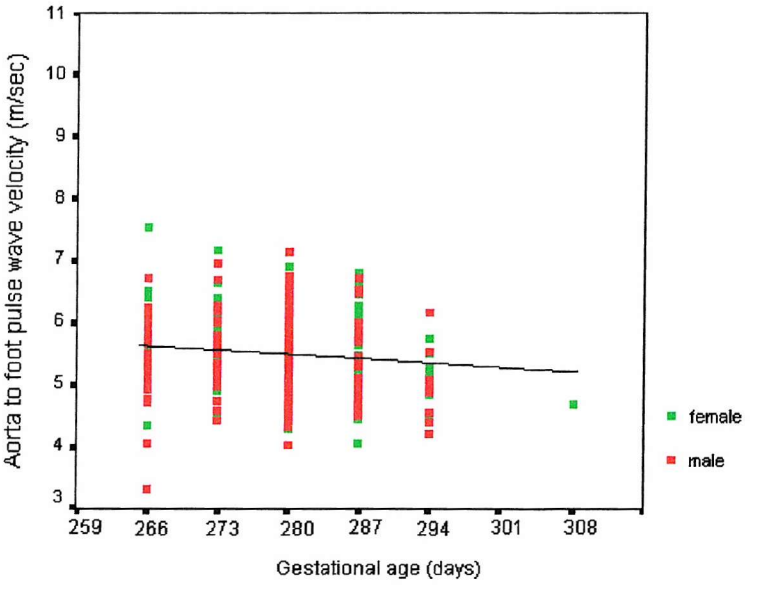
ii age, sex, height, taking the contraceptive pill and systolic blood pressure.

iii age, sex, height, taking the contraceptive pill and gestational age.

Birth measurements	**i**		**ii**		**iii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-0.019	(0.218)	-0.011	(0.458)	-0.003	(0.857)
Head circumference (cm)	0.001	(0.990)	-0.004	(0.459)	-0.006	(0.229)
Length (cm)	-0.003	(0.258)	-0.003	(0.276)	-0.001	(0.631)
Head circumference:length	0.180	(0.302)	0.252	(0.132)	0.206	(0.218)
Ponderal index (kg/m^3)	0.055	(0.755)	0.098	(0.559)	0.099	(0.559)
Placental weight (g)	0.001	(0.815)	0.001	(0.546)	0.002	(0.503)
Placenta:birth weight	0.0002	(0.388)	0.0002	(0.319)	0.0001	(0.482)
Gestational age (days)	-0.002	(0.011)	-0.002	(0.061)		

After adjusting for age, sex, current height and taking the contraceptive pill, aorta to foot pulse wave velocity was faster in men and women who were born after a shorter period of gestation (figure 4.5). This relationship was statistically significant. When systolic blood pressure was added into the model (ii) the relationship was weakened and was no longer statistically significant at conventional levels (p=0.05).

Figure 4.5 Scatterplot of aorta to foot pulse wave velocity after adjusting for the effects of age, sex, current height and taking the contraceptive pill against gestational age.



4.7 Discussion

Systolic and diastolic blood pressure tended to be higher in men and women with a larger current body size, whether measured as height, weight or body mass index. These findings are consistent with previous studies, which have reported that people with a larger current body size tend to have raised blood pressure.[21; 137] Therefore, current body mass index was adjusted for in multivariate analysis. Lifestyle factors such as alcohol consumption, smoking and social class were not related to systolic or diastolic blood pressure levels.

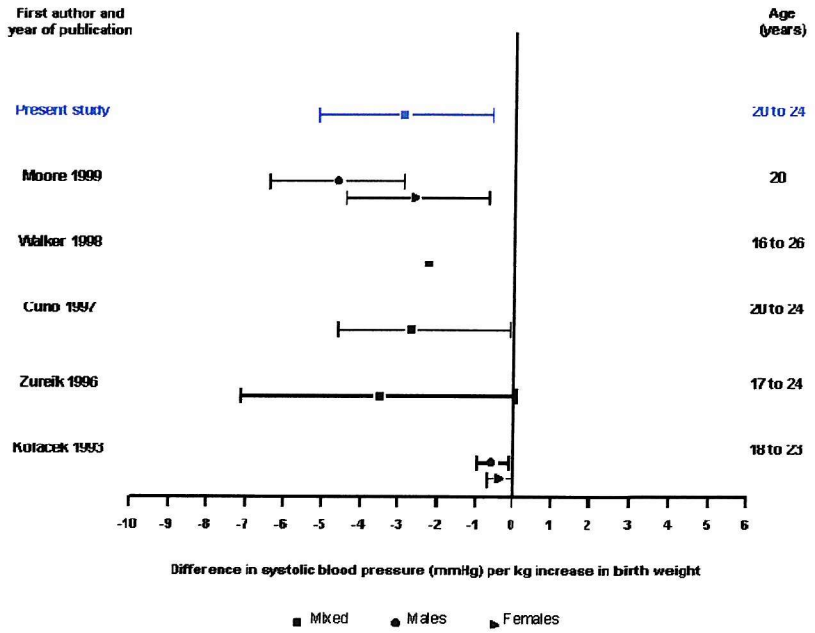
Three of the young adults were taking anti-hypertensive medication (table 4.4). The relationships between systolic and diastolic blood pressure and birth measurements and between pulse wave velocity and birth size measurements were unchanged whether these individuals were included or excluded from the analysis.

4.8 Blood pressure and birth weight

After adjusting for the effects of age, sex, current body mass index and taking the contraceptive pill, for each 1 kg increase in birth weight systolic blood pressure tended to fall by 2.75 mmHg (95% confidence intervals -5.16 to -0.16), and diastolic blood pressure tended to fall by 1.60 mmHg (95% confidence intervals -3.26 to -0.10). Both these relationships were statistically significant.

The results of the present study have been plotted onto figure 4.6 along with those of other studies that have investigated the associations between systolic blood pressure and birth weight in similar age groups.

Figure 4.6 Studies investigating the association between systolic blood pressure and birth weight in young adults (adapted from reference [19]).



From figure 4.6 it can be seen that the regression coefficient of the present study (-2.75) is within the 95% confidence intervals of all the previous studies with the exception of that by Kolacek et al. [162] Therefore, the results of the present study are consistent with most of the previous studies investigating the relationship between birth weight and blood pressure in young adults.[137; 163-165]

4.9 Blood pressure and birth measurements

In the present study it was found that men and women who had had a smaller head circumference at birth tended to have raised systolic and diastolic blood pressure at 20 to 24 years old, although only the relationship with systolic blood pressure was statistically significant. People who were born after a shorter period of gestation also tended to have raised systolic blood pressure and this relationship was statistically significant.

The results of the present study show some consistency with previous studies that have investigated blood pressure in relation to size at birth. For example, 6 other studies of both adults and children have shown that people who had had a smaller head circumference at birth tended to have higher systolic blood pressure in later life. [25; 28; 29; 31; 166; 167] The finding that a shorter gestational age at birth was related to raised blood pressure is not consistent with most of the previous studies.[28; 168] However, one other study has reported a similar inverse relationship between length of gestation and systolic blood pressure.[169]

4.10 Pulse wave velocity

The average pulse wave velocities in the 20 to 24 year old men and women were 4.67 m/sec in the aorta to radial segment and 5.50 m/sec in the aorta to foot segment. Previous studies that have reported mean pulse wave velocities in people of a similar age are summarised in table 4.12.

Table 4.12 Mean pulse wave velocities (m/sec) in young adults.

First author and Year of publication	Age (years)	Pulse wave velocity (m/sec)	Segment
Eliakim 1971	21 to 30	5.7	Aorta to foot
Present study	**20 to 24**	**5.5**	**Aorta to foot**
Avolio 1985	22	8.5	Arm
Avolio 1985	22	11.0	Arm
Present study	**20 to 24**	**4.7**	**Aorta to radial**

Arm = brachial artery to radial artery

In the study by Eliakim et al., mean aorta to foot pulse wave velocity in healthy 21 to 30 year old men was 5.7 m/sec.[140] The mean pulse wave velocity in the aorta to foot segment in the present study was 5.5 m/sec which is consistent with this previous study. In the present study, the mean pulse wave velocity in the aorta to radial segment (4.7 m/sec) is much slower than in the study by Avolio et al (11.0 m/sec).[118] Much of this difference is likely to result from variations in the

methodologies used (detailed in chapter 3, section 3.10). After an estimate of left ventricular contraction time had been subtracted from the pulse wave transit times in the present study, mean aorta to radial pulse wave velocity was 10.8 m/sec, which is comparable with the findings of Avolio et al.[118]

In the present study, pulse wave velocity tended to be faster in men, in people who were heavy, or in men and women who had raised systolic or diastolic blood pressure or a faster heart rate. These findings are consistent with previous studies (detailed in chapter 3, section 3.10).[70; 141-143] In the present study pulse wave velocity was faster in women who were taking the contraceptive pill. Systolic blood pressure was also higher in women taking the contraceptive pill. Therefore, because pulse wave velocity increases as systolic blood pressure rises, women taking the contraceptive pill may have had faster pulse wave velocities because their systolic blood pressure tended to be higher.

4.11 Pulse wave velocity and birth size

4.11.1 Proportionately small

After adjusting for age, sex, current height and taking of the contraceptive pill, aorta to radial and aorta to foot pulse wave velocities tended to be faster in men and women who had been lighter or who were shorter in length or who had had a larger head circumference at birth. However, none of these relationships were statistically significant. Therefore, there was little evidence to suggest that pulse wave velocity was related to proportionate growth restriction in these 20 to 24 year old men and women.

4.11.2 Disproportionately small length in relation to head size

Pulse wave velocity was not related to head circumference to length ratio at birth in either the aorta to radial or aorta to foot segment in any of the multivariate models.

4.11.3 Low ponderal index

Pulse wave velocity was not related to ponderal index at birth in the aorta to radial segment or the aorta to foot segment in any of the multivariate models.

4.11.4 Placental size

Although aorta to radial and aorta to femoral pulse wave velocity tended to be faster in men and women who had had a heavier placenta, the relationships were not statistically significant. Therefore, there is little evidence to suggest that pulse wave velocity was related to placental weight in the 20 to 24 year old men and women.

4.11.5 Gestational age

After adjusting for age, sex, current height and taking of the contraceptive pill, pulse wave velocity in the aorta to foot segment tended to be faster in men and women who were born after a shorter length of gestation and this relationship was statistically significant. Adding systolic blood pressure into the regression model weakened the relationship, which was of borderline statistical significance. Therefore, there is evidence to suggest that pulse wave velocity in a segment containing both elastic and muscular arteries is related to gestational age at birth.

4.12 Birth size in relation to 'type' of artery

In the segment containing muscular arteries with high elastin content (aorta to radial), pulse wave velocity was not related to size at birth. In the segment containing both elastic and muscular arteries (aorta to foot), pulse wave velocity was related to a shorter length of gestation. The results provide weak support for the hypothesis that indicators of restricted fetal growth in the latter stages of pregnancy may lead to permanent reductions in arterial elasticity, although this was only shown in the aorta to foot segment.

4.13 Length of gestation

The relationship between a faster pulse wave velocity in the aorta to foot segment and gestational age at birth was unexpected because previous studies of cardiovascular risk factors, such as raised blood pressure and non-insulin dependant diabetes mellitus, have shown relationships with size at birth that are independent of gestational age.[3; 15-17] However, the findings of the present study can be interpreted.

Major alterations take place in the cardiovascular system at birth. The umbilical circulation ceases and pulmonary blood flow increases on ventilation of the lungs.[170] In the perinatal period physiological changes occur in order to adapt the fetus for the haemodynamic changes that take place at birth. Levels of metabolic, endocrine and neurogenic factors and locally controlled growth modulators alter [171] and these alterations may influence vascular tissue growth, and in particular effect elastin synthesis. Indeed, it has been shown that there is a rapid accumulation of elastin in the days prior to birth. It was previously suggested that this increase in elastin synthesis is related to blood flow changes that occur at birth.[98] However, a recent study of fetal sheep suggested that other physiological factors, such as the late gestational surge in cortisol levels, are instead responsible.[159] A previous study has shown that cortisol can stimulate embryonic and day old chick aortic smooth muscle cells to synthesise elastin.[157] Another study has shown that cortisol levels increase with gestational age of the fetus.[172] Therefore, babies born at a younger gestational age could have stiffer arteries because their lower levels of cortisol, or any other gestational age related factors, may result in a reduced rate of elastin synthesis in the perinatal period that is not rectifiable later.

4.14 Comparison with the published study

The results of the present study were not consistent with those of the previous published study.[28] In the present study there were no statistically significant relationships with lighter birth weight or smaller head circumference or shorter length at birth as were found in the previous study. However, the arterial segments being compared were not the same. In the previous study the aorta to femoral and femoral to foot segments were studied. In the present study the aorta to femoral and femoral to foot segments were studied as one segment (aorta to foot). The aorta to foot segment contains a section of elastic artery (aorta to femoral) and a section of muscular artery with low elastin content (femoral to foot). Therefore, by studying these sections together as one segment, any relationships that exist in one section but not the other may have been obscured. This might account for some of the difference.

4.15 Conclusions

In the present study, after adjusting for the effects of age, sex, current body mass index and taking the contraceptive pill, men and women who were light, or who had had a smaller head circumference at birth or who were born after a shorter length of gestation tended to have raised systolic blood pressure. People who were light at birth also tended to have raised diastolic blood pressure.

In the present study, young adults who were born after a shorter length of gestation tended to have faster pulse wave velocities in the aorta to foot segment. These results provide little support for the hypothesis that fetal growth restraint in the latter stages of pregnancy may lead to a permanent reduction in arterial elasticity and the genesis of raised blood pressure in later life.

Chapter 5

Arterial compliance in ten year old children

This chapter describes a study of ten year old children who were born in the Princess Anne Hospital, Southampton in 1987 where they were measured in detail at birth, and in whom recent arterial compliance measurements were made.

5.1 Subjects and methods

The study subjects consist of a group of children who were selected from a cohort of 380 singletons born consecutively on weekdays during the first four months of 1987 at the Princess Anne Hospital, Southampton. Detailed anthropometric measurements, which were made within three days of delivery under the supervision of Mr T Wheeler (consultant obstetrician) are available for each child. The measurements made included birth weight, length, chest, head and abdominal circumference at birth, placental weight and gestational age.

In 1996, 323 of the children were traced from the details provided in their mothers hospital records. Each child was invited to participate in an echocardiography study. 182 of the children agreed. Due to time and money constraints only 70 of these children could be studied. In order to select 70 subjects, the 182 children were divided in to 5 groups according to their birth weights. 24 children were randomly selected from the lowest birth weight group and 23 from the middle and highest birth weight groups. 4 of the 70 children had congenital heart defects that were not known of at the time of subject selection. These 4 children were excluded from the study leaving a total of 66 children.

Ethical approval was received from the South and West Local Research Ethics Committee. Mr T Wheeler then supplied a database containing the children's names and addresses along with their mothers name and the details of their General Practitioner. A letter was sent to each General Practitioner requesting approval to approach their patient. Once General Practitioner approval had been given, a letter was sent to the mother of each subject. The letter outlined the purpose of the study, described the measurements that would be made and requested permission for their child to participate. A couple of days after anticipated receipt of the letter, a follow up telephone call was made to each mother and a home visit was arranged.

At the home visit written consent was requested from both the mother and child. The child's height was then measured using a portable stadiometer and their weight was measured using a portable digital scale. Arterial compliance and blood pressure measurements were made.

Arterial compliance was measured using the technique outlined in chapter 2. Pulse waveforms were recorded over a 20 second measurement period. The infra red probe was placed firstly over the radial artery of the left wrist, then over the femoral artery below the inguinal ligament on the left hand side and finally over the posterior tibial artery or the dorsalis pedis of the left foot. From

these measurements the arterial segments investigated were the aorto-iliac, aorto-brachial and aorto-femoro-popliteal-tibial segments. An estimate of pulse wave velocity in the femoro-politeal-posterior tibial segment was also possible using the equation detailed in chapter 3, section 3.2.1.

Following each 20 second trace, blood pressure measurements were made at the brachial artery on the left hand side using an Omron HEM711 automated sphygmomanometer.

5.2 Analysis

Tabulation of means and univariate and multivariate linear regression analysis were used to examine the relationships between measurements of size at birth, current body size, blood pressure and pulse wave velocity.

5.3 Results

1 of the 66 children in the original study had moved from the study area (30 mile radius of Southampton General Hospital). Of the remaining 65 children, 64 (98%) agreed to take part in the follow up study. 29 (45.3%) were male and 35 (54.7%) were female. Mean birth and current body size measurements of the 64 children are summarised in table 5.1.

Table 5.1 Mean birth and current body size measurements of the 10 year old children.

	Boys (n=29)	**SD**	**Girls** (n=35)	**SD**	**P value of difference**	**All** (n=64)
Current measurements						
Height (cm)	142.97	6.29	143.36	5.63	0.818	143.18
Weight (kg)	37.19	5.63	38.45	8.25	0.488	37.88
Body mass index (kg/m^2)	18.14	2.13	18.57	2.95	0.521	18.38
Birth measurements						
Birth weight (kg)	3.56	0.56	3.42	0.66	0.385	3.48
Head circumference (cm)	35.76	1.50	35.07	1.67	0.090	35.38
Chest circumference (cm)	33.85	1.82	33.51	2.36	0.527	33.67
Abdominal circumference (cm)	37.81	2.15	36.76	2.28	0.066	37.24
Length (cm)	50.19	2.18	49.60	2.23	0.291	49.87
Head:abdominal circumference	105.75	3.86	104.8	4.84	0.438	105.28
Head circumference:length	0.71	0.02	0.77	0.02	0.338	0.71
Ponderal index (kg/cm^3)	28.01	2.68	27.79	3.27	0.770	27.89
Placental weight (g)	667.31	157.44	614.27	154.90	0.197	637.25
Placental:birth weight	0.18	0.03	0.18	0.03	0.713	0.18
Gestational age (days)	278	12.93	279	9.84	0.402	279

Current body size was similar in boys and girls in the present study. On average, girls were taller, heavier and had a higher body mass index than the boys. However, these differences were very

small and did not reach statistical significance. Boys tended to be larger as babies and weighed on average 0.14 kg more at birth than girls. However, none of the differences in birth measurements were statistically significant.

5.4 Blood pressure

The mean systolic and diastolic blood pressure measurements of the 10 year old children are summarised in table 5.2.

Table 5.2 Mean systolic and diastolic blood pressure measurements (mmHg).

Blood pressure	Boys (n=29)	SD	Girls (n=35)	SD	P value of difference	All (n=64)
Systolic blood pressure	99.61	7.77	98.58	8.40	0.616	99.05
Diastolic blood pressure	60.16	5.23	60.74	5.86	0.680	60.48

Systolic and diastolic blood pressure levels were similar in the boys and girls. Boys had an average systolic blood pressure that was 1.03 mmHg higher and an average diastolic blood pressure that was 0.58 mmHg lower than that of the girls. Neither of these differences were statistically significant.

Linear regression analysis was used to investigate the relationships between blood pressure and current and birth size measurements. The results are summarised in table 5.3.

Table 5.3 Univariate analysis of systolic and diastolic blood pressure (mmHg) with current body size and birth measurements.

	Systolic pressure		Diastolic pressure	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Current body size				
Height (cm)	0.287	(0.022)	0.147	(0.154)
Weight (kg)	0.495	(<0.001)	0.239	(0.013)
Body mass index (kg/m^2)	0.454	(<0.001)	0.734	(0.011)
Birth measurements				
Birth weight (kg)	0.467	(0.781)	-0.277	(0.810)
Head circumference (cm)	-0.004	(0.995)	-0.283	(0.517)
Chest circumference (cm)	0.084	(0.862)	0.210	(0.527)
Abdominal circumference (cm)	-0.113	(0.834)	-0.194	(0.744)
Length (cm)	0.148	(0.751)	0.039	(0.903)
Head:abdominal circumference	-0.078	(0.738)	0.036	(0.822)
Head circumference:length	-20.953	(0.640)	-31.082	(0.312)
Ponderal index (kg/cm^3)	0.058	(0.867)	-0.080	(0.736)
Placental weight (g)	-0.005	(0.430)	-0.004	(0.416)
Placental:birth weight	-50.297	(0.181)	-23.402	(0.362)
Gestational age (days)	0.022	(0.806)	-0.020	(0.750)

In univariate analysis systolic and diastolic blood pressure tended to be higher in boys and girls who were taller in height, heavier in weight and who had a larger body mass index. All of these relationships were statistically significant, except for that between diastolic pressure and height. There were no statistically significant relationships between systolic or diastolic blood pressure and birth measurements.

In multivariate analysis the potential confounding effect of current weight was adjusted for. Gestational age was added into a second model along with current weight.

5.4.1 Systolic blood pressure

The results of the multivariate analysis for systolic blood pressure are summarised in table 5.4.

Table 5.4 Multivariate analysis of systolic blood pressure (mmHg) and birth size adjusted for: -

i current weight.

ii current weight and gestational age.

Birth measurement	i		ii	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-2.051	(0.188)	-1.746	(0.308)
Head circumference (cm)	-0.693	(0.227)	-0.567	(0.375)
Chest circumference (cm)	-0.703	(0.120)	-0.642	(0.207)
Abdominal circumference (cm)	-0.749	(0.807)	-0.675	(0.950)
Length (cm)	-0.406	(0.339)	-0.288	(0.542)
Head:abdominal circumference	0.186	(0.379)	0.154	(0.476)
Head circumference:length	-0.052	(0.683)	-15.419	(0.695)
Ponderal index (kg/cm^3)	-16.062	(0.412)	-0.214	(0.494)
Placental weight (g)	-0.011	(0.064)	-0.012	(0.067)
Placenta:birth weight	-43.615	(0.188)	-42.755	(0.200)
Gestational age (days)	-0.077	(0.350)		

After adjusting for the effects of current weight, systolic blood pressure tended to be higher in children who had had a lighter placenta at birth, although this relationship was not statistically significant at conventional levels (p=0.05). Adding gestational age into the model had little effect on this relationship.

5.4.2 Diastolic blood pressure

The results of the multivariate analysis for diastolic blood pressure are summarised in table 5.5.

Table 5.5 Multivariate analysis of diastolic blood pressure (mmHg) with birth size adjusted for: -

i current weight.

ii *current weight and gestational age.*

Birth measurement	**i**		**ii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-1.419	(0.227)	-1.103	(0.391)
Head circumference (cm)	-0.598	(0.166)	-0.495	(0.302)
Chest circumference (cm)	-0.586	(0.085)	-0.533	(0.163)
Abdominal circumference (cm)	-0.600	(0.720)	-0.531	(0.875)
Length (cm)	-0.200	(0.533)	-0.069	(0.847)
Head:abdominal circumference	0.155	(0.331)	0.128	(0.431)
Head circumference:length	-29.010	(0.325)	-28.475	(0.334)
Ponderal index (kg/m^3)	-0.218	(0.347)	-0.185	(0.430)
Placental weight (g)	-0.006	(0.161)	-0.006	(0.209)
Placenta:birth weight	-20.539	(0.405)	-19.708	(0.200)
Gestational age (days)	-0.065	(0.297)		

After adjusting for weight (model i) and then weight and gestational age (model ii), children who were lighter and smaller at birth tended to have higher diastolic blood pressure. However, none of the relationships were statistically significant.

5.5 Pulse wave velocity

Mean pulse wave velocities for the ten year old boys and girls are summarised in table 5.6.

Table 5.6 Mean pulse wave velocities (m/sec) in ten year olds.

Arterial Segment	**Boys** (n=29)	**SD**	**Girls** (n=35)	**SD**	**P value of difference**	**All** (n=64)
Aorta to femoral	2.72	0.37	2.82	0.44	0.157	2.78
Aorta to radial	3.95	0.46	4.19	0.57	0.014	4.08
Aorta to foot	4.60	0.45	4.82	0.61	0.027	4.72
Femoral to foot	11.14	0.85	11.84	0.92	0.249	11.52

On average, pulse wave velocity was 0.10 m/sec faster in the aorta to femoral, 0.24 m/sec faster in the aorta to radial, 0.22 m/sec faster in the aorta to foot and 0.7 m/sec faster in the femoral to foot segments in girls compared to boys. These relationships were statistically significant in the aorta to radial and aorta to foot segments.

The univariate analysis of pulse wave velocity and current body size, blood pressure and birth size measurements is summarised in table 5.7

Table 5.7 Univariate analysis of pulse wave velocity (m/sec) with current body size and birth measurements.

	Aorta to femoral		**Aorta to radial**		**Aorta to foot**		**Femoral to foot**	
	Regression coefficient	(p value)	Regression coefficient	(p value	Regression coefficient	(p value)	Regression coefficient	(p value)
Current body size								
Height (cm)	0.000	(0.998)	0.005	(0.604)	0.011	(0.302)	-0.015	(0.747)
Weight (kg)	0.011	(0.123)	0.004	(0.645)	0.014	(0.148)	-0.028	(0.503)
Body mass index (kg/m^2)	0.038	(0.055)	0.003	(0.920)	0.031	(0.259)	-0.075	(0.522)
Blood pressure								
Systolic pressure (mmHg)	0.012	(0.013)	0.002	(0.819)	0.0003	(0.959)	-0.024	(0.361)
Diastolic pressure (mmHg)	0.004	(0.566)	0.022	(0.019)	-0.003	(0.729)	0.006	(0.878)
Heart rate (beats per minute)	0.011	(0.036)	0.014	(0.022)	0.012	(0.062)	-0.018	(0.536)
Birth measurements								
Birth weight (kg)	-0.044	(0.482)	-0.077	(0.342)	0.020	(0.866)	0.388	(0.432)
Head circumference (cm)	-0.049	(0.132)	-0.092	(0.029)	-0.012	(0.779)	0.200	(0.285)
Chest circumference (cm)	-0.028	(0.266)	-0.044	(0.175)	-0.020	(0.543)	0.050	(0.698)
Abdominal circumference (cm)	0.001	(0.277)	-0.001	(0.066)	0.001	(0.847)	0.002	(0.239)
Length (cm)	-0.020	(0.398)	-0.013	(0.675)	0.020	(0.527)	0.170	(0.195)
Head:abdominal circumference	0.001	(0.924)	-0.004	(0.821)	0.010	(0.532)	0.038	(0.582)
Head circumference:length	-2.240	(0.328)	-7.268	(0.014)	-3.877	(0.210)	-2.705	(0.838)
Ponderal index (kg/cm^3)	0.004	(0.853)	-0.026	(0.259)	-0.014	(0.567)	-0.023	(0.819)
Placental weight (g)	-0.001	(0.221)	-0.0002	(0.643)	0.0001	(0.412)	0.004	(0.080)
Placenta:birth weight	-0.856	(0.639)	2.910	(0.243)	4.903	(0.048)	22.741	(0.037)
Gestational age (days)	0.007	(0.107)	-0.003	(0.636)	-0.008	(0.192)	-0.007	(0.790)

Children with a larger current body size tended to have faster pulse wave velocities in all arterial segments except for the femoral to foot segment. In particular boys and girls who were heavier tended to have faster aorta to femoral and aorta to foot pulse wave velocities, although neither of these relationships were statistically significant. Children with a faster heart rate tended to have a faster pulse wave velocity in all arterial segments with the exception of the femoral to foot segment. Boys and girls with higher systolic blood pressure tended to have a faster aorta to femoral pulse wave velocity and children with higher diastolic blood pressure levels tended to have a faster aorta to radial pulse wave velocity. Both these relationships were statistically significant.

In general, children who were smaller as babies tended to have faster pulse wave velocities in the aorta to femoral, aorta to radial and aorta to foot segments. In particular, boys and girls who had had a smaller head circumference at birth tended to have faster pulse wave velocities in their aorta

to femoral and aorta to radial segments. Children who were born with a heavier placenta tended to have faster femoral to foot pulse wave velocities. On average, boys and girls with a heavier placenta in relation to their birth weight had faster pulse wave velocities in the aorta to foot and femoral to foot segments. Boys and girls who were born after a shorter length of gestation tended to have faster aorta to femoral and aorta to foot pulse wave velocities. However, not all of these relationships were statistically significant at conventional levels (p=0.05).

Linear regression was used to investigate the simultaneous relationship between pulse wave velocity, birth size measurements and potential confounding factors. Sex and current body weight were related to pulse wave velocity in univariate analysis, therefore, these variables were added into the multivariate model. Sex, current weight and systolic blood pressure were added into a second model. Gestational age was then added into a third model with sex and current weight.

5.5.1 Elastic arteries : aorta to femoral segment

Multivariate analysis of pulse wave velocity in the aorta to femoral segment and birth measurements of the ten year old children are summarised in table 5.8.

Table 5.8 Multivariate analysis of pulse wave velocity (m/sec) in the aorta to femoral segment and birth measurements adjusted for the following variables: -

i sex and weight.

ii sex, weight and systolic blood pressure.

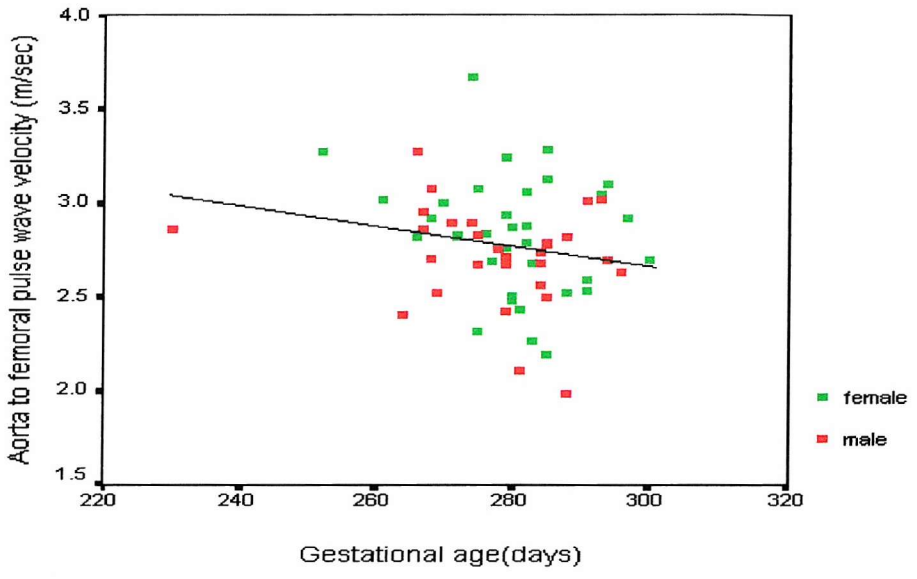
iii sex, weight and gestational age.

Birth measurements	**i**		**ii**		**iii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-0.076	(0.245)	-0.042	(0.524)	-0.008	(0.810)
Head circumference (cm)	-0.059	(0.081)	-0.040	(0.247)	-0.030	(0.425)
Chest circumference (cm)	-0.044	(0.089)	-0.031	(0.238)	-0.022	(0.451)
Abdominal circumference (cm)	-0.002	(0.403)	-0.001	(0.626)	-0.001	(0.721)
Length (cm)	-0.029	(0.243)	-0.017	(0.482)	-0.005	(0.864)
Head:abdominal circumference	0.008	(0.515)	0.006	(0.602)	0.003	(0.800)
Head circumference:length	-1.818	(0.424)	-1.313	(0.554)	-1.676	(0.445)
Ponderal index (kg/cm^3)	-0.010	(0.588)	-0.005	(0.764)	-0.003	(0.846)
Placenta:birth weight	-0.671	(0.713)	-0.171	(0.924)	-0.441	(0.804)
Placental weight (g)	-0.0004	(0.189)	-0.0003	(0.431)	-0.0002	(0.541)
Gestational age (days)	-0.011	(0.023)	-0.010	(0.056)		

After adjusting for the effects of sex and current weight, children who were born with a smaller chest or head circumference at birth tended to have a faster pulse wave velocity in the aorta to femoral segment. However, neither of these relationships were statistically significant. Boys and girls who were born after a shorter length of gestation tended to have faster aorta to radial pulse

wave velocities (figure 5.1) and this relationship was statistically significant. This relationship was changed little when systolic blood pressure was added into the model. Replacing systolic blood pressure with either diastolic blood pressure or heart rate had little effect on the relationship. After adding gestational age into the model, no relationships with pulse wave velocity and birth measurements were present.

Figure 5.1 Scatterplot of aorta to femoral pulse wave velocity adjusted for sex and current weight according to gestational age.



5.5.2 Muscular arteries with high elastin content : aorta to radial segment

The results of the multivariate analysis of aorta to radial pulse wave velocity with birth measurements are summarised in table 5.9.

Table 5.9 Multivariate analysis of aorta to radial pulse wave velocity (m/sec) and birth measurements adjusted for the following variables: -

i sex and weight.

ii sex, weight and systolic blood pressure.

iii sex, weight and gestational age.

Birth measurements	i		ii		iii	
	Regression coefficient	(p value)	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	-0.073	(0.389)	-0.037	(0.395)	-0.023	(0.584)
Head circumference (cm)	-0.083	(0.058)	-0.083	(0.062)	-0.084	(0.063)
Chest circumference (cm)	-0.048	(0.160)	-0.048	(0.165)	-0.044	(0.258)
Abdominal circumference (cm)	-0.004	(0.176)	-0.004	(0.184)	-0.003	(0.236)
Length (cm)	-0.006	(0.852)	-0.005	(0.867)	0.008	(0.822)
Head:abdominal circumference	0.001	(0.934)	0.001	(0.929)	-0.001	(0.941)
Head circumference:length	-6.463	(0.026)	-6.470	(0.027)	-6.396	(0.028)
Ponderal index (kg/cm^3)	-0.027	(0.244)	-0.027	(0.240)	-0.024	(0.300)
Placenta:birth weight	3.163	(0.197)	3.443	(0.175)	3.456	(0.149)
Placental weight (g)	-0.0001	(0.910)	-0.000	(0.934)	0.0003	(0.539)
Gestational age (days)	-0.005	(0.392)	-0.005	(0.395)		

After adjusting for the effects of sex and current weight, children who were small as babies tended to have faster pulse wave velocities in the aorta to radial segment. In particular, boys and girls who had had a small head circumference at birth (figure 5.2), or who had been long in relation to the size of their head circumference (figure 5.3) tended to have faster aorta to radial pulse wave velocities. However, the relationship between head circumference at birth and aorta to radial pulse wave velocity was not statistically significant at conventional levels (p=0.05). Adding systolic pressure or gestational age into the model had little effect on these relationships.

Figure 5.2 Scatterplot of aorta to radial pulse wave velocity adjusted for sex and current weight according to head circumference at birth.

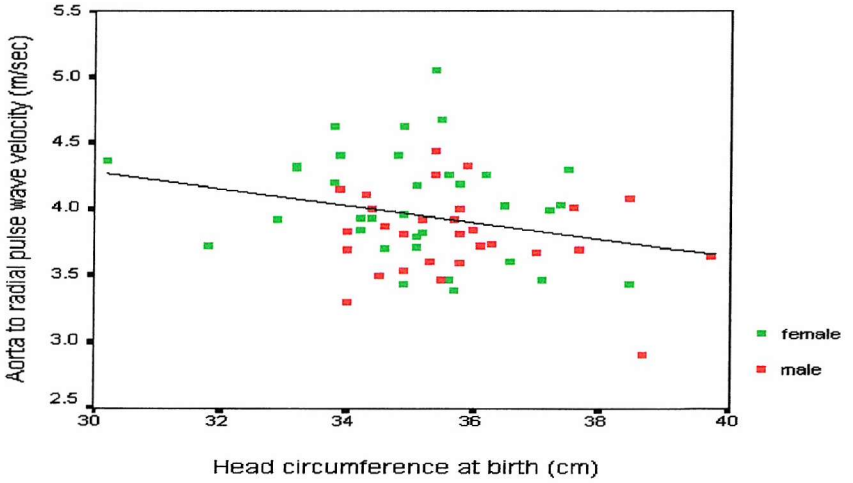
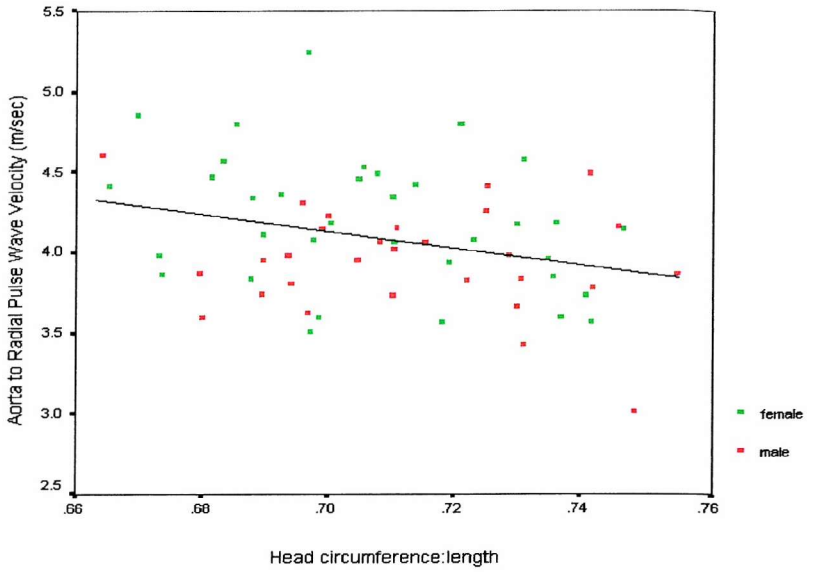


Figure 5.3 Scatterplot of aorta to radial pulse wave velocity adjusted fro sex and current weight according to head circumference to length ratio



5.5.3 Elastic and muscular arteries : aorta to foot segment

The results of the multivariate analysis of aorta to foot pulse wave velocity and birth measurements are summarised in table 5.10.

Table 5.10 Multivariate analysis of pulse wave velocity (m/sec) in the aorta to foot segment and birth measurements adjusted for the following variables :

i sex and weight.

ii sex, weight and systolic blood pressure.

iii sex, weight and gestational age.

Birth measurements	**i**		**ii**		**iii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	0.013	(0.964)	-0.011	(0.926)	0.113	(0.362)
Head circumference (cm)	-0.008	(0.854)	-0.009	(0.841)	0.044	(0.381)
Chest circumference (cm)	-0.036	(0.307)	-0.039	(0.278)	-0.004	(0.919)
Abdominal circumference (cm)	0.002	(0.480)	0.002	(0.463)	0.004	(0.223)
Length (cm)	0.020	(0.547)	0.020	(0.555)	0.063	(0.078)
Head:abdominal circumference	0.021	(0.200)	0.022	(0.177)	0.015	(0.344)
Head circumference:length	-3.035	(0.313)	-3.088	(0.309)	-2.866	(0.328)
Ponderal index (kg/cm^3)	-0.107	(0.408)	-0.022	(0.369)	-0.012	(0.598)
Placental weight (g)	0.0005	(0.281)	0.0005	(0.306)	0.001	(0.027)
Placenta:birth weight	5.254	(0.030)	5.207	(0.033)	5.633	(0.015)
Gestational age (days)	-0.013	(0.042)	-0.013	(0.043)		

After adjusting for the effects of sex and current weight, children who were smaller in size at birth tended to have faster pulse wave velocities in the aorta to foot segment. In particular children who born light in relation to their placental weight tended to have faster aorta to foot pulse wave velocities (figure 5.4). Children born after a shorter period of gestation also tended to have faster pulse wave velocities in the aorta to foot segment (figure 5.5). Both these relationships were statistically significant. Adding systolic blood pressure into the model had little effect on these relationships. Adding gestational age into the model strengthened the relationship between faster aorta to foot pulse wave velocity and placenta to birth weight ratio and a relationship with a heavier placental weight also became apparent.

Figure 5.4 Scatterplot of aorta to foot pulse wave velocity adjusted for sex, current weight and gestational age according to placenta to birth weight ratio.

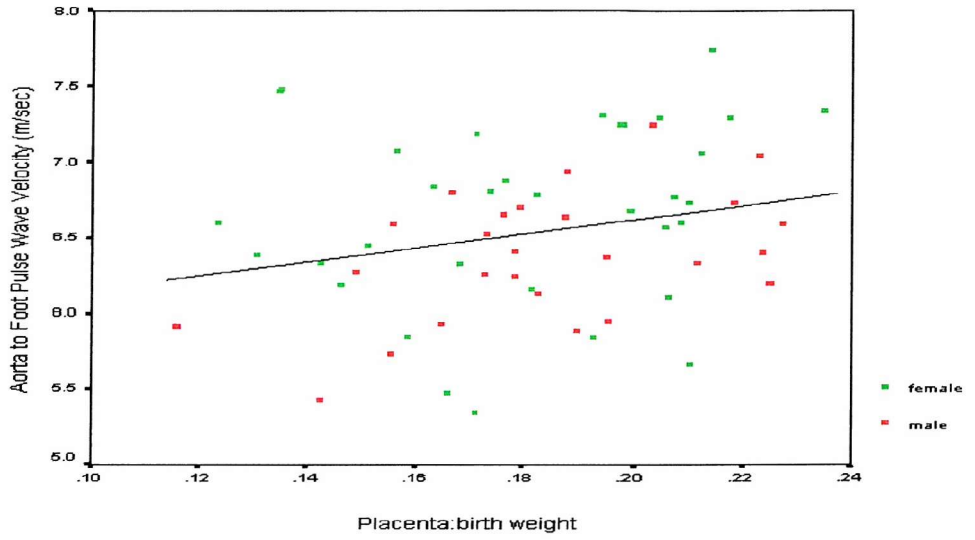
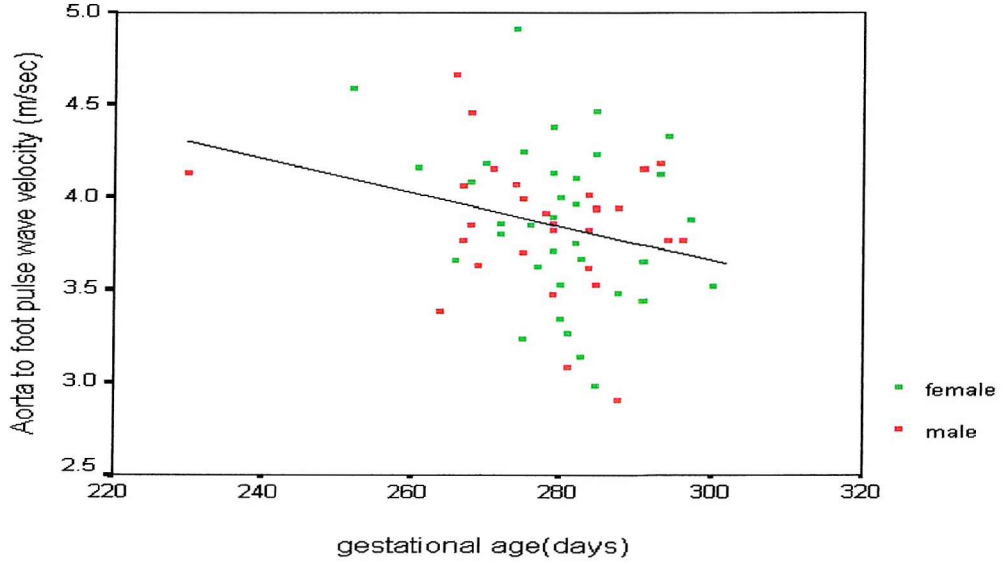


Figure 5.5 Scatterplot of aorta to foot pulse wave velocity adjusted for sex and current weight according to gestational age (days).



5.5.4 Muscular arteries with low elastin content: femoral to foot segment

The results of the multivariate analysis of femoral to foot pulse wave velocity and birth measurements are summarised in table 5.11.

Table 5.11 Multivariate analysis of pulse wave velocity (m/sec) in the femoral to foot segment with birth measurements adjusted for the following variables :

i sex and weight.

ii sex, weight and systolic blood pressure.

iii sex, weight and gestational age.

Birth measurements	**i**		**ii**		**iii**	
	Regression coefficient	(p value)	Regression coefficient	(p value)	Regression coefficient	(p value)
Birth weight (kg)	0.385	(0.197)	0.661	(0.220)	0.865	(0.149)
Head circumference (cm)	0.034	(0.634)	0.041	(0.571)	0.033	(0.657)
Chest circumference (cm)	0.129	(0.402)	0.120	(0.446)	0.183	(0.297)
Abdominal circumference (cm)	0.001	(0.197)	0.001	(0.220)	0.001	(0.131)
Length (cm)	-1.052	(0.937)	-1.348	(0.920)	-0.978	(0.942)
Head:abdominal circumference	0.002	(0.131)	0.002	(0.121)	-0.002	(0.113)
Head circumference:length	-223.992	(0.287)	-214.435	(0.315)	-297.063	(0.205)
Ponderal index (kg/cm^3)	23.125	(0.033)	22.894	(0.037)	23.779	(0.029)
Placental weight (g)	0.005	(0.022)	0.005	(0.026)	0.006	(0.005)
Placenta:birth weight	-0.001	(0.992)	-0.011	(0.921)	0.002	(0.983)
Gestational age (days)	-0.006	(0.844)	-0.006	(0.835)		

After adjusting for the effects of sex and weight, children who had had a larger ponderal index (figure 5.6) or a heavier placenta at birth (figure 5.7) tended to have a faster pulse wave velocity in the femoral to foot segment. These relationships were changed little when systolic blood pressure was added into the model. When gestational age was added into the model, these relationships were strengthened and both were statistically significant.

Figure 5.6 Scatterplot of femoral to foot pulse wave velocity adjusted for sex and current weight according to ponderal index at birth.

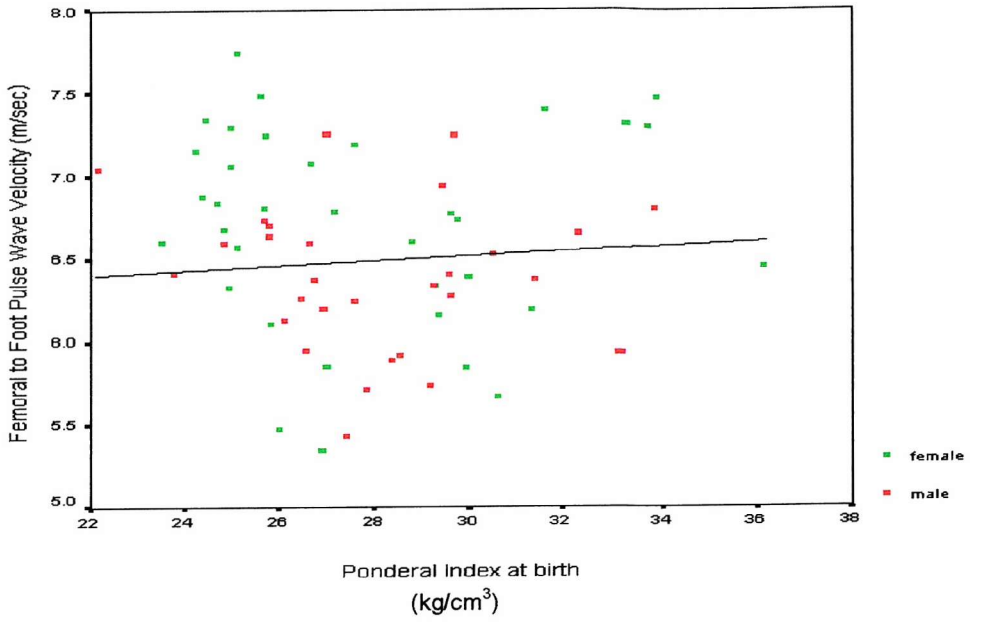
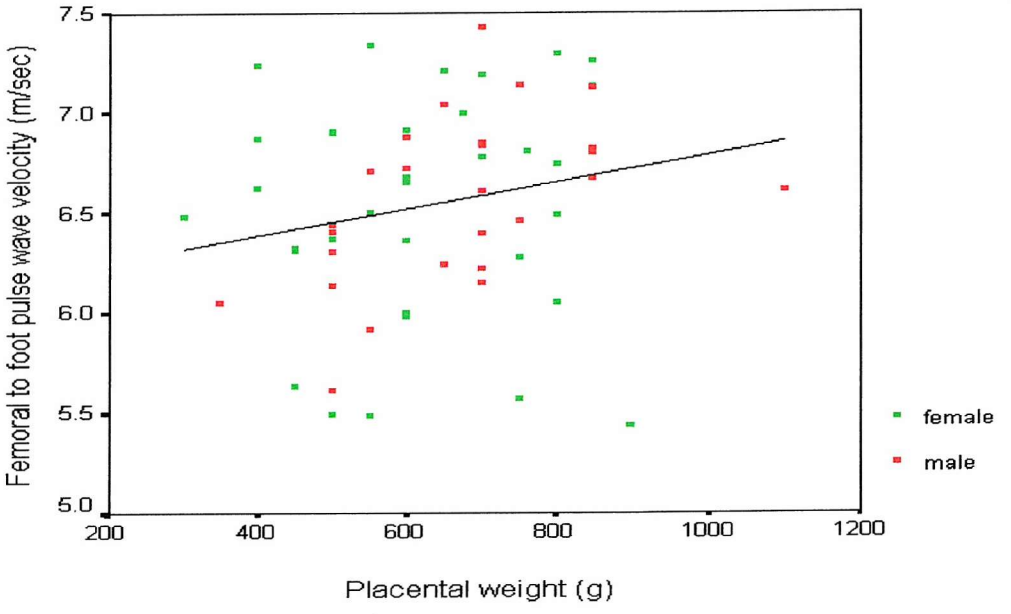


Figure 5.7 Scatterplot of femoral to foot pulse wave velocity adjusted for sex and current weight according to placental weight (g).



5.6 Discussion

Systolic and diastolic blood pressures were higher in children with a larger current body size. In particular, systolic and diastolic blood pressure tended to be higher in boys and girls who were heavy or who had a large body mass index (weight/height^2). These findings are consistent with previous studies which have reported that children with a larger current body size, and in particular a heavier current weight tend to have raised blood pressure levels.[25; 29; 31; 168] Therefore, in order to take account of the effect of current weight, this variable was added into all multivariate models.

5.7 Blood pressure and birth weight

Children who were light at birth tended to have higher systolic and diastolic blood pressure in the present study, although the relationships were not statistically significant. Linear regression analysis showed that after adjusting for the effects of sex and current weight, for each kilogram decrease in birth weight there was a 2.3 mmHg increase in systolic blood pressure (95% c.i. -5.44 to 0.77).

The results of published studies that have investigated associations between systolic blood pressure and birth weight in children of a similar age to those in the present study are presented in figure 5.8.

Figure 5.8 Studies investigating the association between systolic blood pressure and birth weight in children (adapted from [19]).

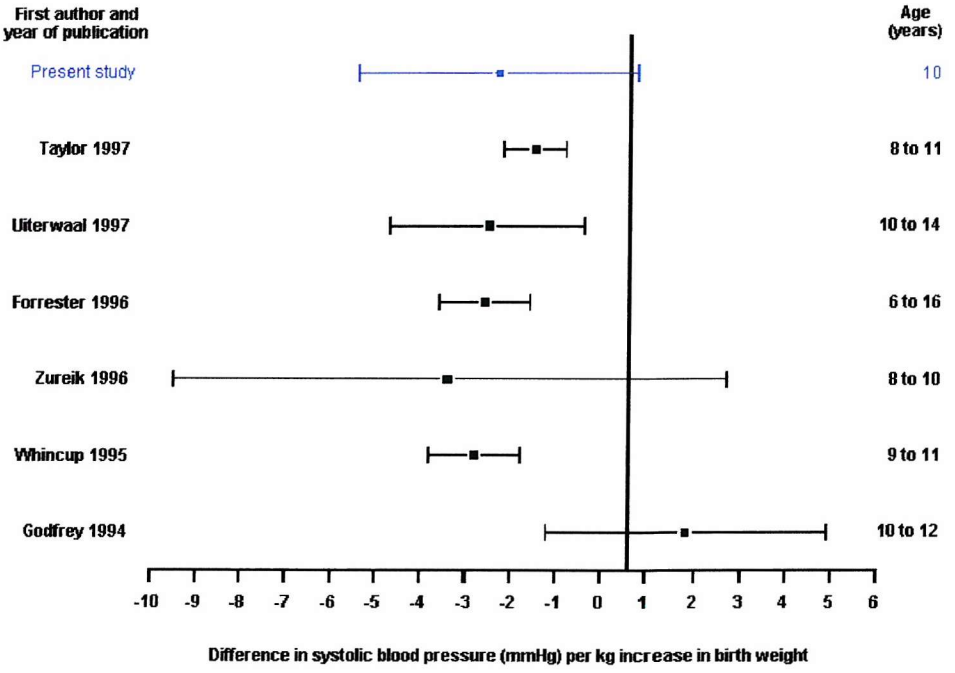


Figure 5.8 shows that the results of the present study are consistent with most of the previously published findings in children. The regression coefficient of the present study (-2.3) is within the 95% confidence intervals of all the studies, with the exception of that by Godfrey et al.[173] A systematic review which assessed the evidence for an inverse relationship between blood pressure and birth weight suggested that in children systolic blood pressure tended to rise by 2 to 3 mmHg for each kilogram decrease in birth weight.[19] The findings of the present study are also consistent with this.

5.8 Blood pressure and birth size

After adjusting for the effects of current weight, children who had had a lighter placenta at birth tended to have higher systolic blood pressure at ten years of age. However, this relationship was of borderline statistical significance (p=0.067).

Body size proportions differ between infants born pre-term (<37 weeks gestation) and those born at term (37 to 42 weeks gestation). Therefore, relationships between size at birth and raised blood pressure may be distorted if pre-term babies are included in the analysis. In the present study there were two subjects who were born before 37 weeks gestation. However, the trends between birth size and raised blood pressure were unchanged whether these two subjects were included or excluded from the analysis. When gestational age was added into the multivariate analysis, the relationship between raised blood pressure and a lighter placenta changed little. This indicates that reduced placental growth, and not premature delivery was responsible for this relationship.

The results of the present study are consistent with some of the previous reports that have investigated childhood blood pressure in relation to size at birth. In 3 studies of 6 to 11 year olds, children born with a lighter placenta also tended to have raised systolic blood pressure.[26; 167; 174] However, 2 other studies of 4 and 8 year olds showed that systolic blood pressure tended to be higher in children with a heavier placenta.[25; 31] A further study of 8 to 11 year olds showed that girls born with a lighter placenta tended to have raised systolic blood pressure whereas boys born with a heavier placenta tended to have higher systolic blood pressure.[29]

The mechanism that links a lighter placenta at birth to raised blood pressure in later life is unknown. Could impaired blood vessel growth resulting in reduced arterial compliance be responsible? Pulse wave velocity was measured in the children to investigate this possibility.

5.9 Pulse wave velocity

In the 10 year old children, mean aorta to femoral pulse wave velocity was 2.78 m/sec. Mean aorta to radial pulse wave velocity was 4.08 m/sec. Mean aorta to foot pulse wave velocity was 4.72 m/sec and mean femoral to foot pulse wave velocity was 11.52 m/sec. Table 5.12 shows the results of the previous published studies that have reported pulse wave velocity values in healthy children of a similar age to those in the present study.

Table 5.12 Mean pulse wave velocities (m/sec) in children.

First author and year of publication	Age (years)	Pulse wave velocity (m/sec)	Segment
Eliakim 1971	11-20	5.5	Aorta to foot
Present study	**10**	**4.7**	**Aorta to foot**
Avolio 1985	10	5.6	Aorta to femoral
Avolio 1985	10	6.2	Aorta to femoral
Present study	**10**	**2.8**	**Aorta to femoral**

Pulse wave velocity in the aorta to foot segment was slower in the 10 year olds in the present study (4.7 m/sec) compared to the group of 11 to 20 year olds (5.5 m/sec) in the study by Eliakim et al.[140] It has previously been shown that pulse wave velocity increases with age.[113] Therefore, the mean aorta to foot pulse wave velocity may have been faster in the subjects in the study by Eliakim et al. because they were older than the people in the present study. The mean pulse wave velocity in the aorta to femoral segment was much slower in the children in the present study compared to those of the two previous studies by Avolio et al.[118] Much of the variation is likely to result from methodological differences (detailed in chapter 3, section 3.10). After an estimate of mean left ventricular contraction time (76.5 miliseconds) had been subtracted from the pulse wave transit times in the present study, the aorta to femoral pulse wave velocity was 5.7 m/sec, which is comparable to the findings of Avolio et al.[118]

In the present study, pulse wave velocity tended to be faster in girls, in children with a larger current body size and in boys and girls with a higher systolic and diastolic blood pressure and a faster heart rate. Previous studies have also shown that pulse wave velocity tends to be faster in adults who are taller, who have a faster heart rate and who have raised blood pressure (detailed in section 3.10).[70; 142; 143] In the present study, the relationship found between a faster pulse wave velocity and female sex is not consistent with previous findings which have shown that males tend to have stiffer arteries than females.[127] However, the faster pulse wave velocities recorded in females in the present study could reflect their tendency to have a larger body size, which is also related to reduced arterial elasticity.[127]

5.10 Pulse wave velocity and birth size

5.10.1 Proportionately small

Only pulse wave velocity in the femoral to foot segment was related to birth weight and children who had been heavier at birth tended to have faster pulse wave velocities in this segment. In the aorta to femoral and aorta to radial segments pulse wave velocity did tend to be faster in children who had had small head, chest or abdominal circumferences or who had been shorter in length at birth. However, the relationships were weak and none were statistically significant. In the aorta to foot and femoral to foot segments pulse wave velocity tended to be faster in children who had had

a larger abdominal circumference at birth, although these relationships were not statistically significant. Therefore, there appears to be little evidence that pulse wave velocity is related to proportional growth restriction in the arterial segments that were studied.

5.10.2 Disproportionately small abdominal circumference or length in relation to head size

There were no relationships between pulse wave velocity and head to abdominal circumference ratio at birth in any of the arterial segments studied. There were no relationships between aorta to femoral, aorta to foot or femoral to foot pulse wave velocity and head circumference to length ratio at birth either. However, aorta to radial pulse wave velocity did tend to be faster in children who were born long in relation to their head circumference and this relationship was statistically significant.

5.10.3 Low ponderal index

There were no relationships between aorta to femoral, aorta to radial or aorta to foot pulse wave velocities and ponderal index at birth. However, pulse wave velocity in the femoral to foot segment was related to ponderal index, although children with a larger ponderal index at birth tended to have faster pulse wave velocities in this segment. When birth weight and length at birth were added into the regression model together, the relationship between femoral to foot pulse wave velocity and birth weight (regression coefficient =0.28, p=0.21) was stronger than the relationship between femoral to foot pulse wave velocity and length at birth (regression coefficient =0.01, p=0.89). Therefore, the relationship between a faster femoral to foot pulse wave velocity and a larger ponderal index at birth may be explained by the greater birth weight of these children, rather than fatness at birth. Therefore, there was little evidence to suggest that pulse wave velocity was related to a low ponderal index in any of the arterial segments studied.

5.10.4 Placental size

After adjusting for the effects of sex, current weight and gestational age, children who had had a heavier placenta at birth tended to have faster pulse wave velocities in their aorta to foot and femoral to foot arterial segments. These relationships were statistically significant. In both these segments pulse wave velocity also tended to be faster in children who had been light in relation to their placentas.

5.10.5 Gestational age

After adjusting for sex and current weight, children who were born after a shorter length of gestation tended to have a faster pulse wave velocities in the aorta to femoral and aorta to foot segments. Both these relationships were statistically significant. Pulse wave velocities in the aorta to radial and femoral to foot segments were not related to gestational age at birth.

5.11 Birth size in relation to 'type' of artery

The results of the present study showed that pulse wave velocity in the elastic arterial segment (aorta to femoral) tended to be faster in children who were born after a shorter length of gestation. Pulse wave velocity in the segment containing muscular arteries with a high elastin content (aorta to radial) was related to disproportionate fetal growth indicating alterations in the fetal growth rate in the latter half of pregnancy. Pulse wave velocity in the segment containing muscular arteries with a low elastin content (femoral to foot) was related to a heavier placenta, which might indicate changes in the fetal growth pattern in mid pregnancy. These results appear to be consistent with the hypothesis that indicators of intrauterine growth restriction in the mid to latter half of pregnancy may reduce elastin synthesis and lead to permanently stiffer arteries.

The question that follows is how could a shorter length of gestation, or alterations in the fetal and placental growth rate lead to stiffer arteries in childhood.

5.12 Length of gestation

As detailed in section 4.13, major alterations occur in the circulatory system at birth. These alterations adapt the fetus to the haemodynamic changes that take place at birth. Changes in metabolic and endocrine factors, such as cortisol alter [171] and could lead to a reduction in elastin synthesis.

A pulse wave velocity value was calculated for the femoral to foot segment using the equation cited in chapter 3 (section 3.2.1). No relationship was found between a faster pulse wave velocity and a shorter gestational age in the femoral to foot segment. Therefore it would appear a shorter length of gestation only led to increased pulse wave velocity in the aorta to femoral segment. A similar relationship was only present in the aorta to foot segment because it includes the aorta to femoral section.

In a study of pig aortas, a temporal gradient of elastin synthesis was found in accordance with distance from the heart.[149] In the days surrounding birth elastin production was greatest in the thoracic aorta. This maximal level of synthesis became progressively distal in the weeks following birth.[93] If the rapid increase in elastin synthesis just prior to birth is reduced by a shorter length of gestation, it is the elastin content of the thoracic aorta that is most likely to be affected at this time. The more distal arteries which have probably not yet reached their maximal synthesis rates may be less affected or unaffected.

5.13 Placenta and blood flow redistribution

The enlargement of the placenta in intrauterine growth restricted pregnancies may represent a response to an adverse intrauterine environment caused by factors such as maternal hypertension, anaemia or undernutrition (section 1.3).[49; 175] As a result there may be adaptive

changes in the distribution of blood flow or in the concentration of fetal and placental hormones and in the sensitivity of different tissues to the hormones.[176-178]

The placenta may enlarge during pregnancy in response, for example, to hypoxia to maintain oxygen supplies.[48] Studies have suggested that the fetus responds to a reduced oxygen supply by redistributing blood flow to the brain at the expense of the trunk.[176; 179] Indeed Doppler blood flow waveforms have demonstrated increased blood flow to the head in growth restricted fetuses.[179; 180] The adaptive response of the growth restricted fetus in reducing its blood flow could have long term effects through irreversibly altering arterial structure. The structure of the artery wall is sensitive to blood flow. Animal studies have shown that reduction in local blood flow in for example, immature rabbits, results in a decrease in vessel elastin content.[181] Elastin is largely responsible for the elastic properties of the arterial wall. Therefore, the reduction of blood flow could lead to stiffer arteries through reduced elastin synthesis. There is some evidence that reduction in blood flow does affect arterial structure in humans. This comes from a study of infants who were born with a single umbilical artery. In a group of these infants, the iliac artery on the side of the present umbilical artery was found to be elastic, whereas the iliac artery on the side of the missing umbilical artery, which had lower blood flow, was thin walled and muscular.[108] A redistribution of blood flow in favour of the brain would result in a reduction of blood flow to the legs. Therefore, the finding that children with a heavier placenta tended to have faster pulse wave velocities in their femoral to foot segments only is consistent with this idea, although there was no evidence of disproportionate growth restriction.

Studies in rats have shown that the activity of the placental hormone 11-β hydroxysteroid dehydrogenase correlates positively with birth weight and negatively with placental size.[182] This hormone acts as a barrier to protect the fetus from the harmful effects of maternal glucocorticoids by converting them to inactive cortisones.[178] Therefore, rats born light in relation to their placental weight are likely to have had a greater exposure to maternal glucocorticoids through reduced 11-β hydroxysteroid dehydrogenase activity. It is possible that fetal exposure to maternal glucocorticoids could lead to alterations in the structure of the vasculature,[178] which may result in the development of stiffer arteries.

5.14 Comparison with the previous published study

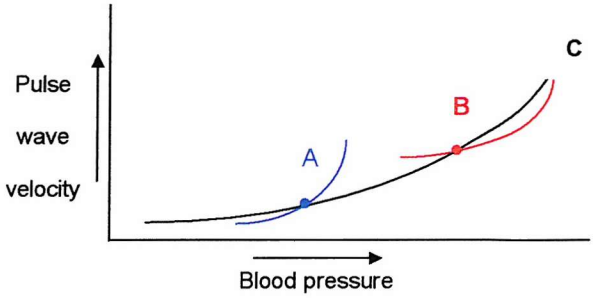
The results of the present study were not consistent with those of the earlier published study.[28] In the present study there were no relationships between lighter birth weight, smaller abdominal or head circumference or shorter length at birth in either the aorta to femoral or femoral to foot segments as were found in the previous published study.

5.15 Arterial compliance and blood pressure

In the adult populations in chapters 3 and 4 it was difficult to determine whether people with indicators of intrauterine growth restriction in mid to late pregnancy had faster pulse wave

velocities due to poor fetal growth, or due to their tendency to have higher blood pressure. In order to control for the effects of adult blood pressure, systolic and diastolic blood pressure were added into multivariate models in these studies. However, this is not an entirely satisfactory thing to do because it assumes that the increase in pulse wave velocity with rising blood pressure is the same for everyone in the study population (figure 5.9 line C), which may not be the case. The relationship may be different for each individual (for example, figure 5.9 lines A and B).

Figure 5.9 Example of the differences that might exist in the arterial pulse wave velocity/blood pressure relationship between individuals (A and B) compared to the whole population (C).



In the children in the present study, the relationships between a faster pulse wave velocity and smaller birth size were changed little whether or not systolic and diastolic blood pressure were added into multivariate analysis. This suggests that the relationships in children are independent of blood pressure, and therefore it is poor fetal growth that is responsible for the trends seen with faster pulse wave velocity and smaller birth measurements and not a tendency to have higher blood pressure. However, this interpretation is speculative.

5.16 Strength of the results

A number of statistically significant results were found in the present study. However, careful interpretation is necessary because the analysis was based on data from a small number of subjects and there were a large number of test variables. Analysis of multiple variables increases the probability that statistically significant results can occur by chance.

There is some evidence to suggest that caution is necessary in interpreting the results of the present study. For example, the analysis of pulse wave velocity and birth measurements showed an opposing association with the results from the systolic blood pressure and birth size analysis. Children born with a lighter placenta tended to have higher systolic blood pressure, whereas boys and girls with a heavier placenta at birth tended to have faster aorta to foot and femoral to foot pulse wave velocities. As systolic blood pressure rises, pulse wave velocity increases. Therefore,

the relationships between systolic blood pressure and placental weight and pulse wave velocity and placental weight might have been expected to be in the same direction.

5.17 Conclusions

The results of the present study showed that children who were light at birth or who had a lighter placenta tended to have higher systolic blood pressure at ten years of age. However, these results were not statistically significant at conventional levels.

Pulse wave velocity was measured to investigate whether reduced arterial compliance might be a mechanism linking small size at birth to raised blood pressure. The results of the present study, although not conclusive, showed that children who were born after a shorter length of gestation tended to have faster aorta to femoral pulse wave velocities. Boys and girls with a small head circumference to length ratio at birth tended to have faster aorta to radial pulse wave velocities. Children with a heavier placenta tended to have faster aorta to foot and femoral to foot pulse wave velocities at ten years old. All these relationships were independent of current blood pressure levels. This adds weak support to the hypothesis that intrauterine growth restriction in mid to late pregnancy is important in the programming of arterial compliance and may provide a mechanism linking reduced fetal growth to raised blood pressure in adult life.

Chapter 6

Quantification of aortic scleroprotein in two animal models of intrauterine growth restriction

6.1 Introduction

The results of the studies detailed in chapters 3 to 5 show that decreased arterial compliance is related to indicators of intrauterine growth restriction in mid to late gestation in humans. This suggests that the uterine environment is important in determining arterial elasticity. Estimates of arterial compliance from measurements of pulse wave velocity provide an indirect estimate of the elastin content of the arteries. The aim of the present study was to make a direct quantification of the aortic scleroprotein content. It was not possible to estimate elastin concentrations in samples of human aorta therefore, the aortas of rats from two established animal models of intrauterine growth restriction were used.

In both the animal models, rat pups born to nutritionally restricted mothers are born lighter in weight and have reliably higher blood pressure compared to rat pups born to mothers fed a control diet.[24; 183] The same process that causes blood pressure to rise in the maternal dietary restricted rat pups may also lead to raised blood pressure in intrauterine growth restricted humans. Therefore, these animal models provide a good basis for studying the mechanisms involved in fetal programming of raised blood pressure. Examples of mechanisms that have been investigated using these models include reduced nephron number, [184] fetal exposure to maternal glucocorticoids, [185] altered hypothalamic-pituitary-adrenal axis function[186] and in this study, reduced aortic scleroprotein synthesis.

6.2 Animal models

6.2.1 Maternal low protein model

In the first of the animal models, rats were fed either a low protein diet (9% casein) or a control (18% casein) diet during pregnancy. Previous studies have shown that offspring of the low protein mothers were lighter at birth and had significantly higher systolic blood pressure measured at 9 weeks of age compared to the offspring of control diet fed mothers.[24]

Eight female virgin Wistar rats were housed individually and maintained at 24°C on a 12-12 hour light-dark cycle. The rats were divided into two equal groups. The first group were designated the low protein group and were habituated to a diet containing 9% protein. The second group were habituated to an isocaloric diet containing 18% protein. The protein source was casein and all diets contained 5 g/kg methionine to avoid sulphur deficiency. All animals were given free access to water. After 14 days the animals were mated by caging them with virgin males until a vaginal plug was observed. Low protein or control diets were continued during the mating period until birth of the pups. Following birth the dams were fed a standard rat chow diet (20% protein). Litters of 9

or more were culled to 8 pups (4 male, 4 female where possible). The birth weight of each rat pup was measured. At 21 days of age the litters were weaned onto the standard rat chow.

At 28 days of age, Dr S Langley Evans, of the Department of Human Nutrition, Southampton University, recorded systolic blood pressure in each rat pup. Systolic blood pressure was measured in the caudal artery using an IITC model 229 tail cuff system. The animals were habituated to the blood pressure measurement every day for 7 days prior to measurement at 28 days so that blood pressure readings could be made in conscious animals with minimal restraint.

Following systolic blood pressure measurement, the rat pups were killed by asphyxiation with CO_2. The thoracic and abdominal segments of the aorta were then removed. The diaphragm defined the boundary between the thoracic and abdominal aortic segment. After cutting through the aorta while it was still in-situ, the separate segments were removed by dissecting them free from the surrounding tissues and cutting all visible side branches. Each segment was then stored separately at -80 °C until scleroprotein analysis could be carried out.

6.2.2 Maternal calorie restricted diet model

In the second animal model, rats were fed either a calorie restricted diet (70% of control diet) or a control diet (standard rat chow) during pregnancy. Previous studies have shown that the rat pups born to calorie restricted mothers were smaller in birth size and had systolic blood pressure that was significantly elevated at 30 weeks of age compared to rat pups born to the control diet fed mothers.[183]

Nineteen 12-14 week old Wistar rats were maintained under standard conditions (room temperature 24 °C, humidity 55% and 12-12 hour light-dark cycle) with free access to water and standard rat chow (crude oil 2.6%, protein 14.7%, and fibre 5.3%). After mating, female rats were randomly divided into two groups, a control group (n=9) fed standard rat chow and a nutritionally restricted group (n=10) fed 70% of the gestation matched dietary intake. Animals were housed individually and fed the prescribed diet daily. Litter size was standardised to 10 pups at day 1. The birth weights of the rat pups were not measured in this animal model.

Two rat pups from each litter were randomly chosen at 60 days, 100 days and 200 days. After an overnight fast systolic blood pressure was measured in the chosen rat pups by Dr Takashi Ozaki at the Department of Obstetrics and Gynaecology, University College, London. Each rat pup was anaesthetised with 60 mg of sodium pentobarbitone. A polythene catheter was inserted into the iliac artery via the left femoral artery. The catheter was tunnelled under the abdominal skin, exteriorised and connected to a pressure transducer. The animals were then placed in a dark box to allow free movement. 3 hours after recovery, systolic blood pressure was recorded in the conscious unrestrained animals.

At 20 days, two rats from each litter were randomly chosen. Each rat pup was killed by overdose of sodium pentobarbitone (200 mg/kg) and aortas were removed from each animal and stored at -80 °C until scleroprotein analysis could be carried out. The same procedure was carried out directly after blood pressure had been measured in the rats who had been selected at 60, 100 and 200 days.

6.3 Methods

6.3.1 Gravimetric scleroprotein measurement

The gravimetric method used to estimate aortic elastin was adapted from the procedure originally described by Neuman and Logan.[187] This method relies on the insolubility of mature cross-linked elastin and the solubility of all the other components of the aorta, such as collagen and ground substance. Samples were boiled long enough to solubilise all the components of the aorta apart from elastin. The remaining solid material that was left was then assumed to be elastin.

The aortas were freeze dried and then weighed. Each aorta sample was then boiled at 126 °C for 6 hours. The solid material that was left from each aorta was freeze dried and then weighed. The proportion of the original aortic weight attributable to elastin was then calculated.

Each sample was placed in an Eppendorf tube of known weight and the sample was weighed in the tube giving the initial wet weight. Holes were pierced into the lid of each tube and the samples were placed in a freeze dryer for 24 hours. Each sample was then reweighed in its tube to determine its dry weight. In order to isolate the elastin, each sample was placed in a separate glass tube and 1 ml of distilled water was added. The tubes were then autoclaved at 20 psi pressure (126°C) for 6 hours to solubilise all the tissue in each sample that was not elastin. After autoclaving, the solid remains of each aorta were removed from the glass tubes and returned to their original eppendorf tubes. Each sample was then placed in a freeze dryer for 24 hours. The samples were then reweighed in their tubes to determine the dry weight of alkali soluble + alkali insoluble elastin.

Each of the samples (low protein model only) was then placed in a separate tube and 1 ml of 0.1 M sodium hydroxide was added. The samples were heated to 80 °C for one hour. The aortas were then removed from the solution, washed twice in distilled water and placed back into their original eppendorf tubes. All the samples were then placed in a freeze dryer for 24 hours. Each aorta was reweighed in its tube to determine the dry weight of alkali insoluble elastin.

6.3.2 Hydroxyproline analysis

The collagen content of the aortas was estimated by quantifying hydroxyproline. After boiling the aorta samples to isolate elastin as described in section 6.3.1, the supernatant contains all the collagen and ground substance. By evaporating the supernatant to dryness and hydrolysing in 6M hydrochloric acid, the hydroxyproline content can be quantified using colourimetric techniques.

Assuming that 13.5% of the weight of collagen is attributable to hydroxyproline,[188] the collagen content of each aorta sample can then be calculated.

Following the autoclaving of each aorta sample in step 6.3.1, the supernatant from each glass tube was decanted into a separate eppendorf tube. The glass tube was then washed twice with 0.5 ml distilled water and this was added to the eppendorf tube. These samples were sent to Caroline Clifford at the laboratories of the Institute of Orthopaedics, Stanmore, Middlesex where she supervised hydroxyproline analysis for collagen content determination.

The hydroxyproline in each sample was measured using the method of Ho et al.[189] According to this technique hydroxyproline was oxidised by chloramine-T (sodium N-cholor-p-toluenesulphonamine) in near neutral buffer (pH 6.0). The chromogens (pyrrole and pyr-2-carboxylic acid) formed are coupled with p-dimethylaminobenzaldehyde in strong perchloric acid (60%) to produce a chromophore whose absorbance was measured at 570 nm.

Collagen consists of 13.5% hydroxyproline.[188] Therefore, the hydroxyproline concentration in the 2 ml of solution from each aorta sample was multiplied by 6.75 to give the collagen concentration.

6.3.3 Fastin Elastin assay (produced by Biocolor Limited)

The Fastin elastin assay is a quantitative colourimetric dye-binding method. The dye reagent binds to a specific non-polar amino acid sequence found in mammalian elastins. Its absorbance is then measured at 513nm.

Due to time and money constraints only the low protein model samples were analysed using this assay. 500 μl of 0.25 M oxalic acid was added to each aorta in its tube and all the samples were heated at 90°C overnight to generate alpha elastin, a solubilised form of elastin. 0, 20, 40, 60 and 80 μl of alpha elastin standard and 50 μl of each sample were added to separate tubes. 600 μl of Fastin elastin precipitating reagent was then added to each tube and was left overnight at 4°C. The samples were then centrifuged at 5 500 rpm for 10 minutes. Each tube was opened and the liquid was poured to waste leaving the precipitated elastin pellet in the bottom of the tube. 500 μl of elastin dye reagent (5,10,15,20-tetraphenyl-21,23-porphrine) and 100 μl of saturated ammonium sulphate was added and the tubes were shaken gently for one hour. The samples were then centrifuged at 5 500 rpm for 10 minutes. Each tube was opened and the unbound dye was drained off. 500 μl of elastin destain reagent (methanol-ammonia solution) was then added and the samples were shaken gently for 10 minutes to solubilise the dye bound to the elastin pellet. The tubes were then centrifuged at 6 000 rpm for 5 minutes. The recovered elastin dye concentration in each sample was measured using a spectrophotometer set at a wavelength of 513 nm. The concentration of elastin in μg was read from the calibration curve, which was produced from the absorbance values measured from the alpha elastin standards.

6.4 Analysis

Tabulation of means was used to compare the difference in the scleroprotein content of the aortas of protein or calorie restricted rat pups and control rat pups. Multilevel linear regression modelling was then carried out to take into account the fact that rat pups from the same litters may have elastin concentrations that are more similar than randomly selected rats from different litters. Adjusting for genetic similarity is necessary because the variation in the data might be smaller than that expected in a totally random sample. Multilevel modelling partitions between-rat variation into between-family variation and between-rat within-family variation and uses the ratio of these to adjust the estimate of the standard error from the regression slope.

6.5 Results

6.6 Low protein model

Mean weights at birth and systolic blood pressure levels of the rat pups born to mothers fed a low protein or a control diet during pregnancy are summarised in table 6.1.

Table 6.1 Mean weight at birth and systolic blood pressure of the caudal artery measured at 28 days in rat pups born to mothers fed either a low protein or a control diet throughout pregnancy.

	Control Mean (SD) (n=13)	Low Protein Mean (SD) (n=26)	P value of the difference
Birth weight (g)	5.33 (0.15)	4.96 (0.10)	<0.050
Systolic blood pressure (mmHg)	96.5 (10.0)	116.5 (14.5)	<0.001

SD = standard deviation

Rat pups born to mothers fed a low protein diet during pregnancy were on average 0.37 g lighter at birth and had systolic blood pressure that was 20 mmHg higher than rat pups born to mothers fed a control diet during pregnancy. Both these differences were statistically significant.

6.6.1 Alkali soluble + alkali insoluble elastin using the gravimetric method

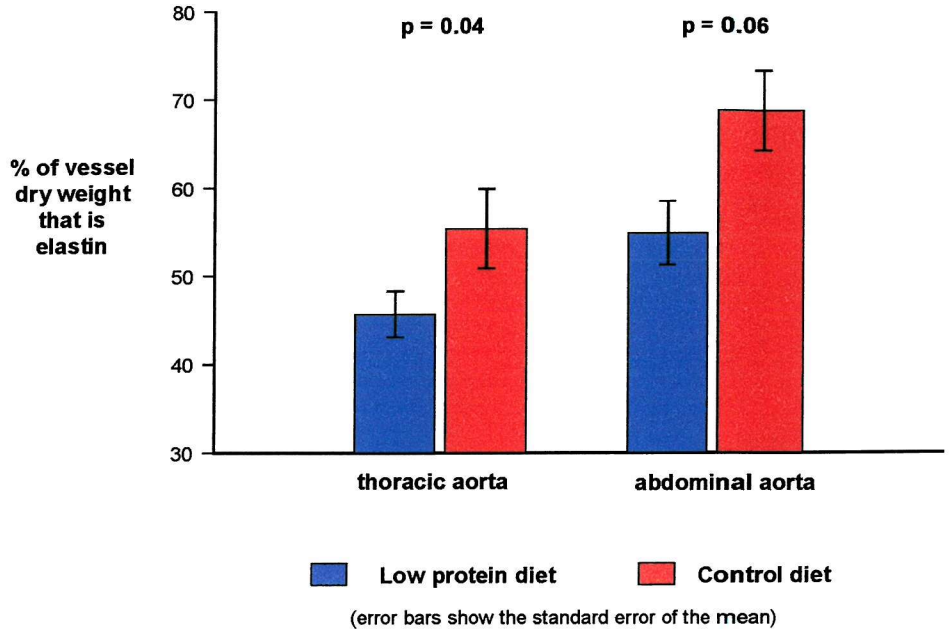
The mean alkali soluble + alkali insoluble elastin content of the abdominal and thoracic aorta segments is summarised in table 6.2 and figure 6.1.

Table 6.2 Mean alkali soluble + alkali insoluble elastin content (%) of tissue samples in 28 day old rat pups whose mothers were fed either a low protein or a control diet during pregnancy.

	Control			Low Protein			P value of
Tissue	Mean	SD	n	mean	SD	n	difference
Thoracic aorta	55.33	16.21	13	45.68	13.36	26	0.04
Abdominal aorta	68.68	14.28	10	54.81	18.41	26	0.06

On average the thoracic aortas of the maternal low protein rat pups contained 9.6% less alkali soluble + alkali insoluble elastin than those of the control pups and this difference was statistically significant. Similarly, the abdominal aortas of the maternal low protein rat pups contained on average 13.9% less alkali soluble + alkali insoluble elastin than those of the control rat pups and this difference was of borderline statistical significance. These results are summarised in figure 6.1.

Figure 6.1 Percent alkali soluble + insoluble elastin content of the aortas of rats whose mothers were fed either a low protein or a control diet during pregnancy.



A multilevel model was used to investigate the effect of a low protein diet on the arterial alkali soluble + alkali insoluble elastin content after adjusting for genetic similarities between rat pups of the same litter. The results are summarised in table 6.3.

Table 6.3 Multilevel linear regression models of the effect of a low protein diet compared to control diet on percentage aortic alkali soluble + insoluble elastin content.

Tissue	Regression coefficient	95% ci	P value
Thoracic aorta	9.66	-0.35 to 19.68	0.059
Abdominal aorta	15.55	- 4.15 to 35.25	0.122

ci = confidence intervals

Rat pups born to mothers who were fed a low protein diet during pregnancy had on average 9.66% less alkali soluble + alkali insoluble elastin in their thoracic aorta compared to those of the rat pups whose mothers were fed a control diet. This relationship was of borderline statistical significance. On average, the abdominal aortas of the maternal low protein rat pups contained

15.55% less alkali soluble + alkali insoluble elastin than those of maternal control diet rat pups, although this difference was not statistically significant.

6.6.2. Alkali insoluble elastin using the gravimetric method

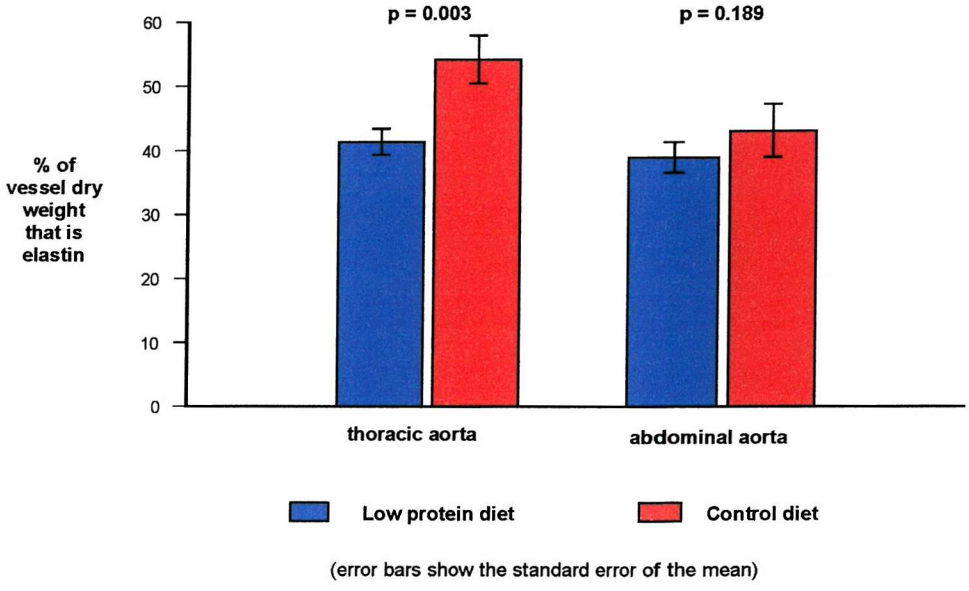
The mean alkali insoluble elastin content of the rat thoracic and abdominal aortas is summarised in table 6.4 and figure 6.2.

Table 6.4 Mean alkali insoluble elastin content of tissue samples in 28 day old rat pups whose mothers were fed either a low protein or a control diet during pregnancy.

	Control			Low Protein			P value of
Tissue	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**difference**
Thoracic aorta	54.20	13.43	13	41.33	10.25	26	0.003
Abdominal aorta	43.04	12.96	10	38.89	12.12	26	0.189

On average the thoracic aortas of the maternal low protein diet rat pups contained 12.9% less alkali insoluble elastin than those of the maternal control diet rat pups. This difference was statistically significant. The abdominal aortas of the maternal low protein diet rat pups contained 4.1% less alkali insoluble elastin on average than maternal control diet rat pups, although this difference was not statistically significant.

Figure 6.2 Percentage alkali insoluble elastin content of the aortas of rats whose mothers were fed either a low protein or a control diet during pregnancy.



(error bars show the standard error of the mean)

To investigate the effect of a low protein diet on arterial alkali insoluble elastin content, multilevel modelling was used to adjust for genetic similarities between rat pups of the same litter. The results are summarised in table 6.5.

Table 6.5 Multilevel linear regression models of the effect of a low protein diet compared to control diet on percentage aortic alkali insoluble elastin content.

Tissue	Regression coefficient	95% ci	P value
Thoracic aorta	13.34	2.37 to 24.31	0.017
Abdominal aorta	9.06	-7.19 to 25.32	0.275

After controlling for the genetic similarities of litter mates, on average the thoracic aortas of the maternal low protein diet rat pups contained 13.34% less alkali insoluble elastin than those of the maternal control diet group. This difference was statistically significant. In the maternal low protein diet rat pups, on average the abdominal aorta contained 9.06% less alkali insoluble elastin than in the maternal control diet rat pups, however, the relationship was not statistically significant.

6.6.3 Alkali insoluble elastin using the Fastin elastin assay

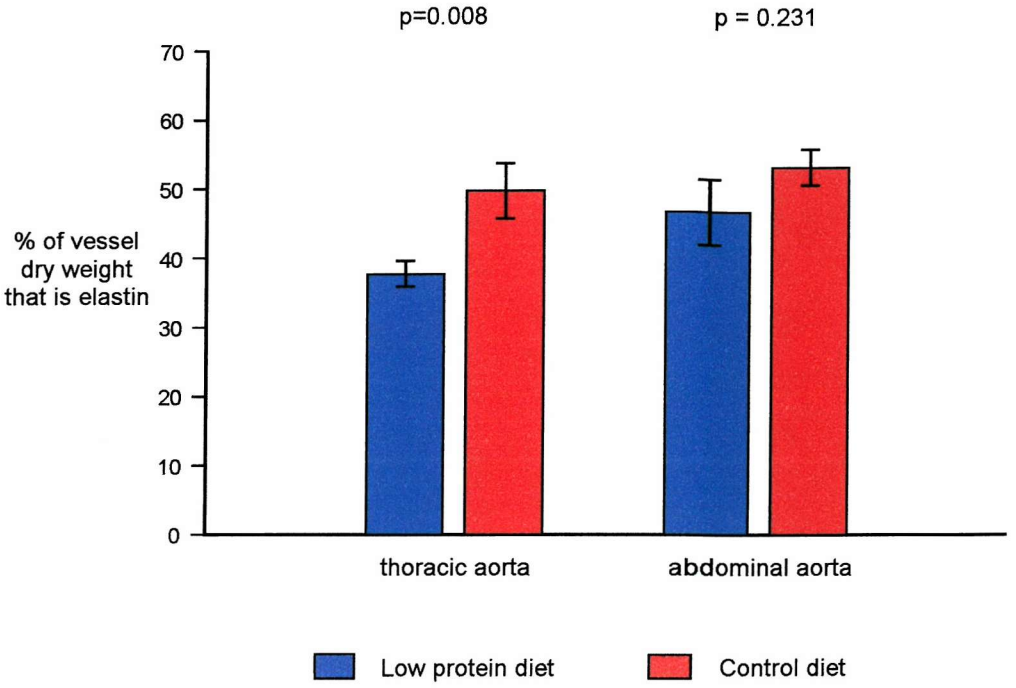
The mean percentage alkali insoluble elastin content of the thoracic and abdominal aortas of low protein and control group rat pups measured using the Fastin elastin assay is summarised in table 6.6 and figure 6.3.

Table 6.6 Mean alkali insoluble elastin content (%) of aorta samples in 28 day old rat pups whose mothers were fed either a low protein or a control diet during pregnancy.

	Control			Low Protein			P value of
Tissue	**Mean**	**SD**	**n**	**mean**	**SD**	**n**	**difference**
Thoracic aorta	49.77	14.26	13	37.73	9.57	26	0.008
Abdominal aorta	53.29	13.24	10	46.69	15.11	26	0.231

The mean alkali insoluble elastin content of the thoracic aorta was 12.04% lower in the maternal low protein diet rat pups compared to the maternal control diet rat pups. In the abdominal aorta the maternal low protein group rat pups had on average 6.6% less alkali insoluble elastin than the maternal control diet group. The difference in the thoracic aorta was statistically significant.

Figure 6.3 Alkali insoluble elastin content (%) of the aortas of rats whose mothers were fed either a low protein on a control diet during pregnancy.



(error bars show the standard error of the mean)

Multilevel modelling was used to investigate the effect of a maternal low protein diet on the arterial alkali insoluble elastin content after adjusting for genetic similarities between rat pups of the same litter. The results are summarised in table 6.7.

Table 6.7 Multilevel linear regression models of the effect of a low protein diet compared to control diet on the percentage alkali insoluble elastin content of the aorta.

Tissue	Regression coefficient	95% ci	P value
Thoracic aorta	14.45	0.67 to 22.15	<0.001
Abdominal aorta	9.45	0.37 to 18.54	0.042

The alkali insoluble elastin content of the thoracic aorta was on average 14.45% lower in maternal low protein group rat pups compared to control diet rat pups and this difference was statistically significant. In the abdominal aorta, the mean alkali insoluble elastin content was 9.45% lower in the maternal low protein rat pups compared to the maternal control diet rat pups and this difference was statistically significant.

6.6.4 Collagen

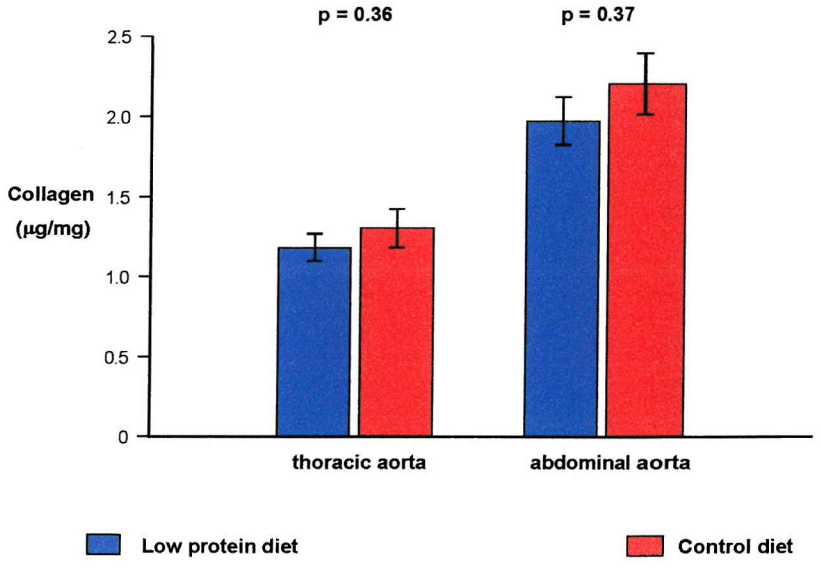
The mean collagen content of the aortas of rat pups whose mothers were fed either a low protein or a control diet during pregnancy are summarised in table 6.8 and figure 6.4.

Table 6.8 Mean collagen content (μg/mg) of tissue samples in 28 day old rat pups whose mothers were fed either a low protein or a control diet during pregnancy.

	Control			Low Protein			P value of
Tissue	**Mean**	**SD**	**n**	**mean**	**SD**	**n**	**difference**
Thoracic aorta	1.30	0.43	13	1.18	0.44	26	0.36
Abdominal aorta	2.20	0.61	10	1.97	0.76	26	0.37

There were no statistically significant differences in the collagen content of either the thoracic or abdominal aortas of maternal low protein diet rat pups compared to the maternal control diet rat pups.

Figure 6.4 Collagen content of the aortas of rats whose mothers were fed either a low protein or a control diet during pregnancy.



(error bars show the standard error of the mean)

Multilevel modelling was used to investigate the effect of a maternal low protein diet on the arterial collagen content after adjusting for genetic similarities between rat pups of the same litter. The results are summarised in table 6.9.

Table 6.9 Multilevel linear regression models of the effect of a low protein diet compared to control diet on tissue collagen content (μg/mg).

Tissue	Regression coefficient	95% ci	P value
Thoracic aorta	0.14	-0.27 to 0.56	0.500
Abdominal aorta	0.16	-0.76 to 1.07	0.736

There were no statistically significant relationships between the collagen content of the thoracic or abdominal aorta between maternal low protein or maternal control diet rat pups in either the multilevel models.

6.7 Calorie restricted model

Birth weight data was not available for the rat pups born to either calorie restricted or control diet mothers. Mean systolic blood pressure levels measured at 60, 100 and 200 days are summarised in table 6.10.

Table 6.10 Mean systolic blood pressure (mmHg) measured by aortic catheterisation according to age in rat pups born to mothers fed either a calorie restricted or a control diet throughout pregnancy.

Age (days)	Control Mean (SD)	Calorie Restricted Mean (SD)	P value of the difference
60	111.8 (5.5)	123.0 (6.0)	<0.001
100	122.0 (5.1)	136.1 (9.4)	<0.001
200	131.6 (7.6)	148.7 (7.7)	<0.001

On average, systolic blood pressure was 11.2 mmHg higher at 60 days, 14.1 mmHg higher at 100 days and 17.7 mmHg higher at 200 days in rats born to mothers fed a calorie restricted diet compared to rat pups born to mothers fed a control diet during pregnancy. All these differences were statistically significant.

6.7.1 Alkali soluble +alkali insoluble elastin using the gravimetric method

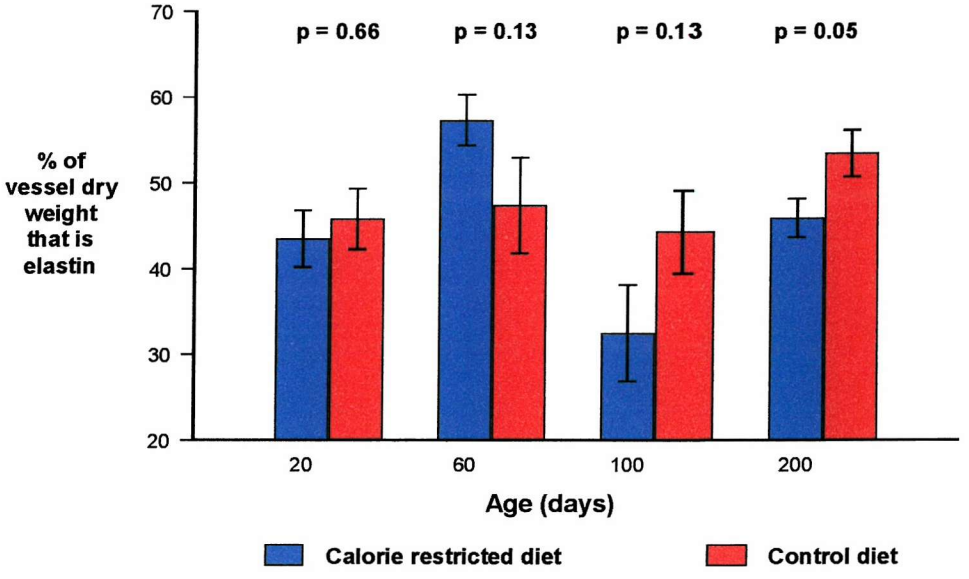
The mean alkali soluble + alkali insoluble elastin content of the rat aortas according to age at death is summarised in table 6.11 and figure 6.5.

Table 6.11 Mean percentage alkali soluble + alkali insoluble elastin content of the aortas of rat pups aged between 20 and 200 days whose mothers were fed either a calorie restricted or a control diet during pregnancy.

Age (days)	Control			Calorie Restricted			P value of
	Mean	SD	n	mean	SD	n	difference
20	45.75	15.94	20	43.42	11.45	12	0.66
60	47.33	19.33	12	57.25	10.26	12	0.13
100	44.21	17.35	13	32.42	16.91	9	0.13
200	53.34	8.99	11	45.85	9.73	19	0.05

On average the alkali soluble + alkali insoluble elastin content of the aortas of rats whose mothers were fed a calorie restricted diet during pregnancy was 2.3% lower in 20 day old animals, 9.9% higher in 60 day old animals, 11.8% lower in 100 day old animals and 7.5% lower in 200 day old animals compared to those whose mothers had been fed a control diet. Only the difference at 200 days was statistically significant.

Figure 6.5 Percentage alkali soluble + insoluble elastin content of the aortas of rats whose mothers were fed either a calorie restricted or a control diet during pregnancy.



(error bars show the standard error of the mean)

Multilevel modelling was used to investigate the effect of a maternal calorie restricted diet on the arterial alkali soluble + alkali insoluble elastin content after adjusting for genetic similarities between rat pups of the same litter. The results are summarised in table 6.12.

Table 6.12 Multilevel linear regression models of the effect of a calorie restricted diet compared to a control diet on the percentage vessel alkali soluble + alkali insoluble elastin content.

Age (days)	Regression coefficient	95% ci	P value
20	2.33	-8.01 to 12.68	0.659
60	-3.93	-25.78 to 17.93	0.725
100	10.25	-6.28 to 26.77	0.224
200	7.49	0.46 to 14.53	0.037

The alkali soluble + alkali insoluble elastin content of the aortas of the maternal calorie restricted diet rat pup group was on average 2.33% lower at 20 days, 3.93% higher at 60 days, 10.25% lower at 100 days and 7.49% lower at 200 days compared to maternal control diet group rat pups. The difference at 200 days was statistically significant.

6.7.2 Collagen

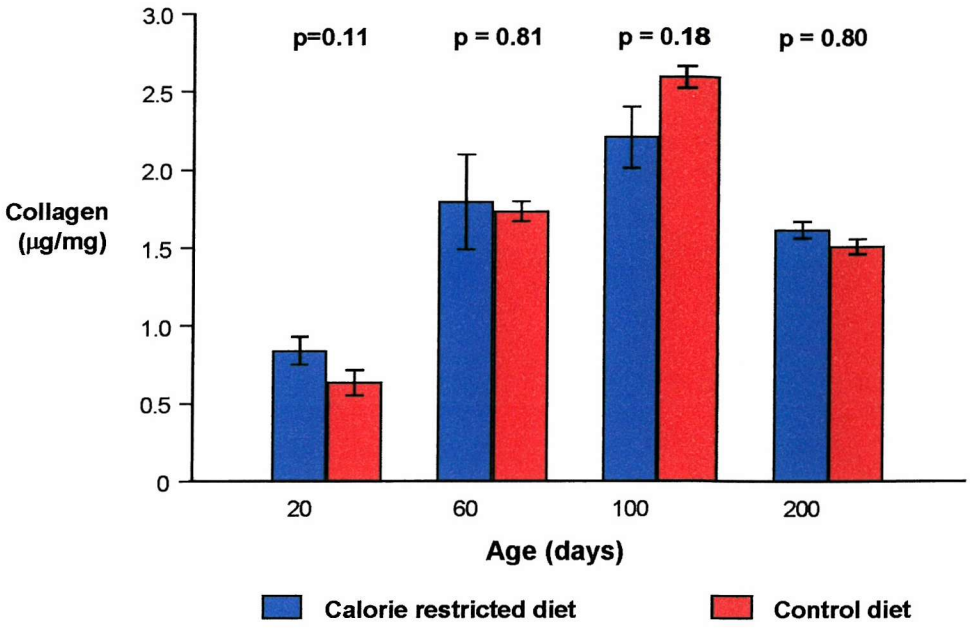
The mean collagen content of the aortas of rat pups born to mothers fed either a calorie restricted or a control diet during pregnancy is summarised in table 6.13 and figure 6.6 according to age at death.

Table 6.13 Mean collagen content (μg/mg) of the aortas of rat pups aged between 20 and 200 days whose mothers were fed either a calorie restricted or a control diet during pregnancy.

Age (days)	Control			Calorie Restricted			P value of
	Mean	SD	n	mean	SD	n	difference
20	0.63	0.37	20	0.84	0.31	12	0.11
60	1.73	0.22	12	1.79	1.05	12	0.81
100	2.59	0.25	13	2.20	0.59	9	0.18
200	1.51	0.16	11	1.61	0.23	19	0.80

On average the collagen content of the aortas of the maternal calorie restricted diet rat pup group was 0.21μg/mg higher at 20 days, 0.06 μg/mg higher at 60 days, 0.39 μg/mg lower at 100 days and 0.10 μg/mg higher at 200 days than maternal control diet rat pups. None of these differences were statistically significant.

Figure 6.6 Collagen content of the aortas of rats whose mothers were fed either a calorie restricted or a control diet during pregnancy.



(error bars show the standard error of the mean)

Multilevel modelling was used to investigate the effect of a maternal calorie restricted diet on the collagen content of the aorta after adjusting for genetic similarities between rat pups of the same litter. The results are summarised in table 6.14.

Table 6.14 Multilevel linear regression models of the effect of a calorie restricted diet compared to a control diet on the vessel collagen content (µg/mg).

Age (days)	Regression coefficient	95% ci	P value
20	-0.21	-0.45 to 0.04	0.103
60	-0.12	-1.18 to 0.95	0.832
100	0.35	-1.14 to 1.85	0.641
200	-0.31	-0.94 to 0.32	0.337

There were no statistically significant relationships between the collagen content of the aortas of maternal calorie restricted or maternal control diet rat pups at any of the ages studied.

6.8 Discussion

The mean birth weight of the rat pups born to mothers fed a low protein diet was statistically significantly lower than the mean birth weight of control group rat pups. Systolic blood pressure was higher in the rat pups born to nutritionally restricted mothers compared to rat pups born to control diet mothers in both models. These differences were statistically significant in each model at all ages studied.

In the maternal low protein model, the gravimetric method showed that the percentage of alkali soluble + alkali insoluble elastin and alkali insoluble elastin were lower in both the abdominal and thoracic aortas of maternal low protein group rat pups compared to controls. After multilevel modelling to take into account genetic similarities between littermates, these differences remained, although they were only statistically significant for alkali insoluble elastin in the thoracic aorta. Using the elastin assay to detect alkali insoluble elastin also showed that elastin concentrations were lower in both the abdominal and thoracic aortas of maternal low protein group rat pups compared to controls, and these differences were statistically significant in multilevel modelling. There was little difference in the collagen levels between maternal low protein and maternal control diet rat pup aortas.

In the calorie restricted model the percentage of alkali soluble + alkali insoluble elastin in the whole aorta sections was lower in the maternal calorie restricted group compared to controls at the ages of 20, 100 and 200 days. Only the difference at 200 days was statistically significant. In the 60 day old animals the percentage elastin content was higher in the maternal control diet group compared to the maternal calorie restricted group, however, the difference was not statistically significant. There was little difference in the collagen levels between maternal calorie restricted and control group rat pup aortas at any of the ages studied.

The results of the present study have shown that in two different models of intrauterine growth restriction and using two different methods of quantifying elastin (in the low protein model) that elastin levels are lower and collagen concentrations are changed little in the aortas of growth restricted rat pups. However, the gravimetric method of elastin determination has been criticised because the collagen content of rat aortas may not be adequately solubilised by aqueous hydrolysis. Therefore, in the present study the hydroxyproline content of the supernatant may not have been exact and the determination of elastin would have been inaccurate because the solid material would also have contained collagen. However, unless collagen solubility differs between the maternal low protein or maternal calorie restricted group aortas and the control group aortas the results should not be affected. This is because the proportion of elastin will be overestimated and the proportion of collagen will be underestimated to the same degree in both the test and control group. Therefore, the mean difference in the proportion of elastin and collagen between groups should be unchanged.

Another less crude method, the Fastin elastin assay was therefore also used to quantify alkali insoluble elastin in the low protein model study. In this method the aortas were treated with oxalic acid to generate alpha elastin, a solubilised form of elastin. A dye reagent was then bound to the alpha elastin in each sample and colourimetric analysis was used to quantify the elastin content. The alkali insoluble elastin measurements were on average 4% lower in the thoracic aorta and 9% lower in the abdominal aorta using the Fastin elastin assay compared to the gravimetric method. These differences could reflect incomplete solubility of collagen in the gravimetric samples. This is suggested by the fact that the mean difference in elastin concentration between the two methods was greater in the abdominal segment (9%) where the collagen concentration is higher compared to the thoracic aorta (4%) where the collagen concentration is lower. Also in the multilevel models, the average percentage reduction in alkali insoluble elastin in maternal low protein group rats was similar in the gravimetric (13.34% thoracic, 9.06% abdominal) and Fastin elastin assay (14.45% thoracic, 9.45% abdominal) methods. This supports the argument that the proportion of elastin is overestimated and the proportion of collagen is underestimated to the same degree in the gravimetric method so the mean difference in the proportion of elastin and collagen between experimental groups is changed little (<1%).

The Fastin elastin assay however, has also been criticised because the dye binding properties of alpha elastin can vary greatly. Variability was controlled for in the present study through the use of a control sample each time the assay was run. An inter-assay coefficient of variation was calculated, which was <10%. Even though both methods used in the present study have limitations, they both yielded similar results. Elastin levels were lower in the maternal low protein group compared to the control group in both the thoracic and abdominal aorta, which suggests that the results are reliable despite the methodological weaknesses.

The number of rats used in the present study was small and therefore the statistical power was low. Also a large number of rats were used from the same litters compared to the total number of litters. Therefore, it was difficult to decipher genetic interactions from the effects of the test diet using comparisons of means and linear regression analysis alone. However, multilevel modelling takes into account genetic similarity between litters. The results of the multilevel modelling still showed aortic elastin to be lower in the low protein or calorie restricted groups (except at age 60 days) compared to controls, although not all of the differences were statistically significant.

There are no other known published studies that have investigated elastin and collagen levels in the aorta in models of intrauterine growth restriction. There are however, several studies that have measured lung elastin and collagen content after maternal protein or calorie restriction in the perinatal period, and in adulthood in rats.[190-192] These studies show that lung elastin content is also reduced in rats born to mother whose diets were nutritionally restricted in the immediate postnatal period compared to controls. In adult life however, dietary restriction had no effect on lung elastin content. Lung collagen content was found to be more resistant to dietary manipulation

both in the perinatal period and during adult life.[190; 192] These studies, together with the results of the present study provide evidence that elastin synthesis in two different rat tissues is sensitive to dietary manipulation during fetal and early postnatal life.

How then could a reduction in maternal nutrition during pregnancy result in lower elastin production in rat aortas? One possibility is that a reduction in Insulin like Growth Factor 1 levels cause a fall in aortic elastin production. Several animal models have shown that serum Insulin like Growth Factor 1 levels fall with maternal undernutrition. For example, lambs born to ewes fed 50% of their recommended energy requirements from mid pregnancy (80 days after conception) had significantly lower Insulin like Growth Factor 1 levels compared to lambs born to non-nutrient restricted ewes.[193] Similarly, the offspring of pregnant rats fed a low protein diet (5%) throughout gestation had serum Insulin like Growth Factor 1 levels that were 30% lower than those born to mothers fed a control diet (20% protein) throughout pregnancy.[194] Insulin like Growth Factor 1 upregulates expression of the tropoelastin gene.[102; 158] Therefore, reductions in Insulin like Growth Factor 1 concentrations as a result of maternal undernutrition could slow elastin synthesis through decreasing tropoelastin mRNA transcription.

6.9 Conclusions

The results of the present study showed that in two different models of intrauterine growth restriction, maternal nutritional deficiency in rats during pregnancy results in a reduction in the content of elastin and little change in the content of collagen in the aortas of the offspring compared to controls.

Although based on small numbers of rats these findings are supporting evidence that the uterine environment influences programming of elastin synthesis in the aorta.

Chapter 7

Discussion and Conclusions

7.1 Summary of the main findings

The work in this thesis describes the relationship between indicators of fetal growth restriction and arterial compliance in three different groups of people who were measured in detail at birth.

The first group of people consisted of elderly men and women who were born in the Jessop Hospital for Women, Sheffield between 1922 and 1930. After adjusting for age, sex and current body mass index, people who were born thin tended to have raised blood pressure, although the relationship was of borderline statistical significance. Diastolic blood pressure level was not related to any of the measures of size at birth. Men and women who were born thin also tended to have faster pulse wave velocities in the aorta to femoral segment and people born long in relation to their head circumference, or with a heavier placenta tended to have faster pulse wave velocities in the aorta to radial segment. There were no relationships between birth measurements and pulse wave velocity in either the aorta to foot or femoral to foot segments.

The second study population was made up of a group of young men and women who were born in the Farnborough Hospital, Kent between 1975 and 1977. Men and women who were light, or who had had a smaller head circumference at birth or who were born after a shorter length of gestation tended to have raised systolic blood pressure after adjusting for the effects of age, sex, current body mass index and taking the contraceptive pill. People who were light at birth also tended to have raised diastolic blood pressure. Young adults who were born after a shorter length of gestation tended to have faster pulse wave velocities in the aorta to foot segment. There were no relationships between birth measurements and pulse wave velocity in the aorta to radial segment.

The third group consisted of a cohort of children born in the Princess Anne hospital, Southampton in 1987. After adjusting for current weight and gestational age at birth, children who were born with a lighter placenta tended to have raised systolic blood pressure, although the relationship was not statistically significant. There were no relationships between diastolic blood pressure and any of the birth measurements. Boys and girls who were born after a shorter length of gestation tended to have faster pulse wave velocities in the aorta to femoral segment. Children who were born long in relation to their head circumference tended to have faster aorta to radial pulse wave velocities. In boys and girls who had had a heavier placenta, a larger ponderal index at birth or who were born after a shorter length of gestation, aorta to foot pulse wave velocity also tended to be faster. In children who had had a heavier placenta at birth, pulse wave velocity in the femoral to foot segment tended to be faster.

7.2 Comparison of the results of the published and present studies

The birth measurements that were associated with faster pulse wave velocities in the studies of elderly people, young adults and children and the previous published study of 50 year old men and women are summarised in table 7.1.

Table 7.1 Summary of the birth measurements that were related to a faster pulse wave velocity in the published and present studies.

Study	Aorta to femoral segment	Aorta to radial Segment	Aorta to Foot segment	Femoral to foot Segment
Published study [28] 50 to 53 year olds	↓ Birth weight ↓ Abdominal circumference ↓ Length	*Not measured*	*Not measured*	↓ Birth weight ↓ Abdominal circumference ↓ Length ↓ Head circumference
Chapter 3 Elderly people	↓ Ponderal index	↓ Head:length ratio ↑ Placental weight	None	None
Chapter 4 Young adults	*Not measured*	None	↓ Gestational age	*Not measured*
Chapter 5 Children	↓ Gestational age	↓ Head:length ratio	↑ Placental weight ↓ Gestational age	↑ Placental weight

↓ = smaller/shorter ↑ = greater green text indicates trends that were statistically significant (p=<0.05)

The results of the present studies were not consistent with those of the published study.[28] Pulse wave velocity was not related to smaller birth weight, abdominal or head circumference or length at birth in any of the segments studied in the elderly men and women, in the young adults or in the children. These inconsistencies could not be explained by differences in the mean birth size between the published and present study populations.

There were similarities between the results of the present studies. For example, in elderly men and women and in ten year old children, pulse wave velocity in the aorta to radial segment tended to be faster in people who were born long in relation to their head circumference. In the young adults and in children, aorta to foot pulse wave velocity tended to be faster in people who were born after a shorter period of gestation. In the elderly people and in the children, pulse wave velocity tended to be faster in those who had had a heavier placenta at birth. However, the arterial segments in which these relationships were found differed (aorta to radial segment in the elderly men and women, aorta to foot and femoral to foot segments in the children).

However, there were also inconsistencies between the present studies. For example, in the study of elderly men and women, aorta to femoral pulse wave velocity was related to a lower ponderal index, this was not found in the children's study. In the study of young adults, no relationships were found between size at birth and pulse wave velocity in the aorta to radial segment. In the elderly and children's studies, a faster aorta to radial pulse wave velocity was related to a low head circumference to length ratio and a heavier placenta (elderly men and women only). In the elderly men and women's study there were no relationships between size at birth and pulse wave velocity in the aorta to foot or femoral to foot segments. In the young adults and children's studies, a faster aorta to foot pulse wave velocity was related to a shorter length of gestation, and also to a heavier placenta in the children's study. These inconsistencies could not be explained by differences in the mean birth sizes between study populations. Instead they may reflect different growth restricting factors acting at different stages during pregnancy in each of the study populations.

If the birth measurements that were related to pulse wave velocity are considered according to the likely timing of the causal growth-restricting factor during pregnancy, rather than the actual body dimensions at birth, a slightly more consistent picture appears across the present studies. Pulse wave velocity in the elastic arteries (aorta to femoral segment) was faster in people born after a shorter length of gestation in the children's study, and also in men and women who had a smaller ponderal index at birth in the elderly men and women's study. Both these are indicators of fetal growth restraint in the latter stages of pregnancy (figure 1.1), a time when elastin is being produced maximally in the aorta.[99] Pulse wave velocity in the muscular arteries (aorta to radial, aorta to foot and femoral to foot) was faster in people who were born with a heavier placenta (elderly and children's study), or who were born disproportionately long in relation to their head circumference (elderly and children's study). Both of these are indicators of growth restriction in mid to late pregnancy (figure 1.1).

7.3 Weaknesses and limitations

In summary, the results of the present studies indicate that people born with indicators of fetal growth restriction in mid to late pregnancy tend to have faster pulse wave velocities in later life. The question that arises is whether these relationships might be explained by reasons other than poor fetal growth causing the aorta and large arteries to be stiffer. Alternative explanations might include biases in the study design, confounding effects or chance associations.

7.4 Bias

Bias is defined as any systematic error that results in an incorrect estimate of the relationship between a disease and the exposure of interest.[195] There are two main sources of bias. The first source is information bias, which occurs from errors in measuring the disease or exposure. The second source is selection bias, where the subjects in the study are not representative of the population from which the sample was derived.

Measurement error can be minimised by using techniques that have been validated. In chapter 2, several validation studies were outlined which show that the optical technique for measuring pulse wave velocity reproduces the characteristics of the pressure wave. In each of the present studies pulse wave velocity traces were analysed blind to subjects' birth measurements, therefore systematic error is unlikely. Random error was examined by making repeat pulse wave velocity measurements after a period of 2 to 14 months (chapter 2) and the technique was shown to be reproducible.

In the study of elderly men and women, the final follow up study sample consisted of only 6.8% of the original cohort of singletons born in the Jessop Hospital between 1922 and 1930 for whom birth records had been kept (n=7118). Therefore, there is potential for selection bias because of the great loss to follow up. The participants in the elderly men and women's study are not representative of all the people born in Sheffield during 1922 to 1930 because they were born in a maternity hospital at a time when many births took place at home. Also these people continued to live in the city in which they were born. However, in the statistical analysis comparisons were only made within the group who participated so selection bias would only arise if the associations between fetal growth and pulse wave velocity were different between those who took part in the study and those who did not. There was less than 2% difference in the mean birth weights of the 481 elderly men and women who participated in the follow up study and those of the original cohort of people who were born in the Jessop Hospital, so it is unlikely that selection bias can account for the findings.

In the young adults and children's study the losses to follow up were lower and 29% and 48% of the original cohorts (respectively) were willing to take part, although the figure was reduced to 16% in the children's study by a stratification sampling method (chapter 5, section 5.1). Again, the mean birth measurements of the 347 young adults and 64 children who participated in these studies were both within 1% of those of the original cohorts so selection bias is not a likely explanation for the results.

7.5 Confounding

Another explanation for the relationships found in the present studies might be confounding by factors that are associated with poor fetal growth and which independently determine arterial compliance. Confounding may cause associations to be confused or spurious relationships to appear. Information on known confounding factors was collected during the studies, and included for example, sex and current blood pressure. It was then possible to use multiple regression analysis to examine the association between birth size and pulse wave velocity whilst controlling for these variables.

Controlling for confounding is limited by the fact that there may be unknown confounding factors, or excessive adjustment in statistical modelling may dilute any real effects or bias the data in the

favoured direction. Several variables were controlled for in the linear regression models in the study of elderly men and women and young adults. However, the children's study had the advantage of fewer known confounding variables because many of the factors that are associated with poor fetal growth and which also determine arterial compliance are related to ageing, for example raised blood pressure and ischaemic heart disease.

7.6 Random error

Even after potential biases and confounding have been considered the results of the present studies may still be subject to errors through chance alone. However, the likelihood of random error is quantified by the p value in hypothesis testing during statistical analysis.

Analysis of multiple variables was carried out in the present studies, which increases the likelihood of finding statistically significant results by chance. For example, in table 3.8 there were 18 tests in each of 4 groups giving a total of 72 tests. Assigning the statistical significance level at a p value of 0.05 means that 1 time in 20 a result may be found to be statistically significant through chance alone. Therefore, 3 to 4 of the tests in table 3.8 might be expected to be statistically significant due to chance. One solution to the problem of multiple comparisons is to use the bonferroni correction.

According to the Bonferroni correction, to achieve an overall type 1 error rate of 0.05 when conducting *n* significance tests, only results where the p value was less than 0.05/*n* would be classed as statistically significant. Thus to test the 72 hypotheses in table 3.8, the results would only be considered statistically significant when the p value for any one of the tests was less than 0.007 (0.5/72). Therefore, applying the Bonferroni correction to the data presented in chapters 3, 4 and 5 would have resulting in fewer of the relationships between blood pressure or pulse wave velocity and birth measurements being considered statistically significant.

7.7 Interpretation

Bias, confounding and chance are not likely explanations for the association between indicators of fetal growth restriction during mid to late pregnancy and a faster pulse wave velocity in the present studies. Therefore, poor fetal growth may well have lead to reduced arterial compliance in the elderly men and women, the young adults and the ten year old children. There is a biologically plausible explanation that can demonstrate how the fetal environment might influence arterial compliance and lead to raised blood pressure and increased risk of cardiovascular disease in adult life.

7.7.1 Critical period during which elastin synthesis must take place

The elastic properties of arteries are largely dependent upon the amount and organisation of the scleroprotein elastin in their vessel walls. Synthesis of elastin begins during fetal life and reaches a maximum during the early postnatal period, then slows thereafter (4 to 6 months postnatal). During postnatal life elastin production ceases and no more elastin is produced in healthy vessels. There appears to be a critical period of time during fetal and early postnatal life when elastin must be synthesised. Failure to produce adequate quantities of elastin during this period would result in the elastic properties of the arterial walls being permanently reduced.

7.7.2 How the fetal environment might affect elastin synthesis

An adverse fetal environment during a critical period of arterial development could alter elastin synthesis levels through reductions in elastin gene expression or by altering local haemodynamic conditions.

Elastin production is regulated at the transcriptional level. Expression of the elastin gene is modulated by a number of factors, such as cortisol, Transforming Growth Factor-β and Insulin like Growth Factor-1, which upregulate tropoelastin production. Levels of these elastin gene modulators are modified by cytokines such as Fibroblast Growth Factor. The enzyme lysyl oxidase catalyses cross-linking of the tropoelastin molecules to form mature elastin. Lysyl oxidase expression is upregulated by effectors such as Fibroblast Growth Factor. An adverse uterine environment might lead to a reduction in elastin synthesis rates if levels of any of these factors are altered as a result of fetal growth restriction. Indeed, Insulin like Growth Factor 1 levels have been shown to be lower in the umbilical cord blood of growth restricted babies compared to non-growth restricted infants [196] and one study has shown that cord serum Fibroblast Growth Factor-2 levels decrease as birth weight falls.[154]

There is evidence to suggest that elastin production is influenced by local haemodynamic conditions. For example, lower elastin contents have been observed in the carotid arteries of immature rabbits after experimentally reducing blood flow.[181] Generation and biological activation of one of the elastin gene modulators, Transforming Growth Factor-β, has been shown to increase in human vascular smooth muscle cells in proportion to the intensity of shear stress, which is the frictional force due to fluid flow.[197] Redistribution of fetal blood flow occurs in disproportionately growth-restricted fetuses where there is a preferential flow of blood to the brain.[180] The resulting reduction in blood flow to the periphery might reduce elastin synthesis through diminished rates of Transforming Growth Factor-β modulated elastin tropoelastin mRNA stabilisation.

7.7.3 Elastin in the aortas of intrauterine growth restricted rats

The results of the studies detailed in chapter 6 showed that the elastin content of the rat aorta was reduced in the growth restricted offspring in two different models of intrauterine growth restriction. Elastin levels were lower in the rat pups of mothers fed either a low protein diet during pregnancy

or mothers who were calorie restricted throughout pregnancy. Although the results can not be directly related to humans, they do provide strong evidence to suggest that elastin is susceptible to nutritional factors that restrict intrauterine growth in rats.

7.7.4 Reduced arterial compliance leads to raised blood pressure

If the arterial elastin content is also lower in growth restricted humans at birth this could lead to the genesis of raised blood pressure in adult life through acceleration of the normal ageing process. During ageing, elastin laminae in the arterial walls fragment and stress is transferred to the less extensible collagen fibres and the arteries become stiffer. Because the arteries are stiffer, pulse pressure rises and the vessel walls are stressed further. The vascular smooth muscle cells respond to greater stretch by synthesising collagen. The increase in collagen results in thickening of the vessel wall and a further loss of elasticity. Pulse pressure increases and a feedback mechanism is set up whereby raised blood pressure levels are maintained (figure 1.5).

7.7.5 Reduced arterial compliance leads to cardiovascular disease

Arterial compliance is an important factor in maintaining the efficient functioning of the circulatory system. Reducing elasticity of the aorta and large arteries impairs their buffering function and has adverse cardiovascular consequences. Systolic blood pressure rises, diastolic blood pressure falls, and pulse pressure increases. The rise in systolic blood pressure is a major contributor to left ventricular hypertrophy, and the increase in pulse pressure promotes development of vascular damage and atherosclerosis.

7.7.6 Left ventricular hypertrophy

Pulse pressure is determined by the interaction of the pressure wave generated by left ventricular ejection and the reflected waves created within the arterial system.[198] In a compliant arterial system reflected waves return to the heart during diastole after the aortic valve has shut and do not contribute to ventricular load.[199] A reduction in arterial compliance causes the pulse waves to propagate along the arterial wall at a faster rate. The increased pulse wave velocity means that reflected waves return to the heart sooner, which augments systolic pressure. The left ventricle responds to a rise in systolic pressure by increasing its mass. Initially this acts as a compensatory mechanism to maintain wall tension. According to the law of Laplace, left ventricular wall tension is directly proportional to ventricular pressure and the cavitary radius, and is inversely proportional to wall thickness (Lamés equation). Therefore, the wall tension remains normal despite elevated ventricular systolic pressure because the thickness of the wall is increased. However, when the rise in systolic pressure persists, the cardiac muscle hypertrophies. Despite the adaptive benefit of left ventricular hypertrophy in maintaining wall tension, if the process continues it confers cardiovascular risk. The increased tissue mass results in a greater myocardial oxygen demand. Failure to meet this demand leads to fibrosis of the myocardium and eventually results in left ventricular failure.[200]

Several studies in humans have shown that reduced arterial compliance is related to left ventricular hypertrophy.[201; 202] An animal study has also shown that increasing aortic stiffness in dogs aggravated myocardial ischaemia when coronary blood flow was impaired.[203] These studies emphasise the importance of the elastic properties of the arteries in moderating the systolic pressure rise, reducing left ventricular afterload and aiding coronary blood flow.[57]

7.7.7 Vascular damage and atherosclerosis

Physical materials will rupture or tear when stresses they cannot withstand are applied. Pulsatile stresses are more likely to cause tears than steady stresses. As the pulsatility of the stress becomes greater, the rate at which the damage will occur also increases. These mechanical concepts can be applied to the arterial system, which undergoes repetitive cyclic stress as it dampens the pulsations from the heart. Indeed, these principles might explain why the degeneration that leads to arterial damage and the initiation of atherosclerotic disease occurs.

A reduction in arterial compliance leads to a rise in pulse pressure (figure 1.5). This will amplify the arterial diastolic-systolic expansion and increase the blood velocity at the vessel wall.[57] Therefore, the likelihood of vascular injury and intimal tears will rise. The arterial wall is stretched to a greater extent in response to the raised pulse pressure and so the endothelial surface area is increased. This may heighten endothelial permeability to albumin and lipoproteins and adherence of leukocytes and as a result atherosclerotic plaque formation is initiated.[61] Indeed, animal studies have shown that arterial compliance is reduced in the aortas of monkeys fed on atherosclerotic diets.[205] In humans, a post-mortem study showed that the degree of atherosclerosis correlated with arterial compliance measured between 60 and 370 days before death.[63]

The atherosclerotic process may begin in the large arteries and progressively involve the smaller vessels including the coronary arteries and thus lead to coronary heart disease. Several epidemiological studies have shown that arterial compliance is reduced in patients with coronary heart disease compared to healthy controls.[64; 65] However, these studies only provide evidence of coexistence of reduced arterial compliance with atherosclerosis and coronary heart disease, they do not demonstrate the direction of the cause effect relationship. Atherosclerotic lesions in the vessel wall could lead to a reduction in arterial elasticity rather than resulting from it.

7.8 Evidence from the studies to support the hypothesis

The theoretical explanation outlined above in section 7.7 demonstrates how the fetal environment might influence arterial compliance through reduced elastin synthesis and lead to raised blood pressure and increased risk of cardiovascular disease in adult life. However, do the results of the present studies support the theory? In order to answer this question, the hypothesis on which the work was based (section 1.21) has been split into three sections. Firstly, the synthesis of arterial elastin is influenced by poor intrauterine growth. Secondly, fetal growth restriction results in lower arterial elastin concentrations leading to persistently stiffer arterial walls and the genesis of raised

blood pressure. Thirdly, growth-restricting factors acting in the latter stages of pregnancy have the greatest effect on reducing arterial elastin synthesis. The strengths and weaknesses of the study results will be discussed for each section separately.

7.8.1 The synthesis of arterial elastin is influenced by poor intrauterine conditions

The studies carried out on the two rat models of intrauterine growth restriction (chapter 6) showed that elastin levels were lower in the aortas of rat pups born to mothers fed either a low protein or a calorie restricted diet compared to rat pups born to mothers fed a control diet. This provides evidence that intrauterine growth restriction can reduce arterial elastin concentrations as the hypothesis predicts. However, the results of these studies need to be confirmed in another study with a larger number of animals from different litters to increase the statistical power and to reduce the likelihood of genetic similarity. The methodological problems involved in the elastin quantification techniques (detailed in section 6.7) also need to be addressed. To overcome the methodological problems it is hoped that a Western Blot method for quantifying the elastin content of the rat aortas will be developed.

In the Western Blot method an antibody specific to elastins, and one specific to alpha elastin will be bound to separate aliquots of the solubilised aortic elastin sample from each of the rats that were used in the study detailed in chapter 6. A secondary antibody with a marker will then be bound to the primary antibody. Using an immunodetection system the bands of elastin will be visualised onto x-ray film and quantified using densitometry. The advantage of this technique will be that different isoforms of elastin and tropoelastin will be identifiable, so it should provide a qualitative as well as a quantitative estimate of the elastin content in the aortas of the maternal dietary restricted and control rat groups.

The results of the animal studies cannot be directly related to humans. If the Western Blot method for quantifying elastin confirms that aortic elastin levels are lower in growth restricted rat pups compared to controls, then the next step would be to measure arterial compliance in human babies to investigate whether pulse wave velocity is faster in infants who are small in size at birth. The difficulty with this study would be deciding at what age to make the pulse wave velocity measurements. At birth elastin is still being synthesised in the arteries and the postnatal age at which elastin synthesis ceases in humans is unknown. One solution to this problem would be to make a series of pulse wave velocity measurements starting at birth, and then at 6 month intervals thereafter in a group of babies whose birth size had been measured in detail. Then it would be possible to see if and how the relationships between birth size and pulse wave velocity differ over time during infancy and childhood. Alternatively the umbilical cord artery elastin concentrations could be measured from babies whose size was measured in detail at birth. First, a validation study would be necessary to investigate whether umbilical cord artery elastin concentrations correlate with aortic elastin levels.

7.8.2 Fetal growth restriction results in lower arterial elastin concentrations and leads to persistently stiffer artery walls and the genesis of high blood pressure through acceleration of the normal ageing process

The studies detailed in chapters 3 to 5 have provided some interesting results lending support, although weak, to the idea that fetal growth restriction leads to persistently stiffer artery walls and the genesis of high blood pressure in later life. The data suggest that pulse wave velocity of the elastic arteries may provide a link between small birth size and raised blood pressure. The data also suggest that a reduction arterial compliance in the muscular arterial segment with high elastin content (aorta to radial) and in the segment containing elastic and muscular arteries (aorta to foot) may be a cardiovascular risk factor in its own right. An important finding in the children's study was that the relationships between birth size and pulse wave velocity were independent of current blood pressure levels. These results are discussed below.

In the study of elderly men and women (chapter 3), people who were thin at birth tended to have raised blood pressure, although the relationship was not statistically significant at conventional levels. Pulse wave velocity also tended to be faster in elderly men and women who were thin at birth, but only in the aorta to femoral segment. These results suggest that reduced compliance of the elastic arteries could provide a mechanism linking small size at birth to raised blood pressure in adult life. There is supporting evidence for this theory in the study of young adults (chapter 4). Systolic blood pressure tended to be higher in men and women who were born after a shorter length of gestation. Pulse wave velocity in the aorta to foot segment, which includes an elastic artery segment (aorta to femoral) and a muscular artery segment (femoral to foot) also tended to be faster in the young adults born at a younger gestational age. However, the results of the children's study (chapter 5) did not support this theory. Children who were born with a lighter placenta tended to have raised blood pressure at ten years old, whereas aorta to femoral pulse wave velocity tended to be faster in boys and girls who were born with a heavier placenta. Therefore, the evidence for this section of the hypothesis is unclear from the human studies.

In the studies detailed in chapters 3 and 5, pulse wave velocity in the segments containing muscular artery with a high elastin content (aorta to radial) and in the segment containing elastic and muscular arteries (aorta to foot segments) was related to birth dimensions that did not show strong relationships with systolic blood pressure. For example, aorta to radial pulse wave velocity was faster in elderly men and women and children who were born long in relation to their head circumference. Relationships with systolic blood pressure and head circumference to length ratio were in the same direction in both these study populations, however, they were weak and not statistically significant. Aorta to radial (elderly men and women) and aorta to foot (children) pulse wave velocities were faster in people who were born with heavier placentas. However, systolic blood pressure was higher in elderly men and women and in children who were born with lighter placentas. This could indicate that faster pulse wave velocity in the muscular arterial segments is

a cardiovascular risk factor independently of raised blood pressure. Indeed, this has been suggested by studies, which have shown that men and women with coronary heart disease, myocardial infarction, left ventricular hypertrophy and stroke have stiffer arteries compared to healthy controls.[69] Another study has suggested that an increase in the pulsatile component of blood pressure, which is determined by arterial elasticity for a given left ventricular ejection is related to cardiovascular disease independently of the steady component, which is influenced by peripheral resistance and cardiac output.[206] Alternatively these results may demonstrate that there is in fact little consistency in the relationship between blood pressure, pulse wave velocity and size at birth.

The direction of the cause effect relationship between poor fetal growth, reduced arterial compliance and raised blood pressure is not straightforward. Arterial compliance is related to blood pressure levels and as blood pressure rises arterial compliance will fall. Therefore, the relationship between indicators of fetal growth restriction in mid to late pregnancy and reduced arterial compliance in each of the studies could reflect these peoples' tendencies to have higher blood pressure rather than changes in arterial wall composition. In order to take into account the effects of current blood pressure levels on arterial compliance, systolic and diastolic blood pressure were added into the multivariate analysis in the studies of elderly men and women and young adults. However, this is not an entirely satisfactory procedure (detailed in chapter 5, section 5.15). In the children's study the relationships between size at birth and reduced arterial compliance were independent of blood pressure level. This suggests that pulse wave velocity was faster in ten year old children because they had been smaller at birth. However, the number of subjects in the children's study was low, particularly in relation to the number of hypothesis tests carried out. Therefore, firm conclusions could not be drawn from the results of this study. The results need to be verified in further children's studies that have greater statistical power, but the present results indicate that poor fetal growth results in a reduction in the elastic properties of the arteries and is not just a consequence of blood pressure level.

There were also some surprising findings in the studies detailed in chapters 3 to 5. For example, the strength of the relationships between pulse wave velocity and size at birth were weak. The method for measuring pulse wave velocity proved unsatisfactory in the elderly men and women with ischaemic heart disease. In the study of young adults pulse wave velocity was not strongly related to any of the birth size measurements and in the children's study the numbers were too small to draw firm conclusions. These findings are discussed below.

The relationships between pulse wave velocity and birth size measurements were weak in each of the studies. However, it is difficult to assess the significance of the changes that the regression coefficients describe in terms of cardiovascular risk. For example, in the study of elderly men and women what does a 0.17 m/sec increase in logged aorta to femoral pulse wave velocity per oz/in^3 x 1000 increase in ponderal index actually mean?

To explore this, regression coefficients from each multivariate analysis were divided by the standard deviation of the study population mean for systolic blood pressure or pulse wave velocity to produce z scores. This enabled comparison of the size of the effect that a one-unit reduction in each birth measurement would have on the population standard deviation of systolic blood pressure and pulse wave velocity. The strength of the z scores for pulse wave velocity could then be compared with those of systolic blood pressure. Z scores for the systolic blood pressure and pulse wave velocity measurements with each birth measurement in the study of elderly men and women are summarised in table 7.2.

Table 7.2 Z scores for systolic blood pressure (mmHg)and pulse wave velocity (m/sec) in the study of elderly men and women.

	Systolic blood pressure	Aorta to femoral	**Arterial** Aorta to radial	**Segment** Aorta to foot	Femoral to foot
Birth weight (lb)	0.052	0.018	0.008	0.043	0.001
Head circumference (in)	0.076	0.008	0.142	0.546	0.002
Chest circumference (in)	0.001	0.001	0.048	0.051	0.004
Abdominal circumference (in)	0.012	0.016	0.015	0.065	0.016
Length at birth (in)	0.041	0.002	0.070	0.047	0.003
Head:abdominal circumference	0.024	0.386	1.378	1.420	0.039
Head:length	1.886	0.146	3.991	1.439	0.058
Ponderal index (oz/in^3 x 1000)	0.792	0.175	0.437	0.693	0.004
Placental weight (oz)	0.008	0.000	0.032	0.001	0.001
Placenta:birth weight	1.746	0.361	3.953	0.331	0.079
Gestational age (days)	0.005	0.000	0.002	0.001	0.000

The z scores for pulse wave velocities are similar to, or greater than those for systolic blood pressure and birth measurements in at least one of the arterial segments in all but the femoral to foot segment. Therefore, the relationships between aorta to femoral, aorta to radial and aorta to foot pulse wave velocities and size at birth may well represent a significant cardiovascular risk. Z scores for the study of young adults and children showed similar patterns.

In the study of elderly men and women (chapter 3) the method used to determine pulse wave velocity was probably inaccurate in people with ischaemic heart disease (detailed in 3.11). In fact the methodology, which incorporated left ventricular contraction times may have been flawed in all the studies because the method assumes that there is a standard aortic ejection time, which may not be the case. Indeed, one study showed that aortic ejection time increased with normal ageing and with presence of ischaemic heart disease.[66] In the elderly men and women's study this problem was taken into account in the analysis by including a term for ischaemic heart disease in

the regression models. However, in future studies of age groups where ischaemic heart disease is likely to be present, the foot of the pulse waveform detected by an infra red probe placed over another large artery, such as the carotid artery could be used to provide the first signal instead of the R wave of the electrocardiogram. However, signals originating from the carotid artery are not easy to measure or interpret as reversal of flow may occur, particularly in the elderly. Measuring the time between the aortic valve opening (visualised using ultrasound techniques) to the arrival of the pulse at a peripheral artery may provide a more satisfactory alternative.

In the study of young adults, pulse wave velocity was not strongly related to any measures of size at birth. Indeed the only relationship that was found was a tendency for men and women born after a shorter period of gestation to have faster aorta to foot pulse wave velocities. However, it was suggested earlier that it is reduced compliance of the elastic arteries that might link raised blood pressure to small size at birth. Pulse wave velocity in the aorta to femoral segment was not measured in these adults. Young adults who were born light, or with a small head circumference or who had been born after a shorter period of gestation tended to have higher systolic blood pressure, after adjusting for age, sex, current weight and taking the contraceptive pill. Men and women who were born after a shorter length of gestation did tend to have faster aorta to foot pulse wave velocities, and this relationship was statistically significant. Also, people who were born light or with a small head circumference tended to have faster aorta to foot pulse wave velocities (which includes the elastic aorta to femoral segment as well as the muscular femoral to foot segment). However, the relationships were weak (table 4.11, model iii: log B = - 0.003 and log B = -0.006 respectively) and not statistically significant (p=0.857 and p= 0.229 respectively). The relationships with birth measurements in the aorta to foot segment may not reflect those in the aorta to femoral segment alone. Indeed this was found to be the case in the study of elderly men and women. Therefore, it would be interesting to visit the subjects who took part in the young adults study again and make aorta to femoral pulse wave velocity measurements in them. It would then be possible to investigate whether pulse wave velocity in the aorta to femoral segment is faster in those people who were born light or with a smaller head circumference or after a shorter length of gestation.

The data from the children's study showed that relationships between birth measurements and pulse wave velocity of the elastic and muscular arteries were present at ten years old before adult blood pressure levels have been reached. However, firm conclusions could not be drawn from the data because of the small number of subjects (n=64). A larger scale study would be necessary to confirm these relationships. Indeed, the way forward with the elastin hypothesis might be to carry out another large scale study of children who were measured in detail at birth and to concentrate on determining pulse wave velocity in the aorta to femoral segment. The methodological and study size problems could be overcome by using an alternative method for measure pulse wave velocity, for example timing the aortic valve opening and the subsequent arrival of the pulse at a peripheral artery, and the study sample size should be derived using power calculations. The hypothesis

would be that elastin formation is adversely affected by poor intrauterine conditions and this leads to permanent structural changes in the elastic arteries leading to persistently stiffer vessel walls and the genesis of raised blood pressure. An extension of the children's study would also be important to confirm that the relationships between size at birth and pulse wave velocity are independent of blood pressure levels. This would provide evidence as to the direction of the cause effect relationship, that is children with indicators of fetal growth restriction have stiffer arteries because they were small at birth and not because they tend to have higher blood pressure.

7.8.3 Growth restricting factors acting in the latter stages of pregnancy will have the greatest effect in reducing arterial elastin synthesis and lead to permanently stiffer arteries

The results of the studies detailed in chapters 3 to 5 showed that all relationships found between measures of birth size and a faster pulse wave velocity (summarised in table 7.1) indicated intrauterine growth restriction in the mid to late pregnancy (figure 1.1), although the relationships were weak and inconsistent. There was no evidence that pulse wave velocity was related to proportionate growth restriction, which is indicative of poor fetal growth in the early stages of pregnancy. In the published study showed that 50 year old men and women with faster aorto-iliac or femoro-popliteal-tibial pulse wave velocities tended to be those who were born light, or with a smaller head or abdominal circumference or who were short in length at birth, which indicates proportionate growth restriction. Therefore, the evidence for this section of the hypothesis is not clear.

7.9 Conclusions

Elderly men and women, young adults and ten year old children whose size at birth suggested growth restriction in mid to late pregnancy tended to have faster pulse wave velocities in the aorta to femoral, aorta to radial and aorta to foot arterial segments which indicates that their arteries were stiffer. However, many of these results were trends rather than statistically significant findings. In view of the serious flaws in the human studies, which include the inadequacy of the method for measuring pulse wave velocity, particularly in elderly men and women with ischaemic heart disease, the small number of subjects in the children's study and the inconsistency of the findings and the borderline and sometimes unexpected directions of the trends, it is concluded that the results of the human studies do not support the starting hypothesis. It is uncertain whether the lack of support for the hypothesis was due to the methodological problems in the study designs or due to no real relationship existing between arterial compliance and intrauterine growth restriction.

The results of the animal studies showed that aortic elastin concentrations, and hence aortic compliance was reduced in rat pups born to mothers who were protein or calorie restricted during pregnancy compared to control group rat pups. These findings do support the starting hypothesis. However, the rat models of intrauterine growth restriction induced by dietary manipulation do not necessarily mimic human intrauterine growth restriction, which is unlikely to be caused by protein or severe calorie reduction. Although the results of the animal studies are not directly comparable to humans, they do provide evidence that arterial elastin concentrations are reduced by intrauterine growth restriction.

The results of the human studies were not entirely negative. The results of the children's study provided some interesting findings. For example, measurement of blood pressure in the children was not elevated and therefore it is proposed that the arterial changes are primary and may result in elevated blood pressure in later life. Also, the relationships that were found between arterial compliance and fetal growth restriction were independent of blood pressure, which suggests a reduction in arterial compliance causes blood pressure rise rather than being a results of increased blood pressure. Further studies in children are required to verify these findings.

In summary, although it was not possible to verify that persistent structural changes in blood vessels provide a mechanism linking small size at birth to raised blood pressure in later life in the human studies, the animal studies did show that formation of elastin, the scleroprotein primarily responsible for the elastic properties of the arteries, is adversely influenced by poor intrauterine conditions. Whether a similar reduction in arterial elastin also occurs in intrauterine growth restricted humans which is then translated in pathology in later life remains unclear.

Appendix A Raw data from the elderly men and women's study

Figure A1 Distribution of height (cm) in the elderly men and women

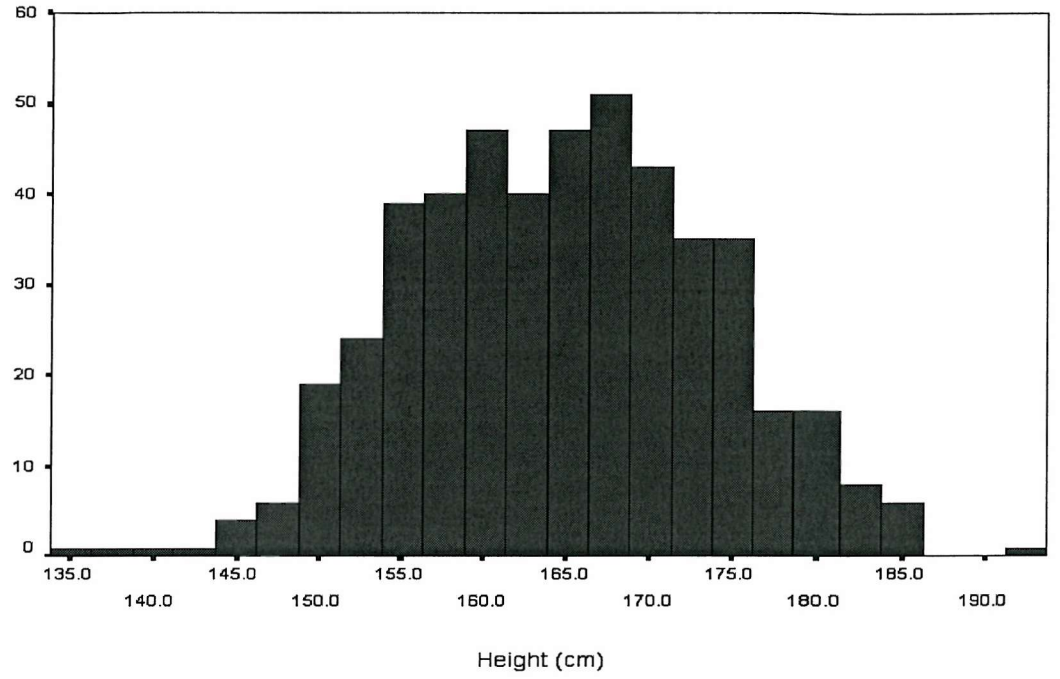


Figure A2 Distribution of weight (kg) in the elderly men and women

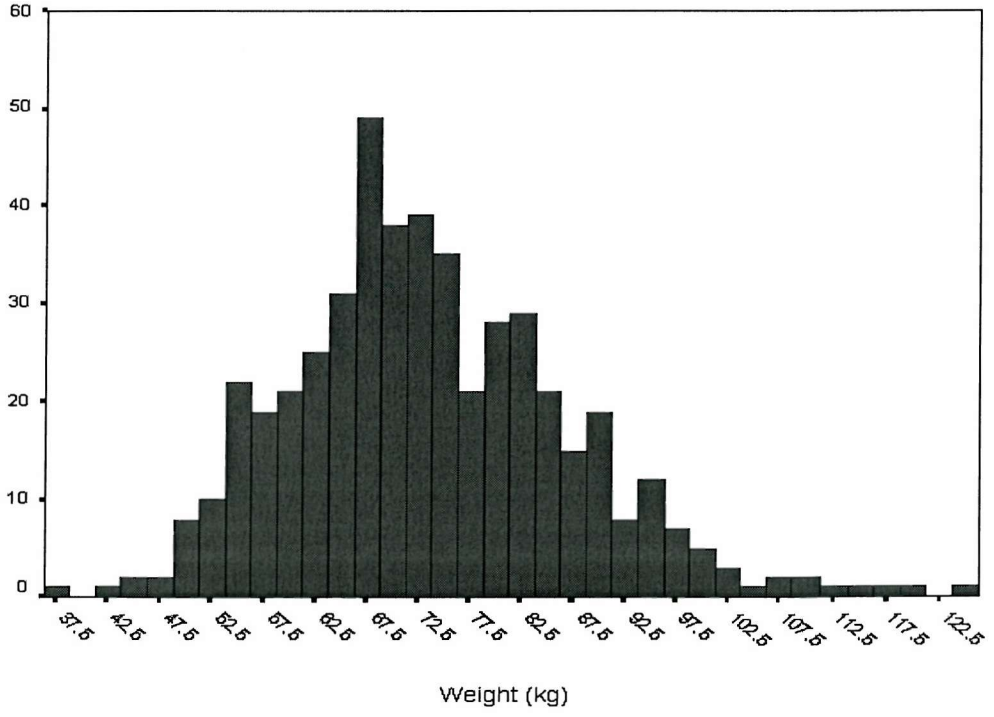


Figure A3 Distribution of body mass index (kg/m^2) in the elderly men and women

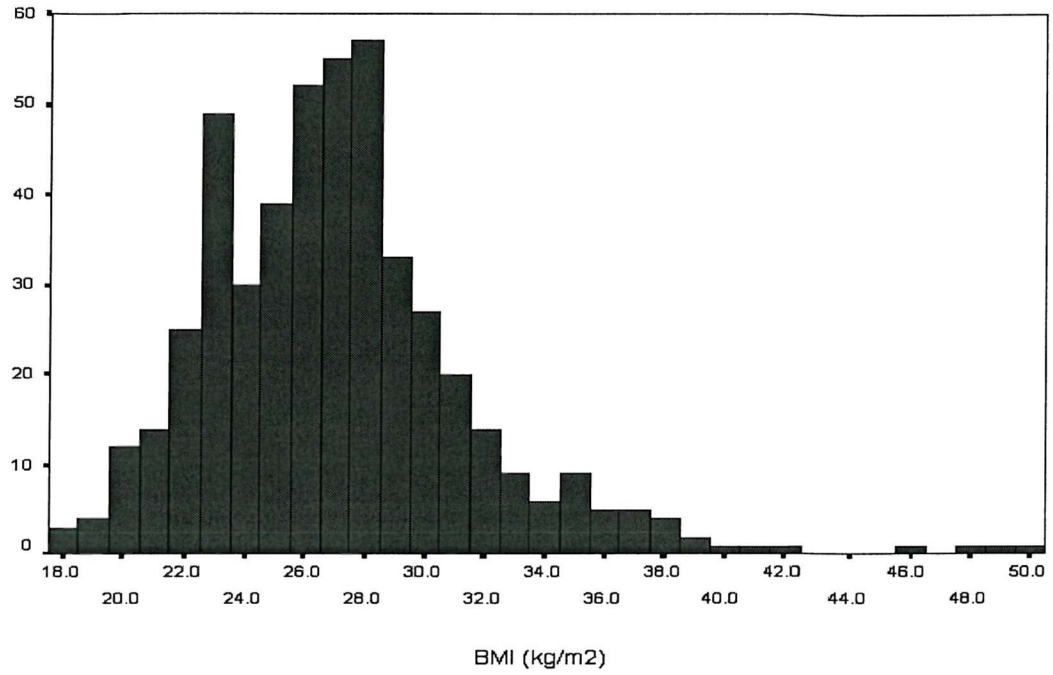


Figure A4 Distribution of age (years) in the elderly men and women

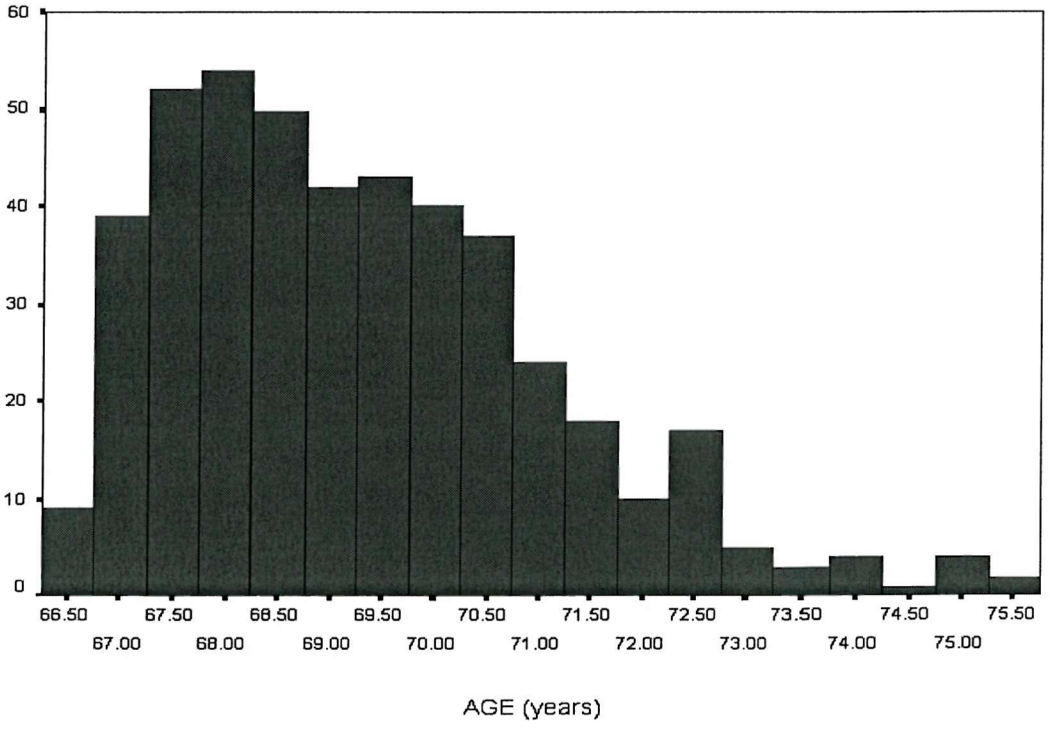


Figure A5 Distribution of weight at birth (lb) in the elderly men and women

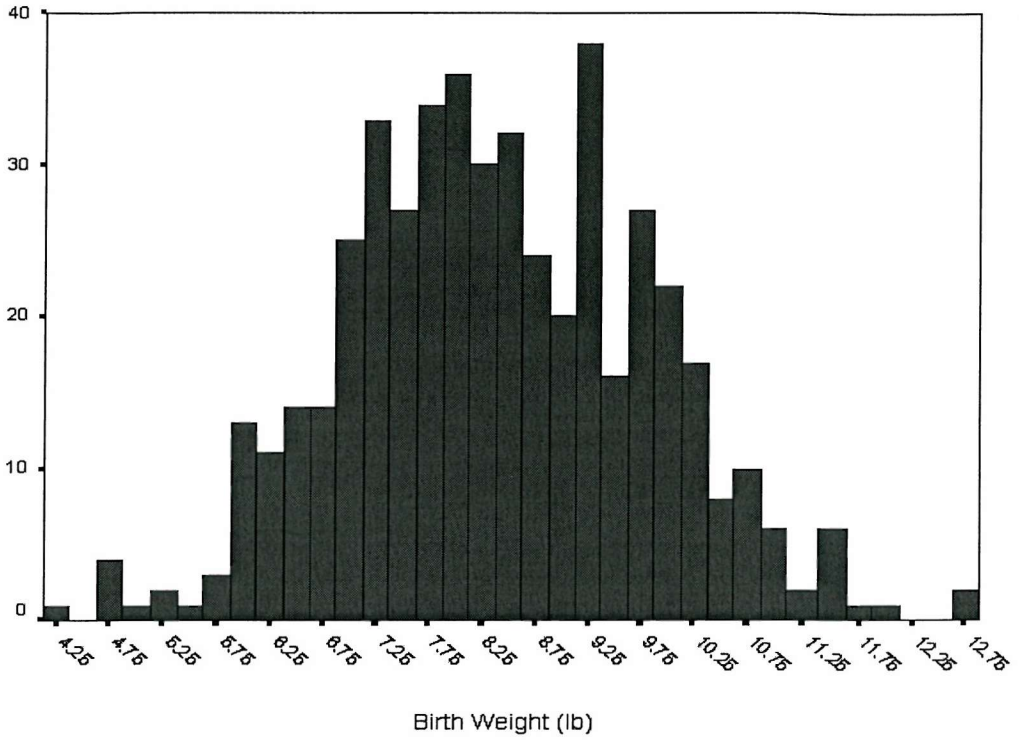


Figure A6 Distribution of head circumference at birth (in) in the elderly men and women

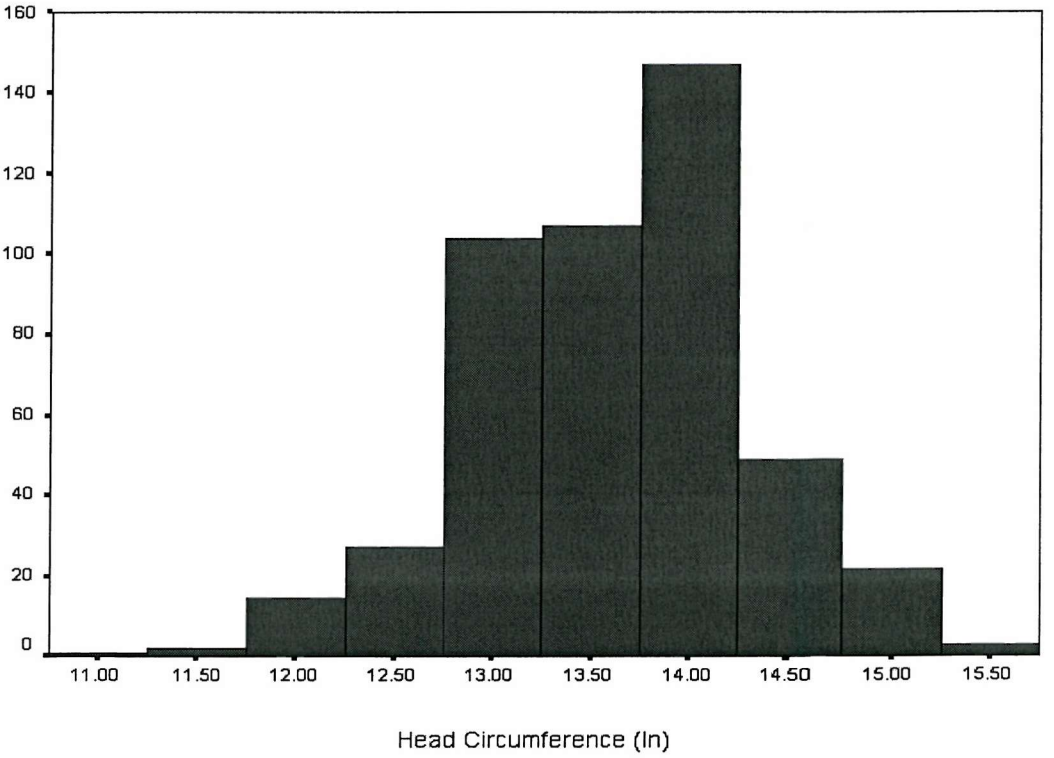


Figure A7 Distribution of chest circumference at birth (in) in the elderly men and women

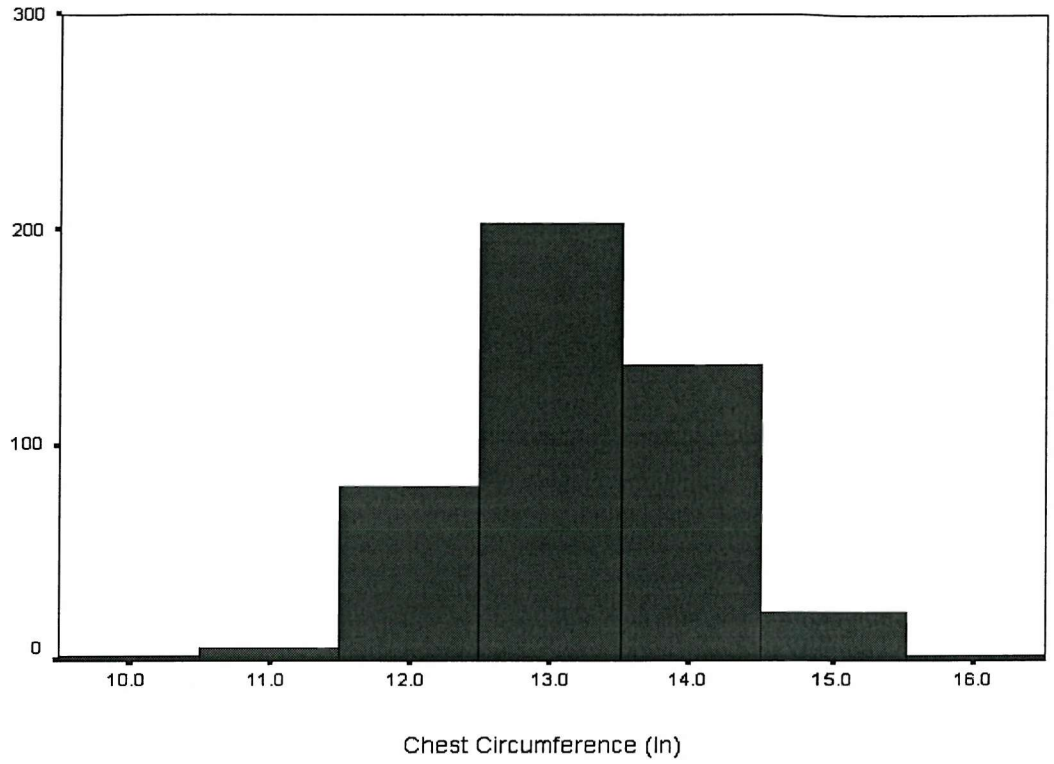


Figure A8 Distribution of abdominal circumference at birth (in) in the elderly men and women

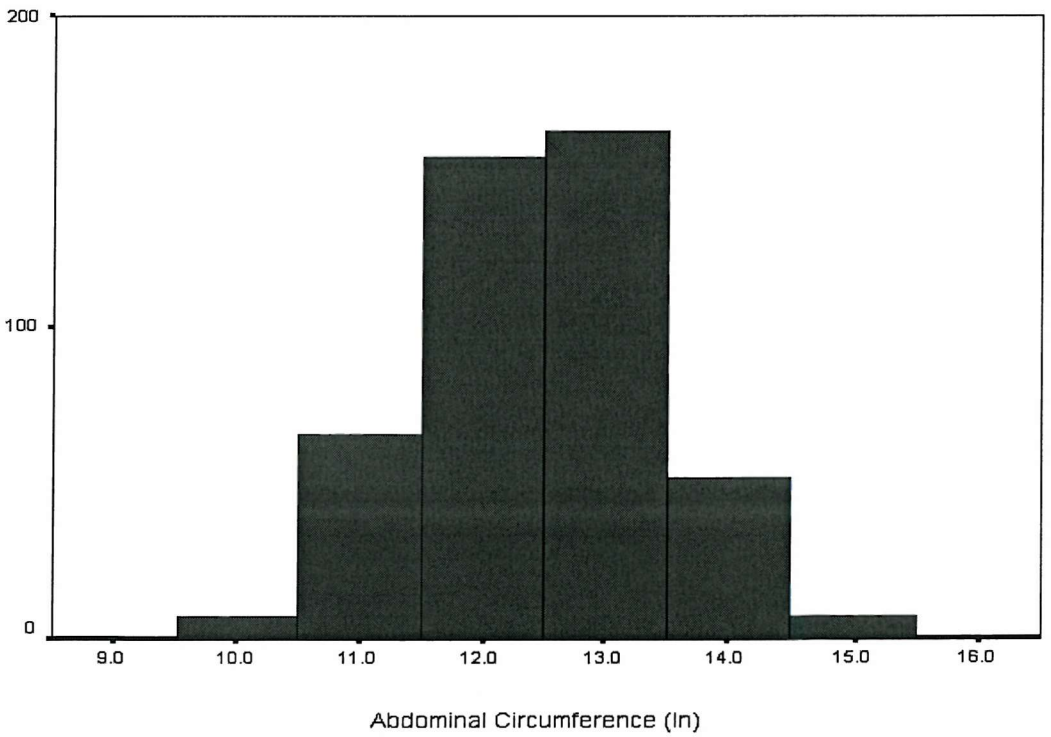


Figure A9 Distribution of length at birth (in) in the elderly men and women

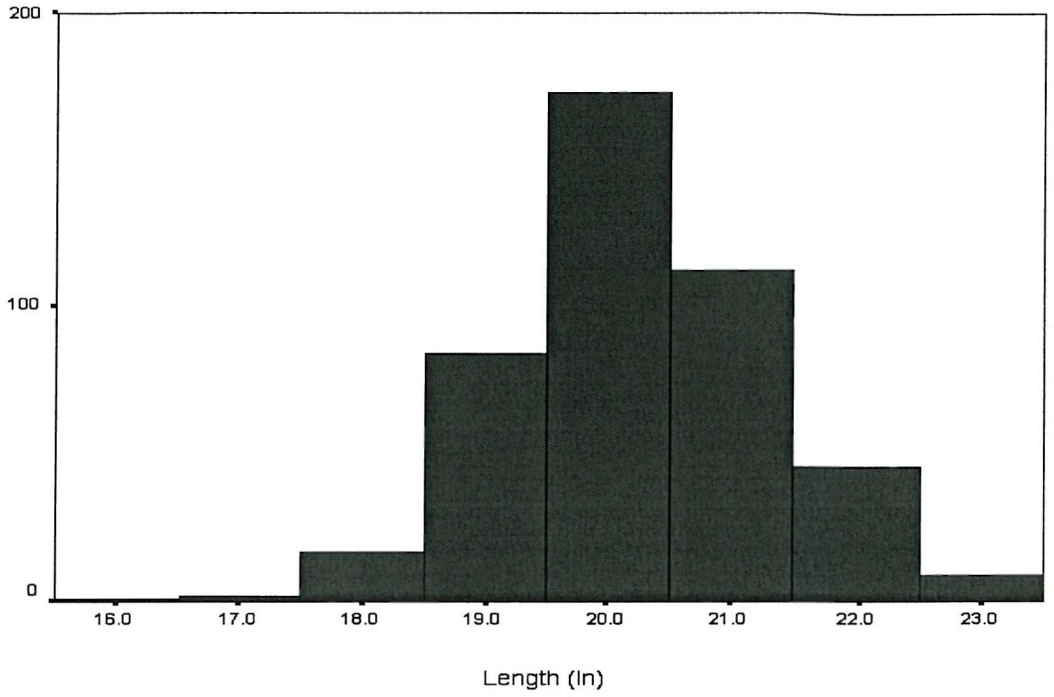


Figure A10 Distribution of head to abdominal circumference ratio at birth in the elderly men and women

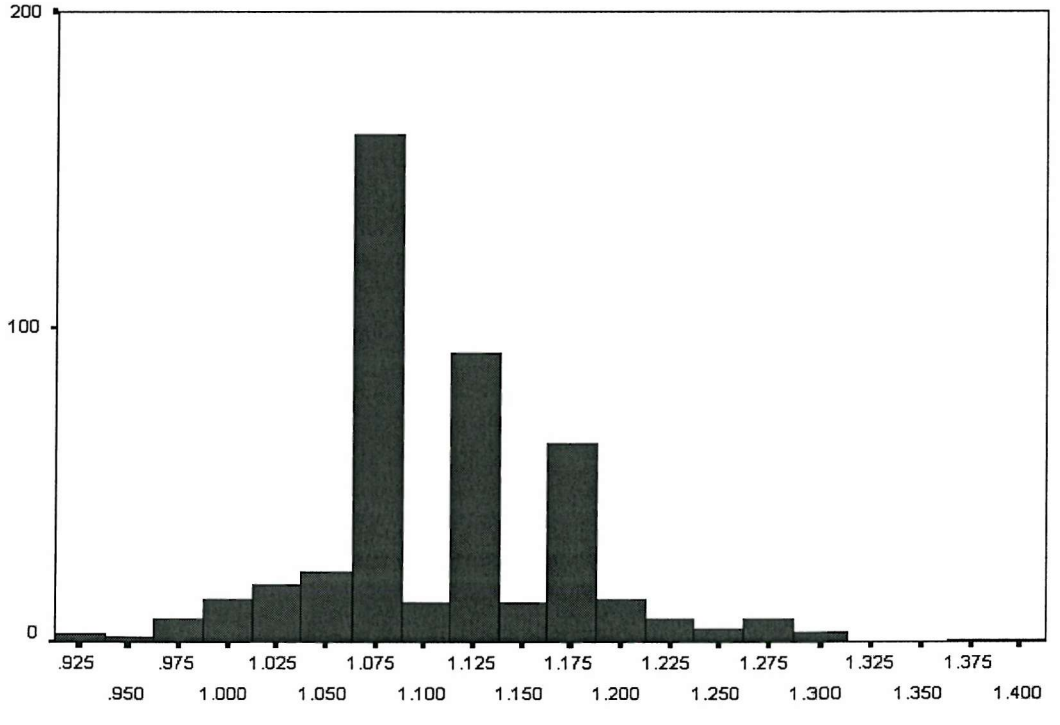


Figure A11 Distribution of head circumference to length ratio at birth in the elderly men and women

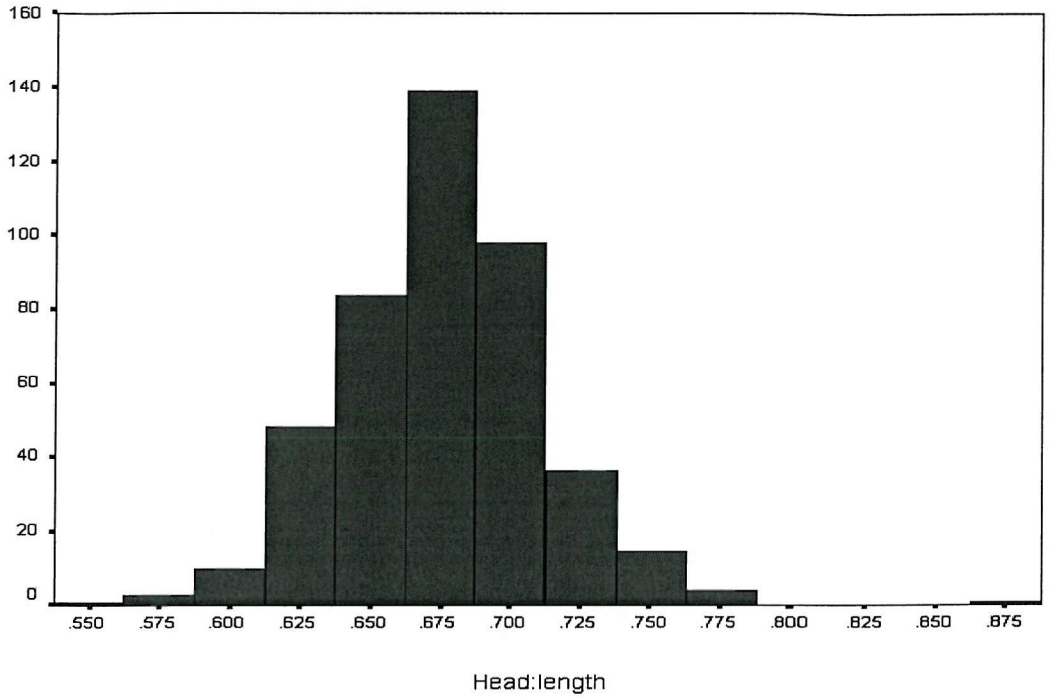


Figure A12 Distribution of ponderal index at birth (oz/in^3 x 10000) in the elderly men and women

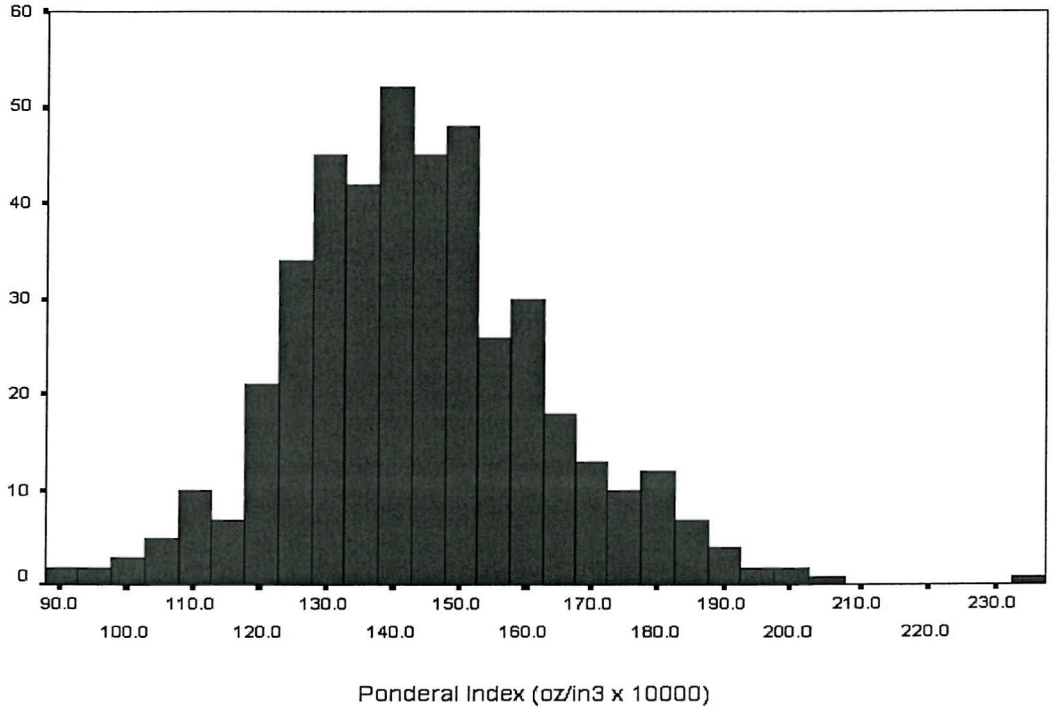


Figure A13 Distribution of placental weight at birth (g) in the elderly men and women

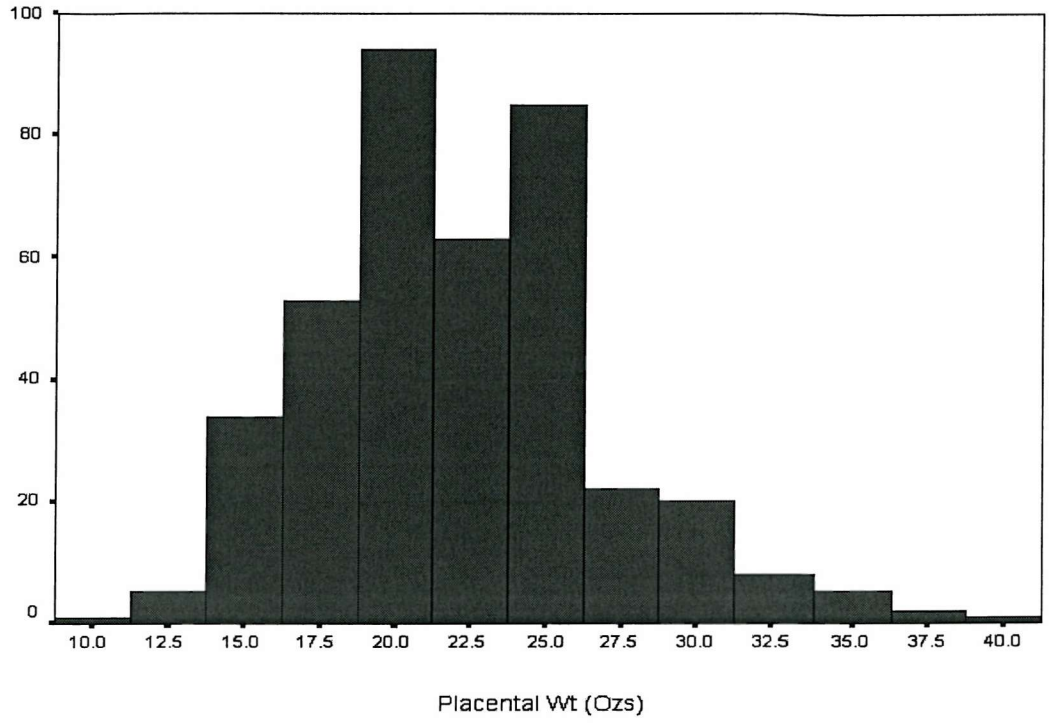


Figure A14 Distribution of placenta to birth weight ratio in the elderly men and women

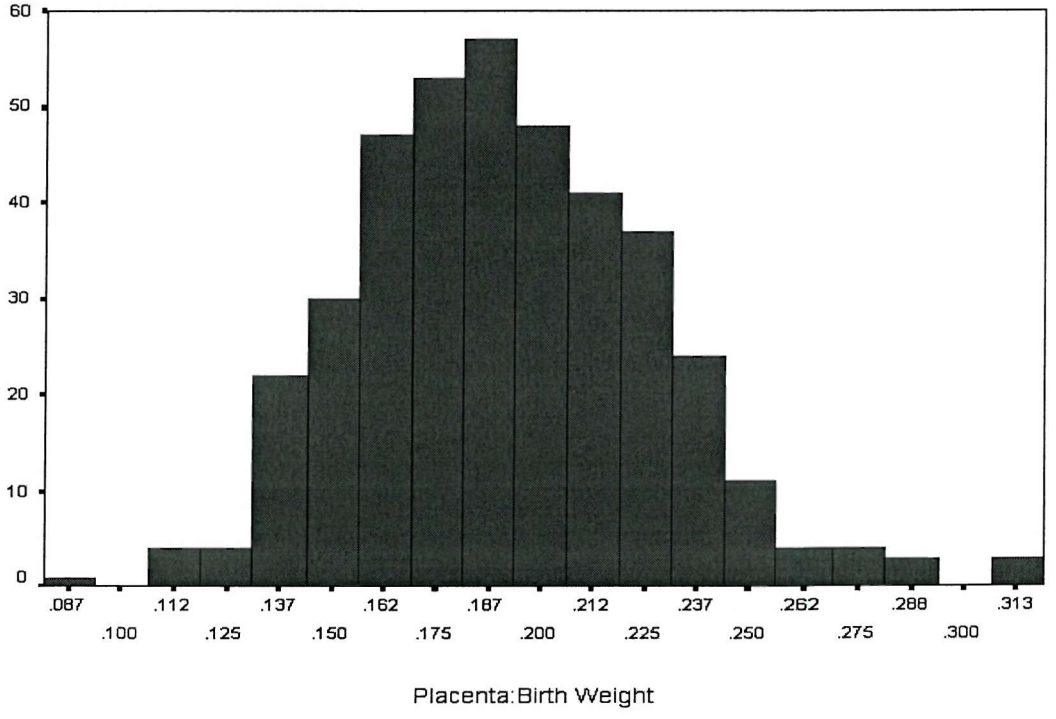


Figure A15 Distribution of gestational age at birth (days) in the elderly men and women

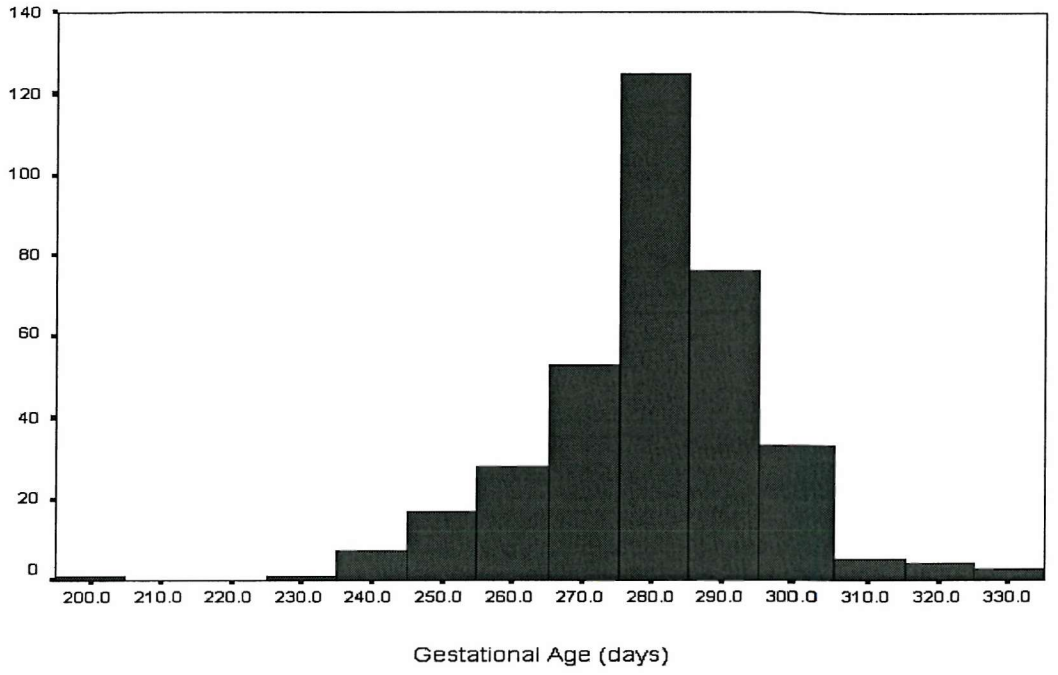


Figure A16 Distribution of systolic blood pressure (mmHg) in the elderly men and women

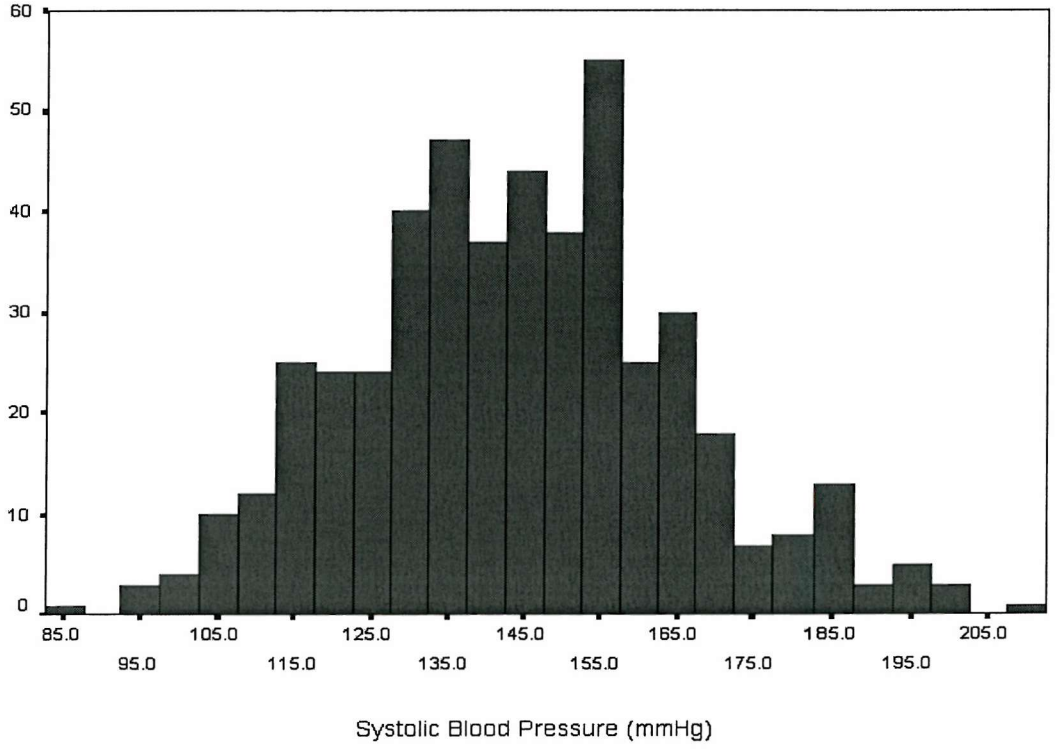


Figure A17 Distribution of diastolic blood pressure (mmHg) in the elderly men and women

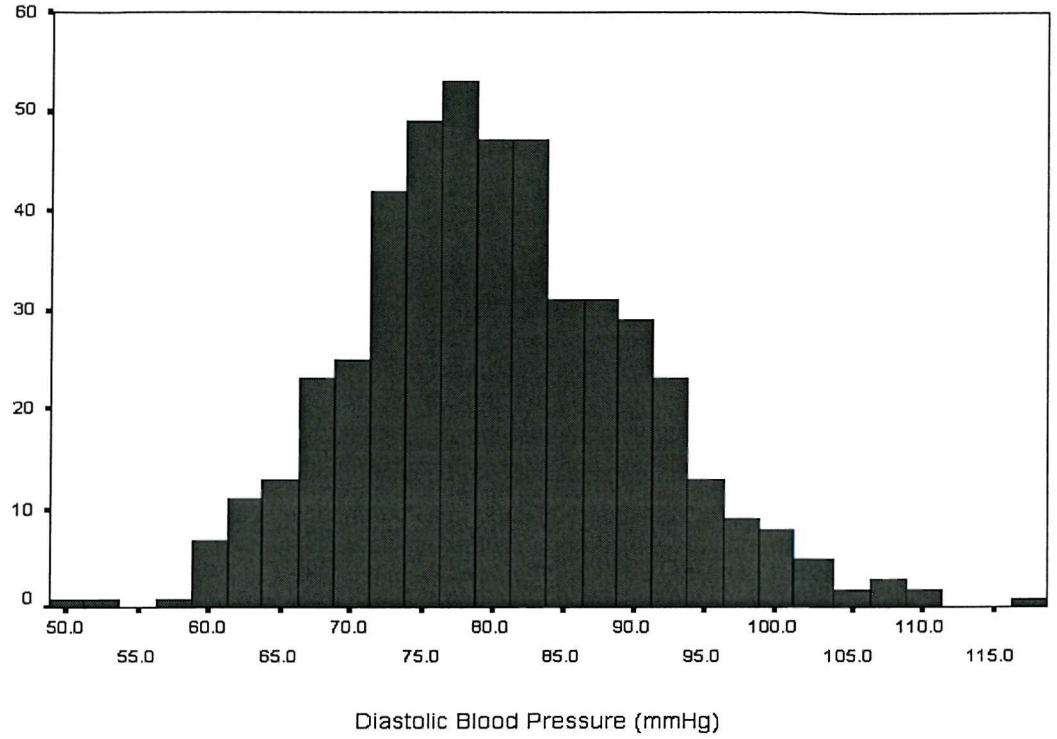


Figure A18 Distribution of aorta to femoral pulse wave velocity (m/sec) in the elderly men and women

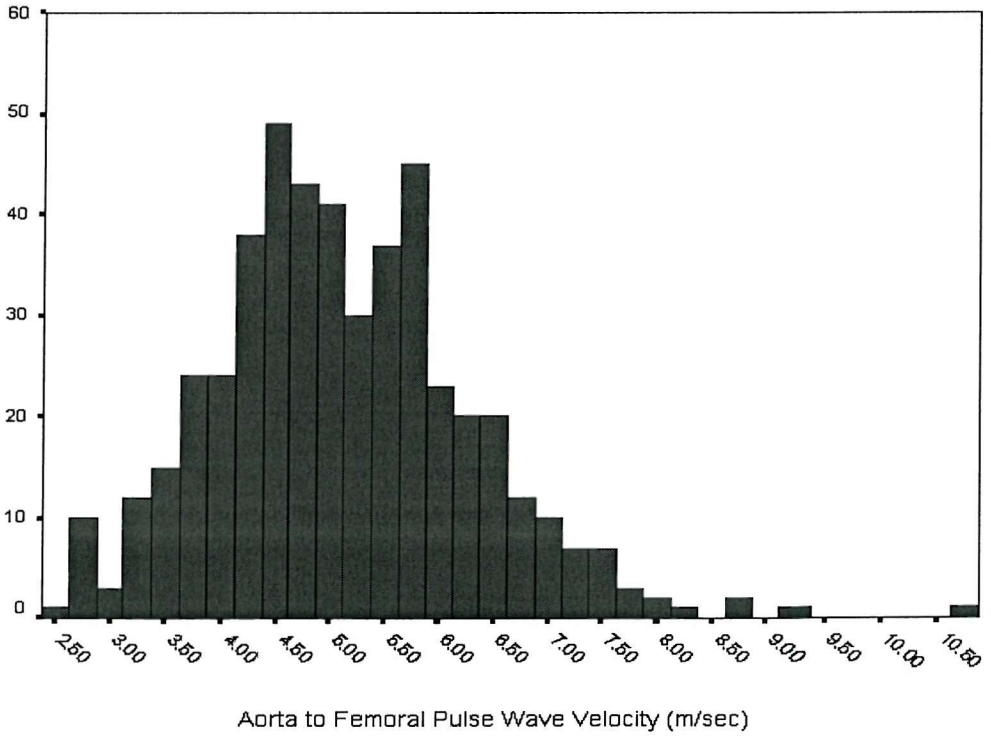


Figure A19 Distribution of aorta to radial pulse wave velocity (m/sec) in the elderly men and women

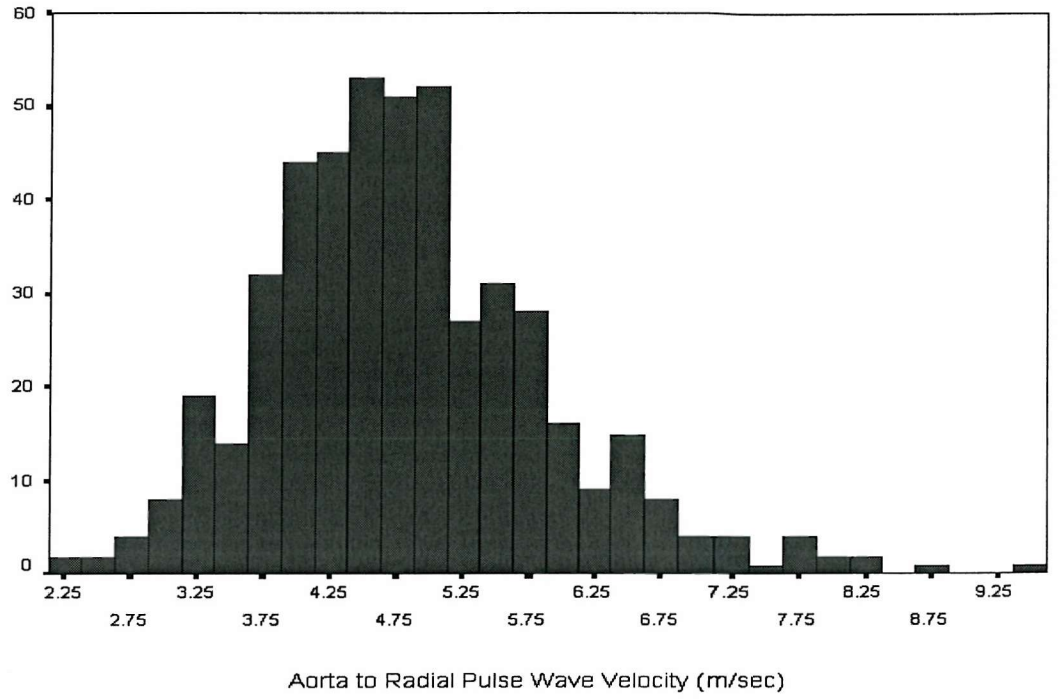


Figure A20 Distribution of aorta to foot pulse wave velocity (m/sec) in the elderly men and women

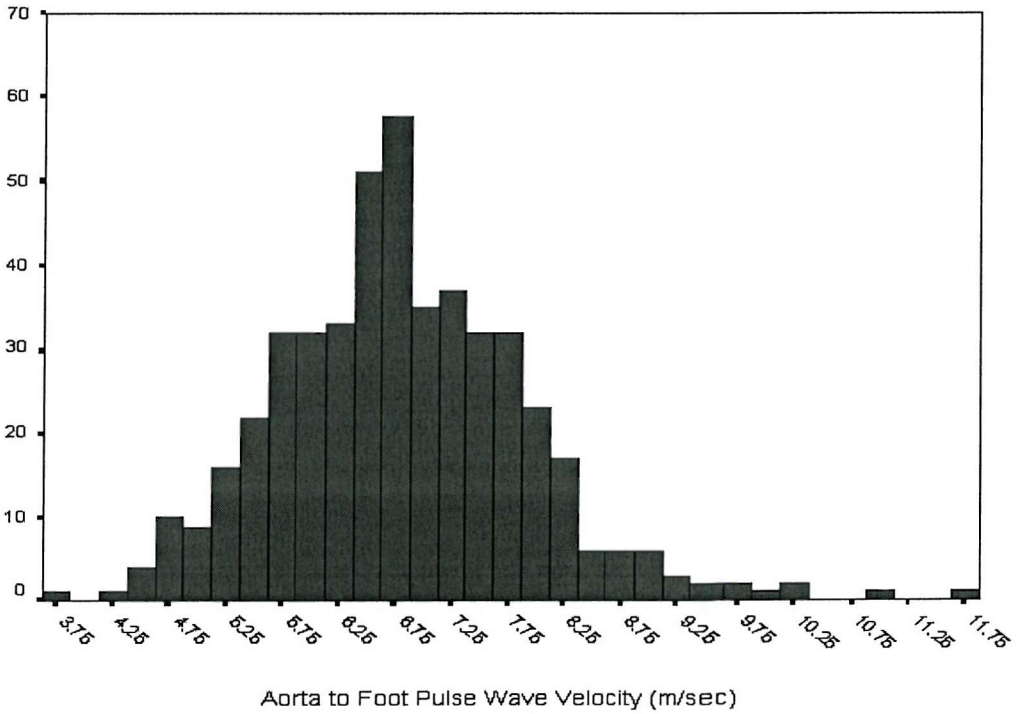
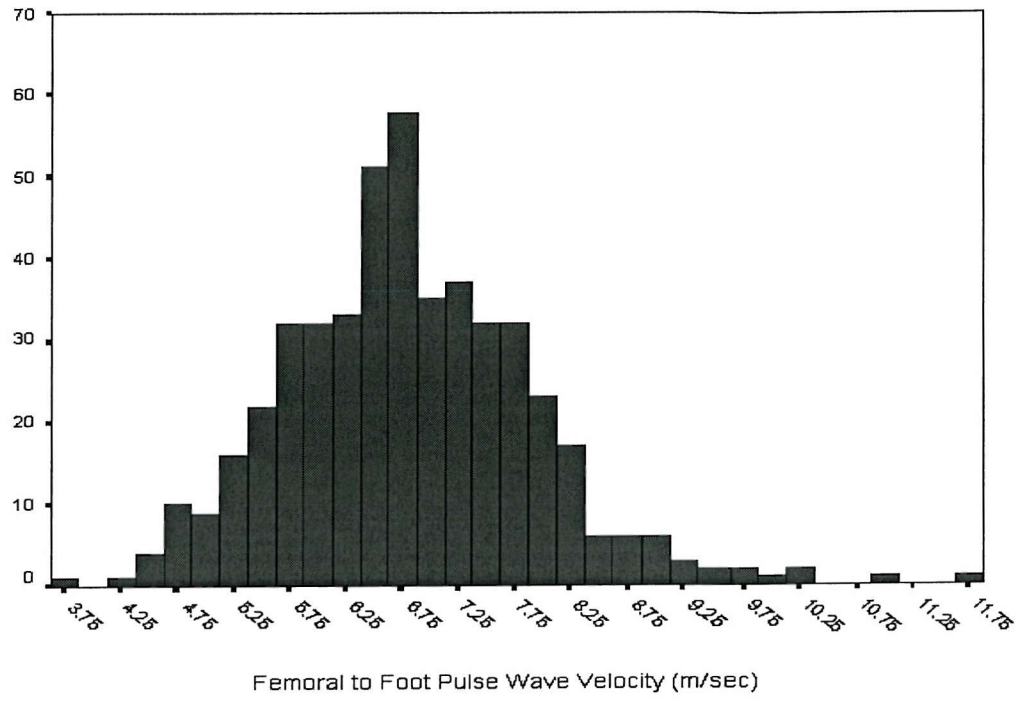


Figure A21 Distribution of femoral to foot pulse wave velocity (m/sec) in the elderly men and women



Appendix B Local Research Ethics Committee Summary

Surrey

North West Surrey Ethics Committee

South West Surrey Ethics Committee

East Surrey Health Authority Research Ethics Committee

Kingston and Richmond Research Ethics Committee

Hampshire

Winchester and North and Mid Hampshire Ethics Committee

Southampton Research Ethics Committee

Portsmouth Research Ethics Committee

Sussex

Hastings Research Ethics Committee

Brighton and Hove Research Ethics Committee

Eastbourne Research Ethics Committee

Worthing Research Ethics Committee

Chichester Research Ethics Committee

Crawley Research Ethics Committee

Haywards Health Research Ethics Committee

Kent

Medway Ethics Committee

Maidstone Ethics Committee

Tunbridge Wells Ethics Committee

Dartford Ethics Committee

Thanet Ethics Committee

Canterbury Ethics Committee

South East Kent Ethics Committee

Appendix C Raw data from the young adult's study

Figure C1 Distribution of height (cm) in the young adults

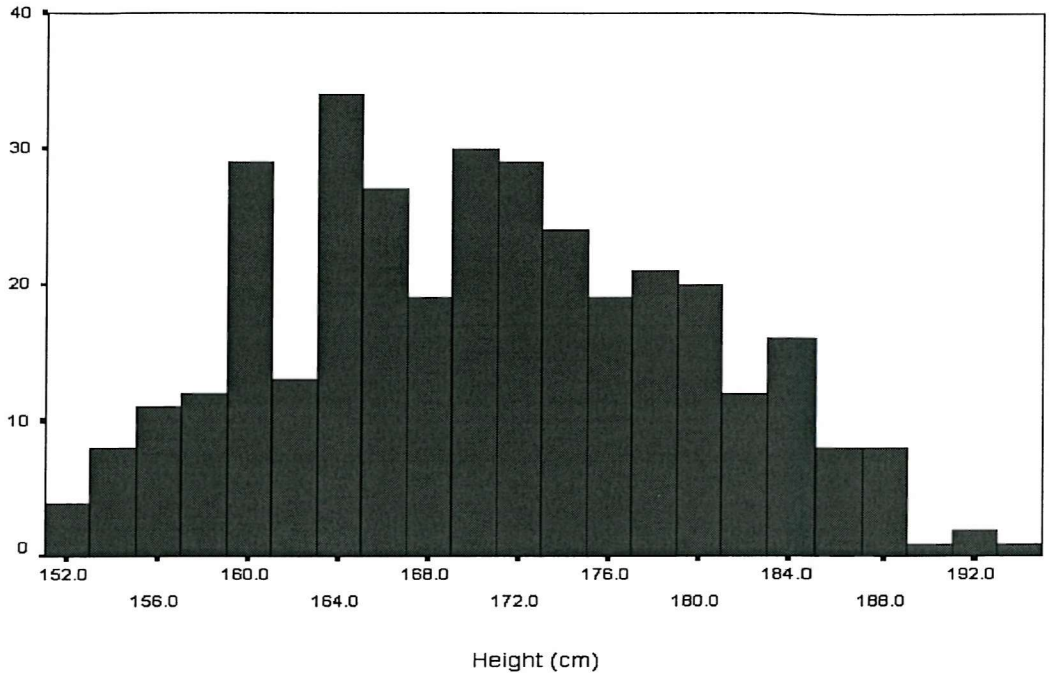


Figure C2 Distribution of weight (kg) in the young adults

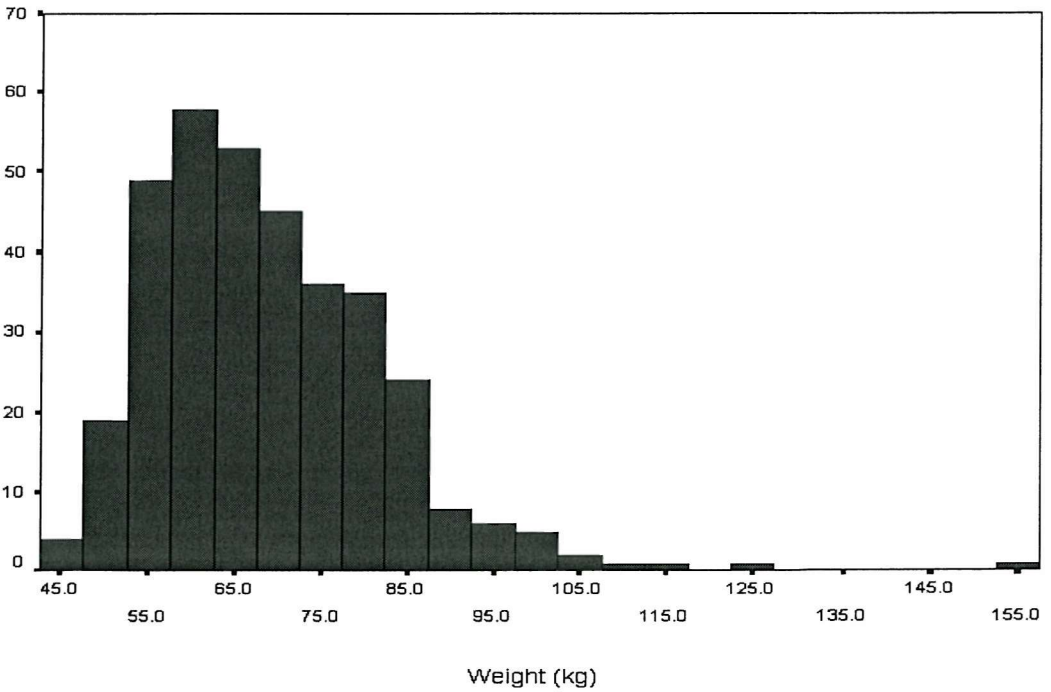


Figure C3 Distribution of body mass index (kg/m^2) in the young adults

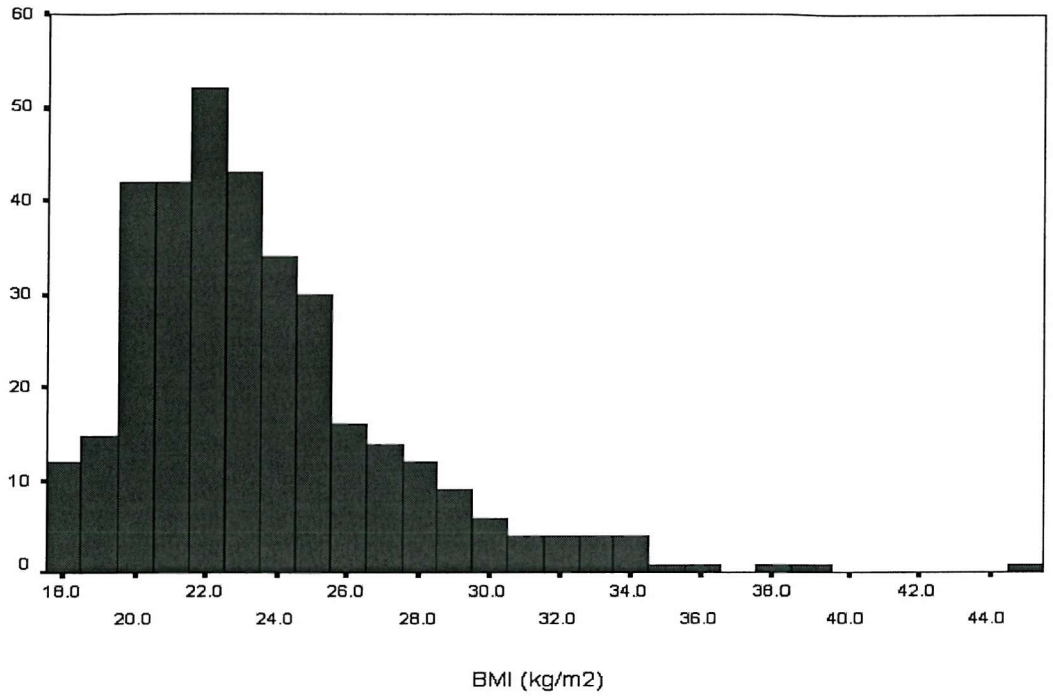


Figure C4 Distribution of age (years) in the young adults

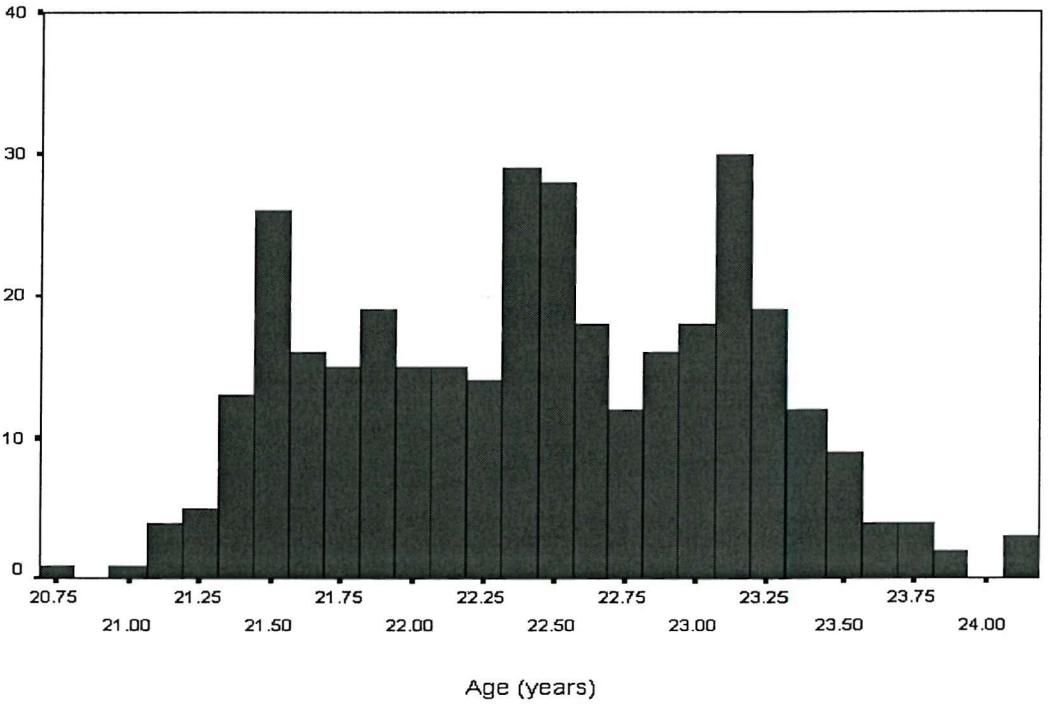


Figure C5 Distribution of weight at birth (kg) in the young adults

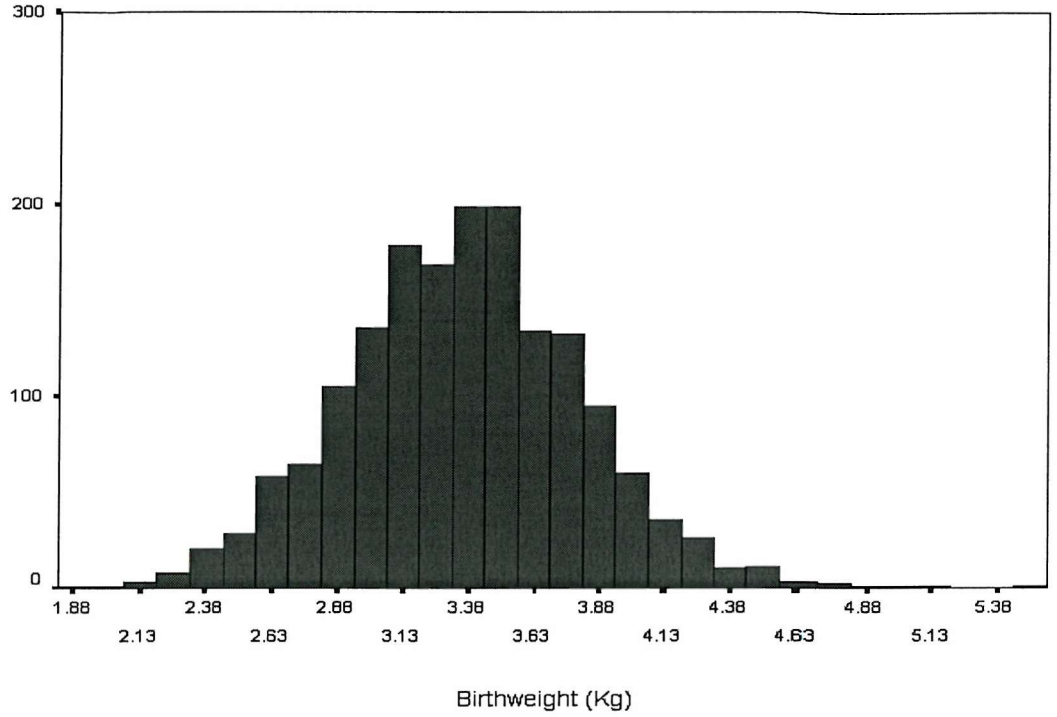


Figure C6 Distribution of head circumference at birth (cm) in the young adults

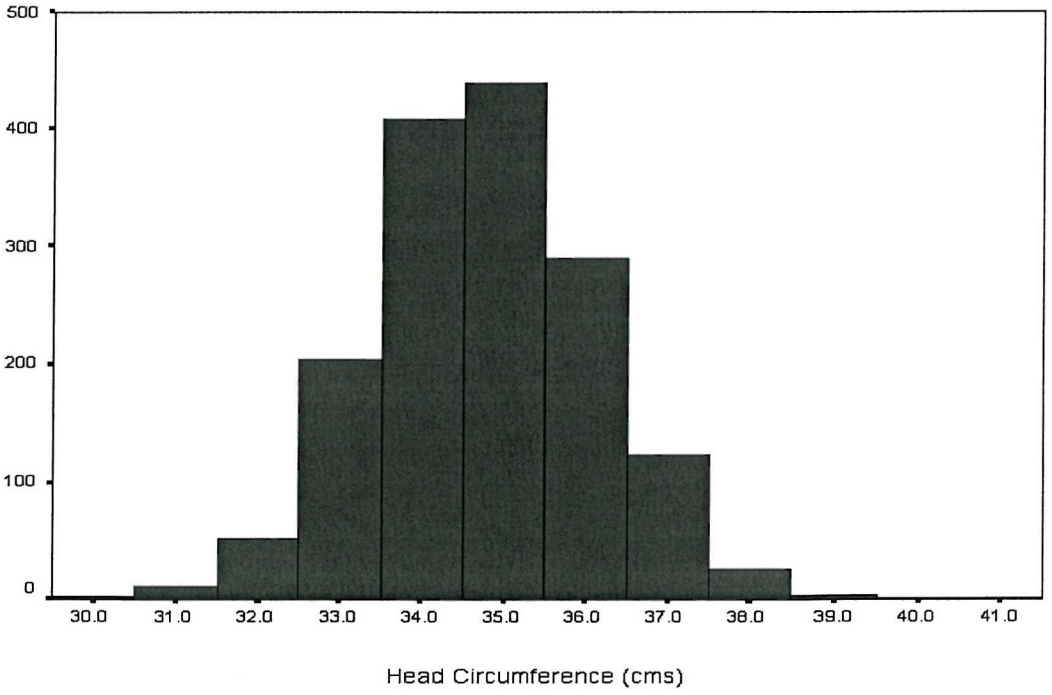


Figure C7 Distribution of length at birth (cm) in the young adults

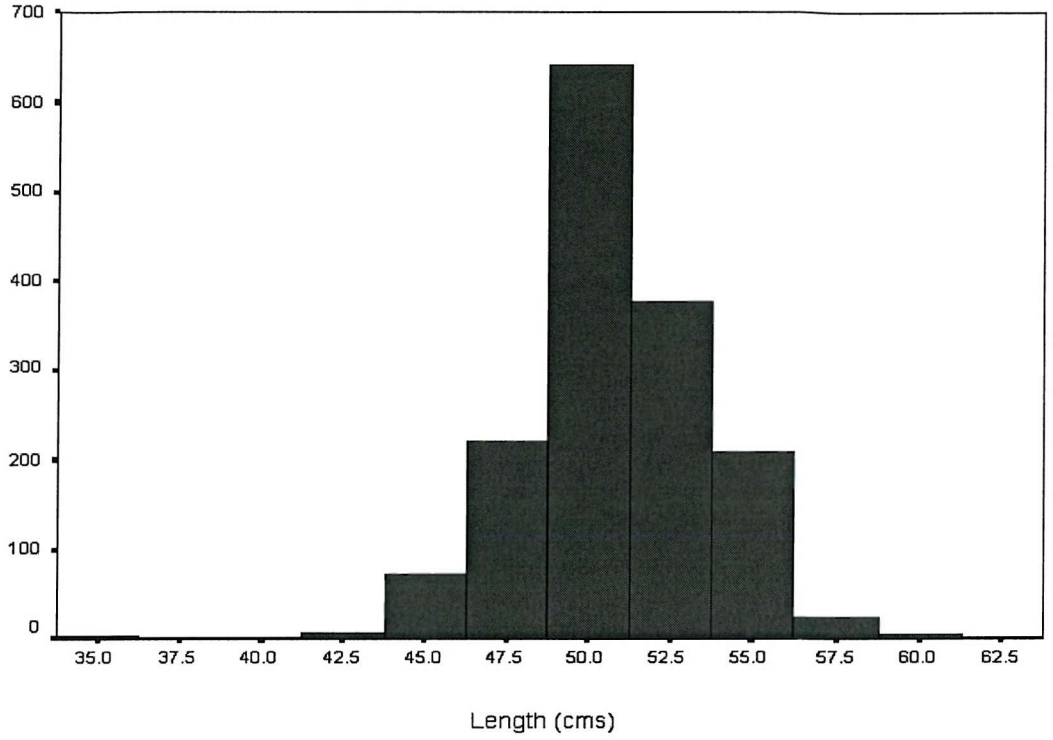


Figure C8 Distribution of head circumference to length ratio at birth in the young adults

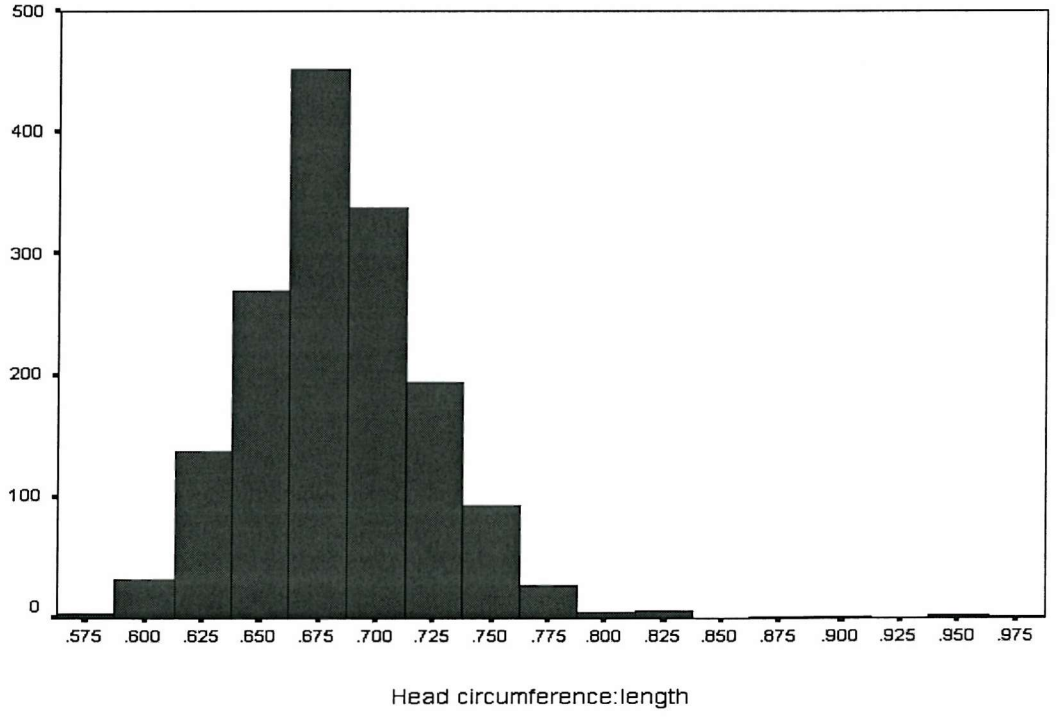


Figure C9 Distribution of ponderal index at birth (kg/m^3) in the young adults

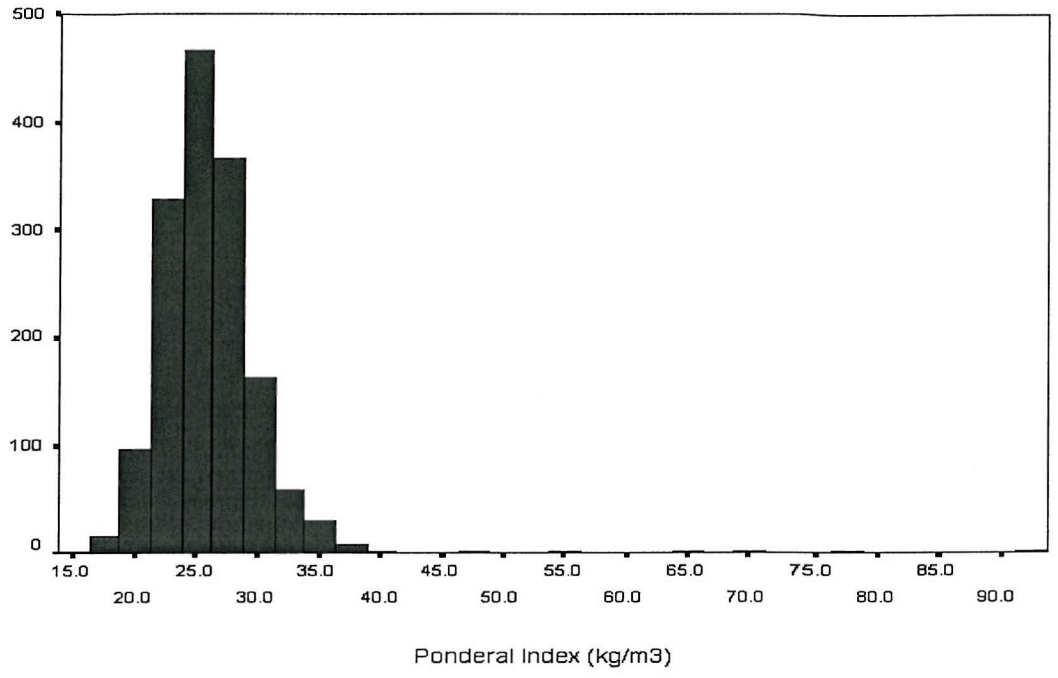


Figure C10 Distribution of placental weight at birth (g) in the young adults

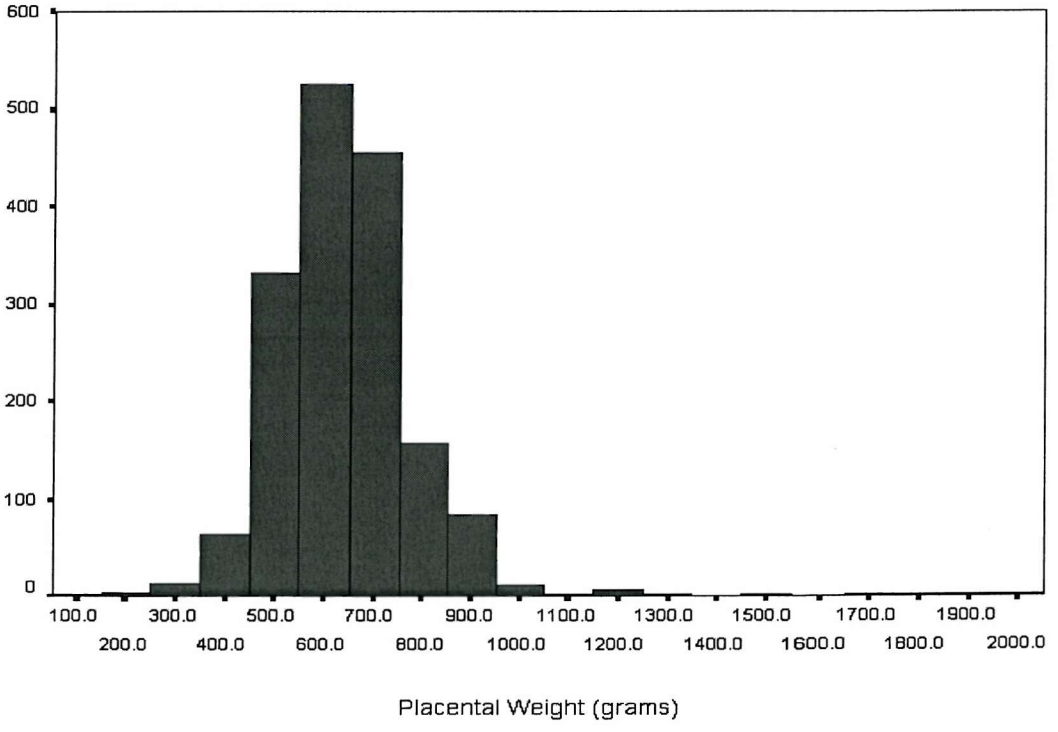


Figure C11 Distribution of placenta to birth weight ratio in the young adults

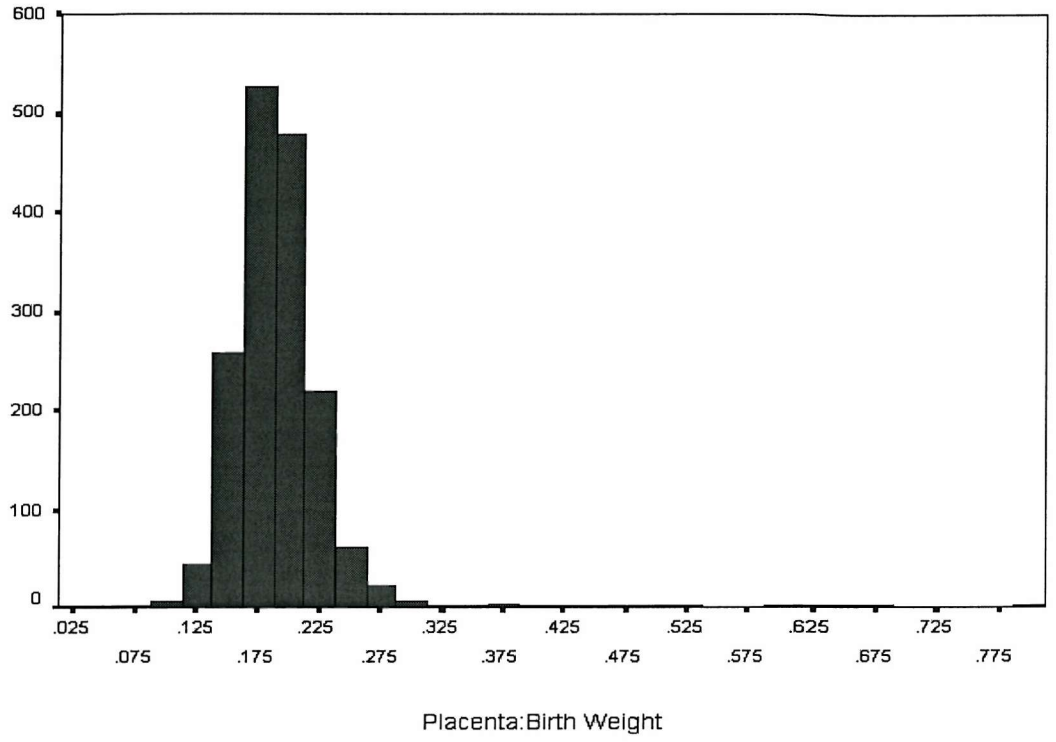


Figure C12 Distribution of gestational age at birth (days) in the young adults

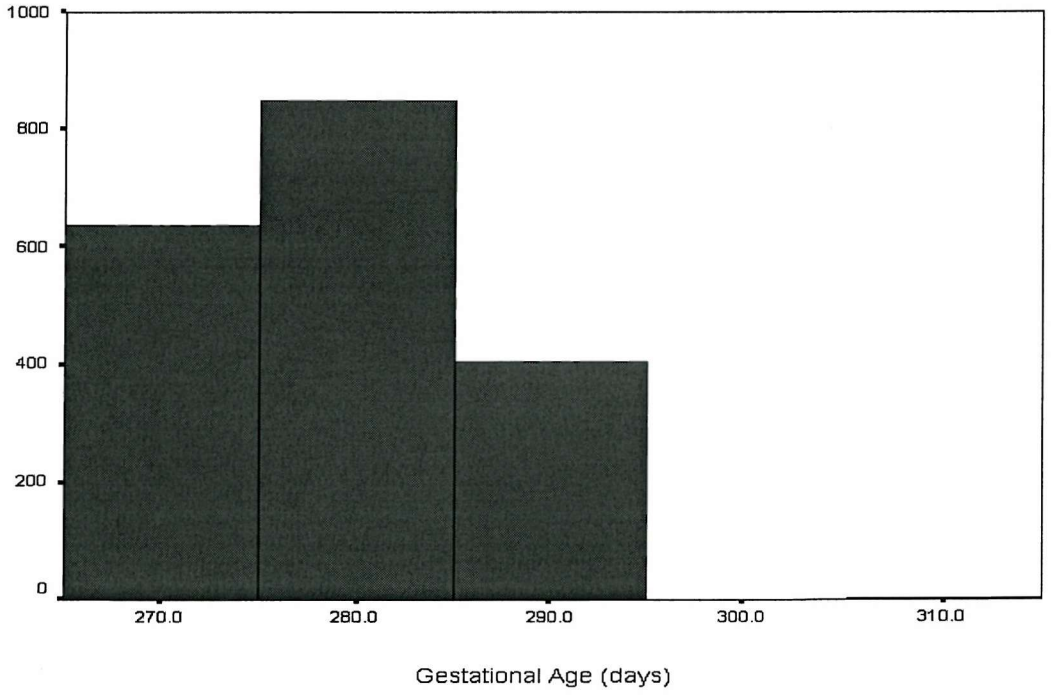


Figure C13 Distribution of systolic blood pressure (mmHg) in the young adults

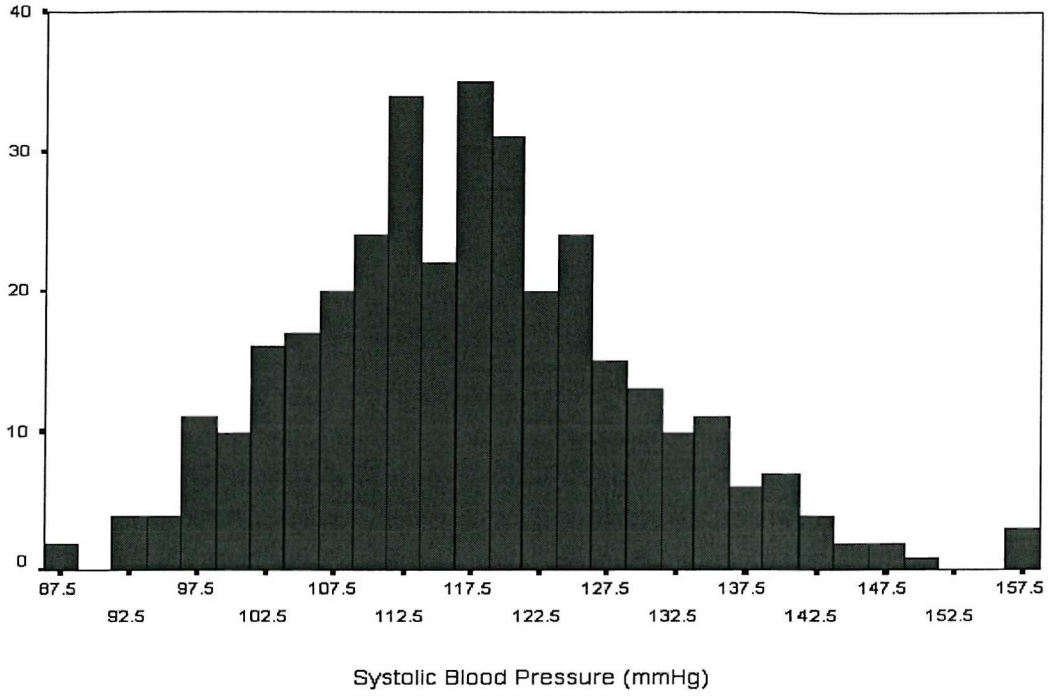


Figure C14 Distribution of diastolic blood pressure (mmHg) in the young adults

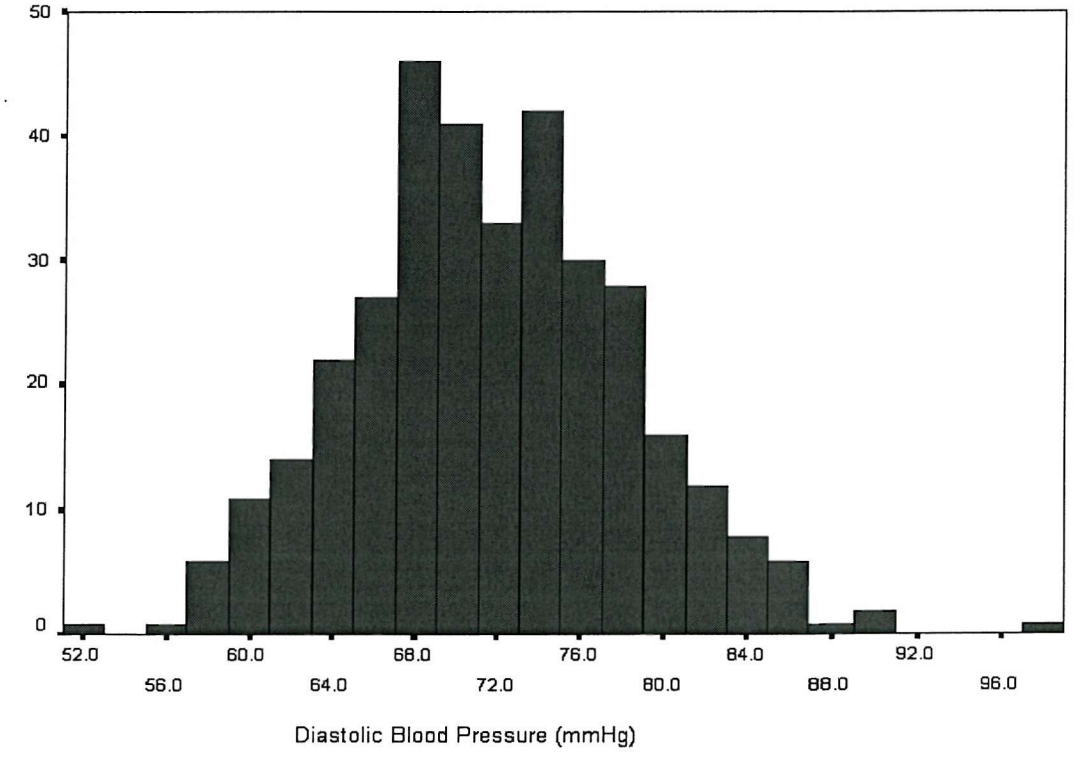


Figure C15 Distribution of aorta to radial pulse wave velocity (m/sec) in the young adults

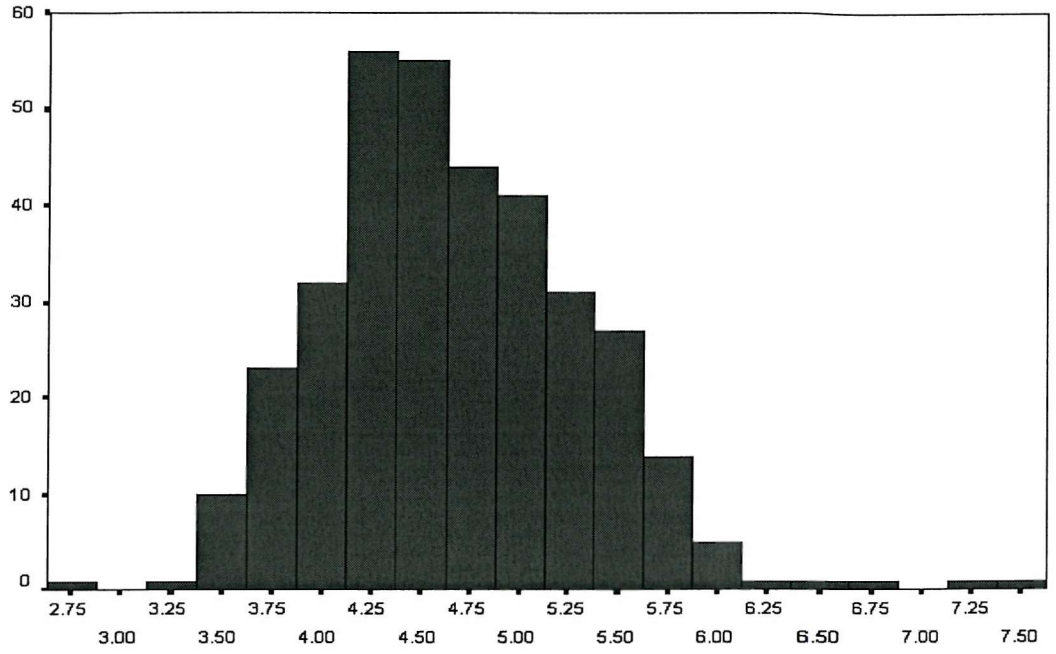
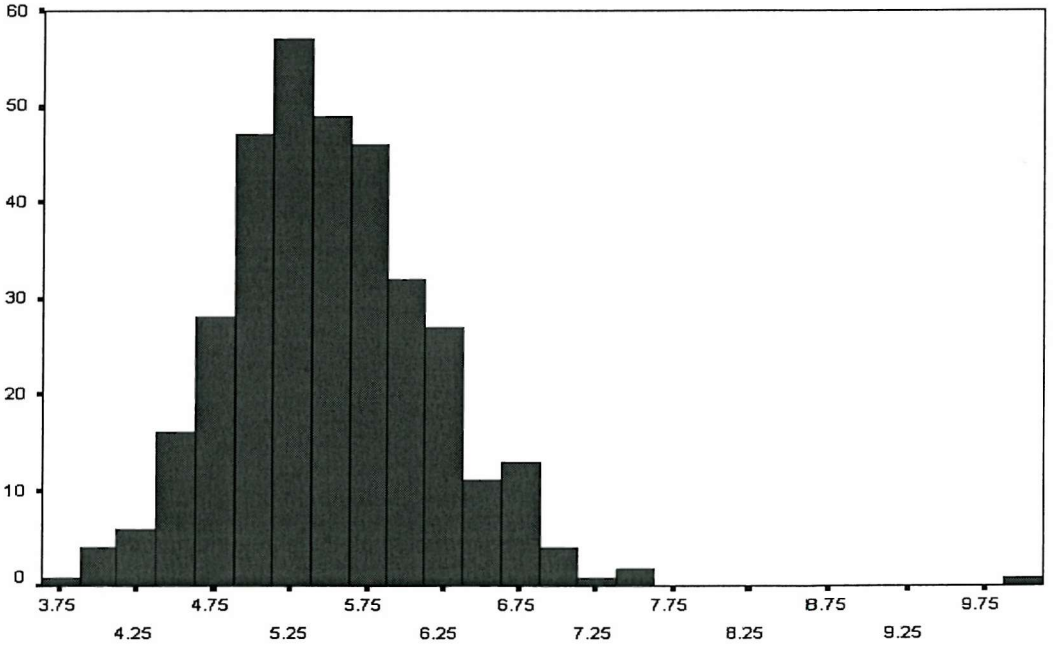


Figure C16 Distribution of aorta to foot pulse wave velocity (m/sec) in the young adults



Appendix D Raw data from the children's study

Figure D1 Distribution of height (cm) in the children

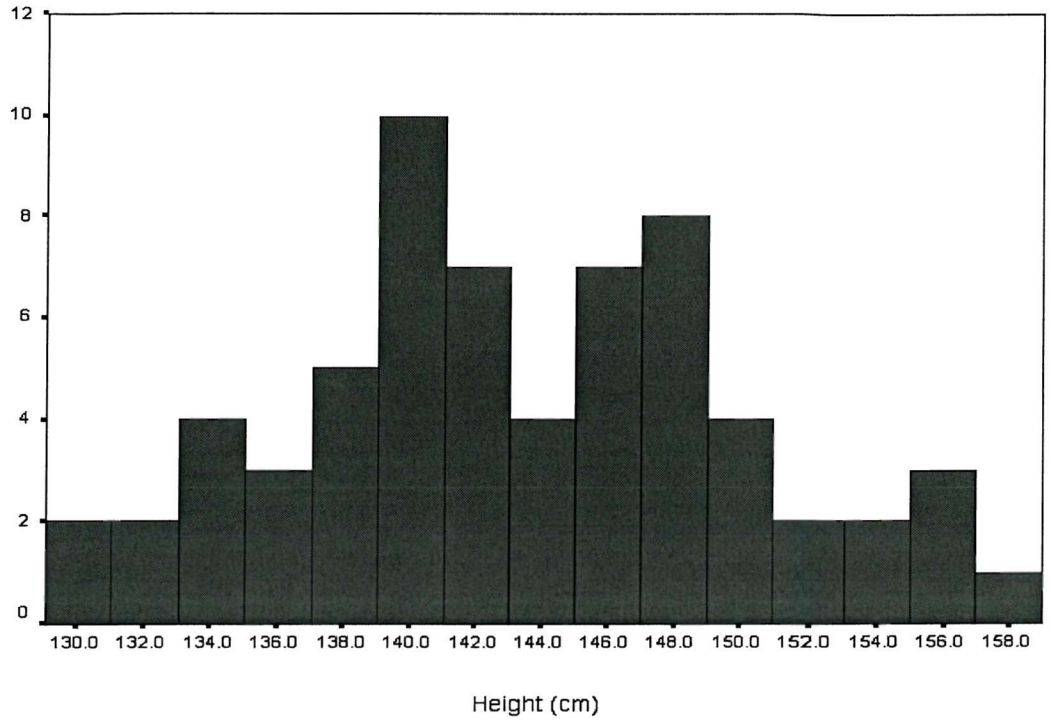


Figure D2 Distribution of weight (kg) in the children

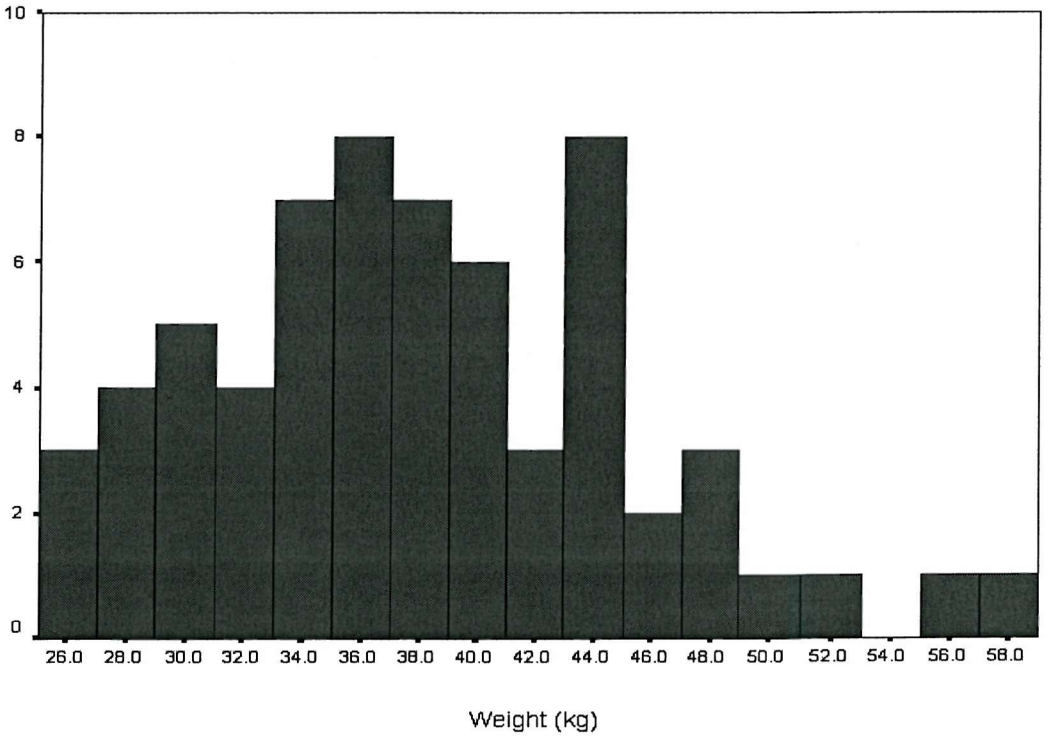


Figure D3 Distribution of body mass index (kg/m^2) in the children

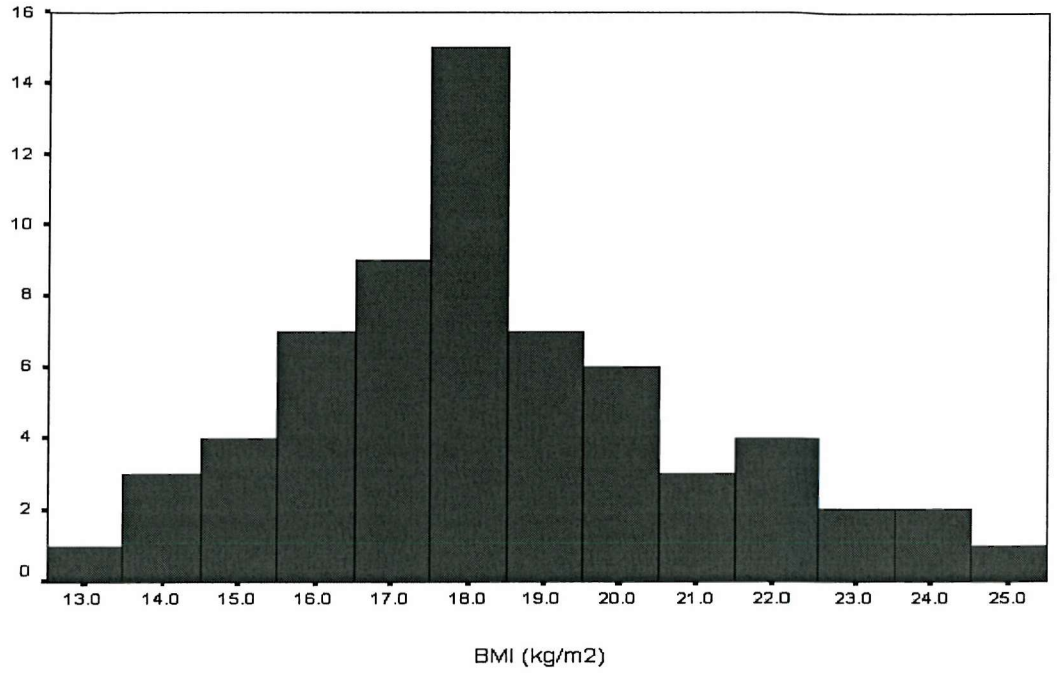


Figure D4 Distribution of weight at birth (kg) in the children

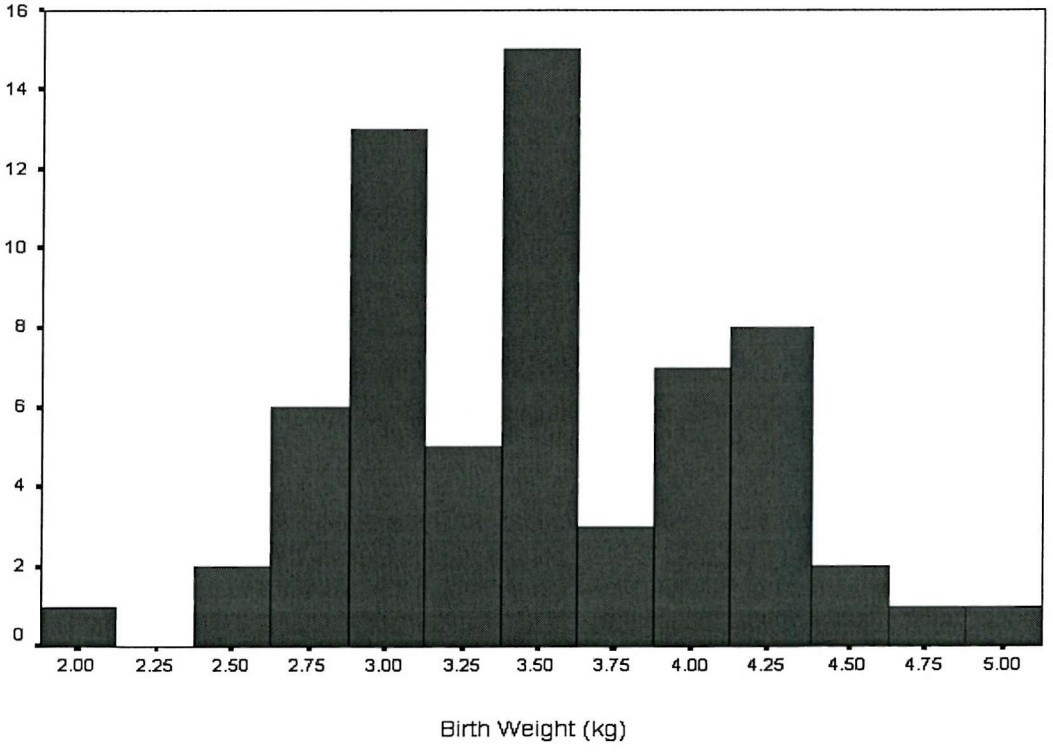


Figure D5 Distribution of head circumference at birth (cm) in the children

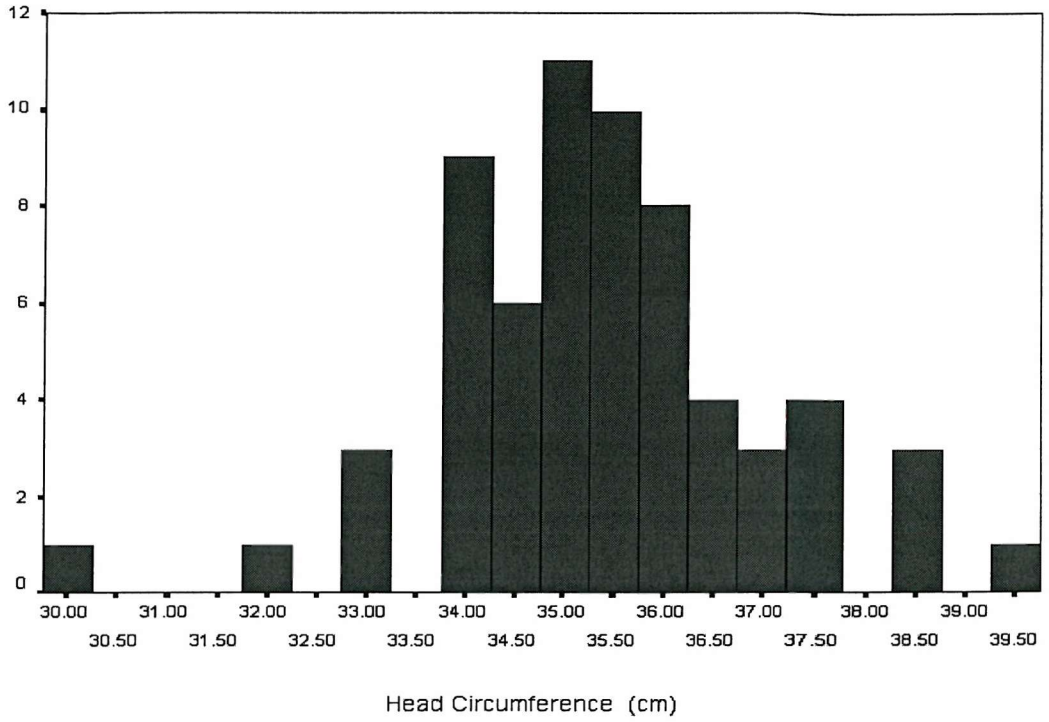


Figure D6 Distribution of chest circumference at birth (cm) in the children

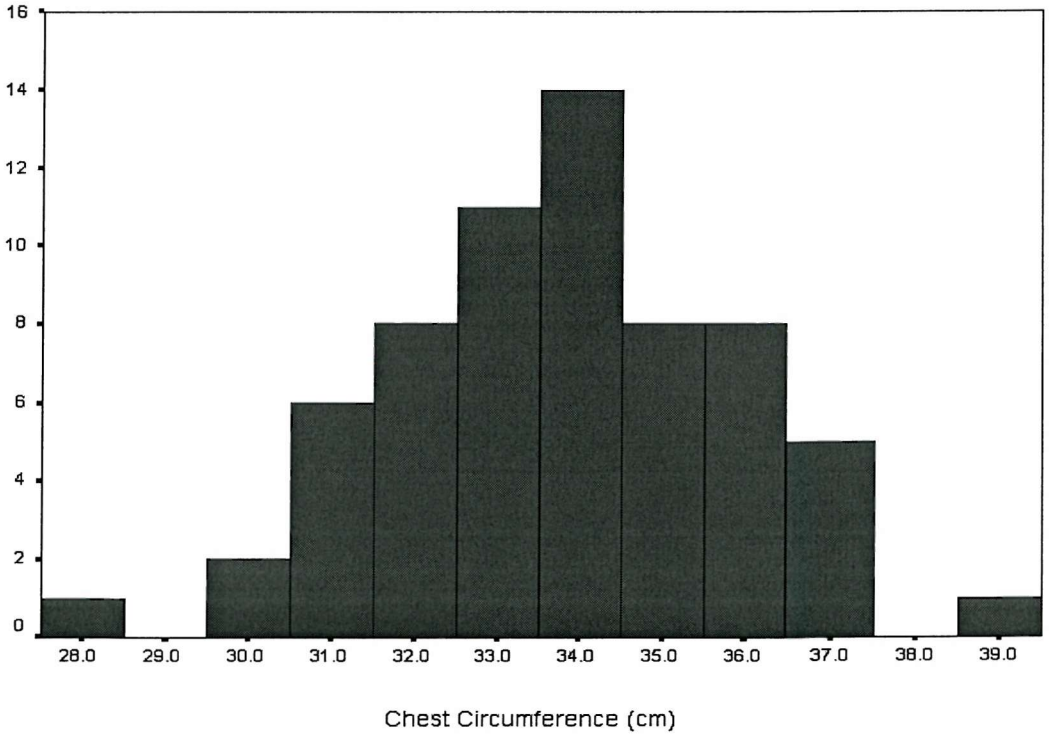


Figure D7 Distribution of abdominal circumference at birth (cm) in the children

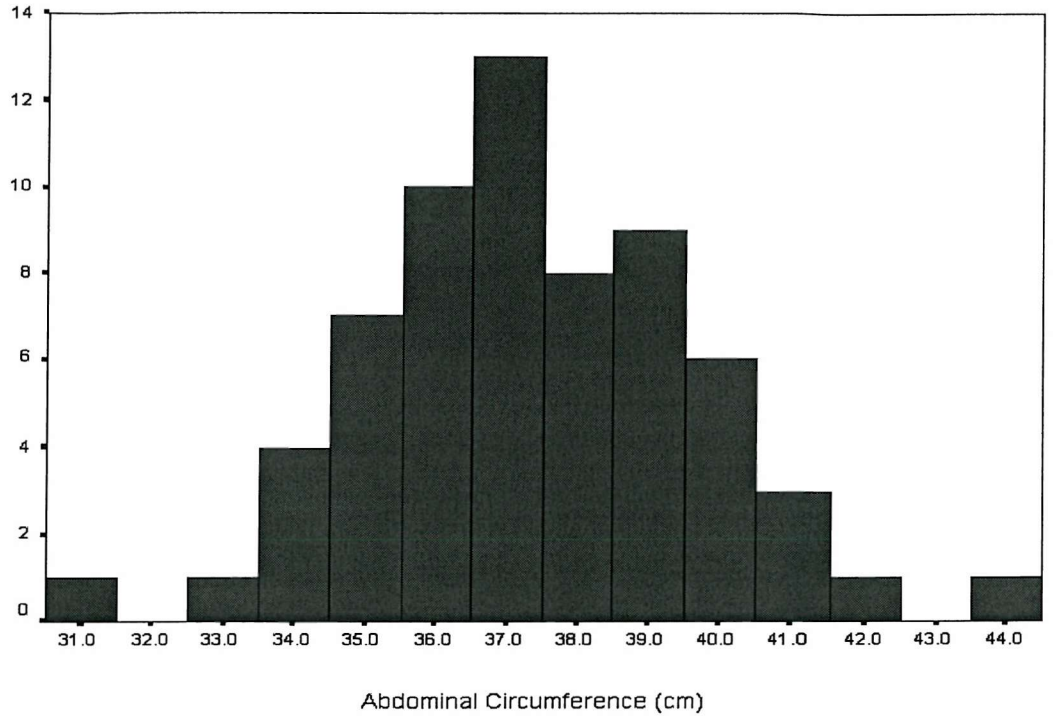


Figure D8 Distribution of length at birth (cm) in the children

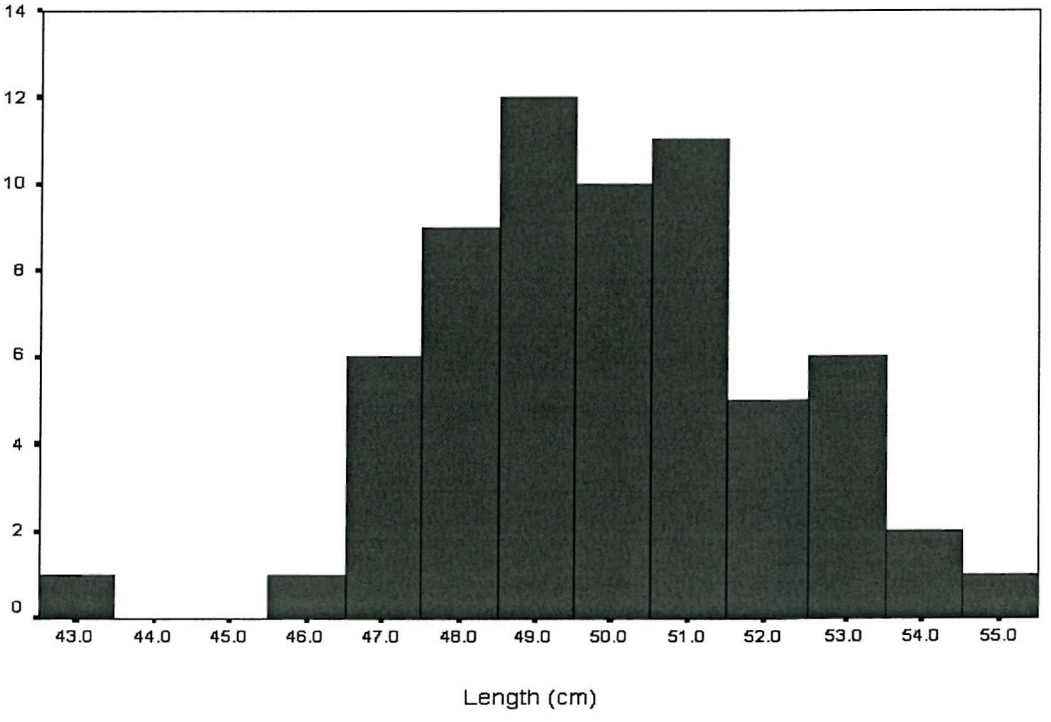


Figure D9 Distribution of head to abdominal circumference ratio at birth in the children

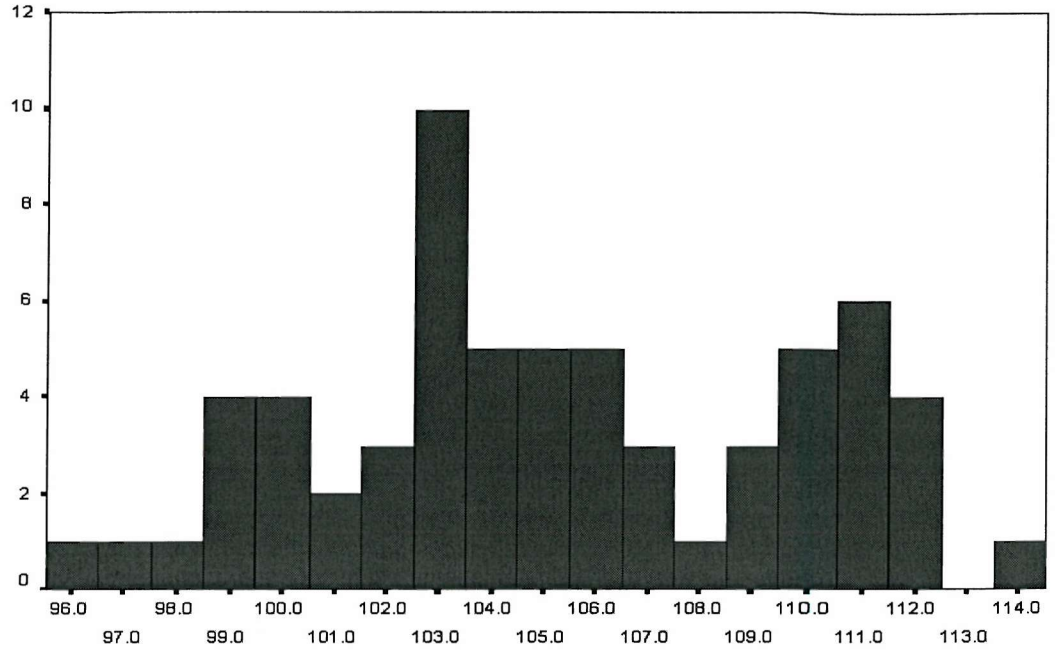


Figure D10 Distribution of head circumference to length ratio at birth in the children

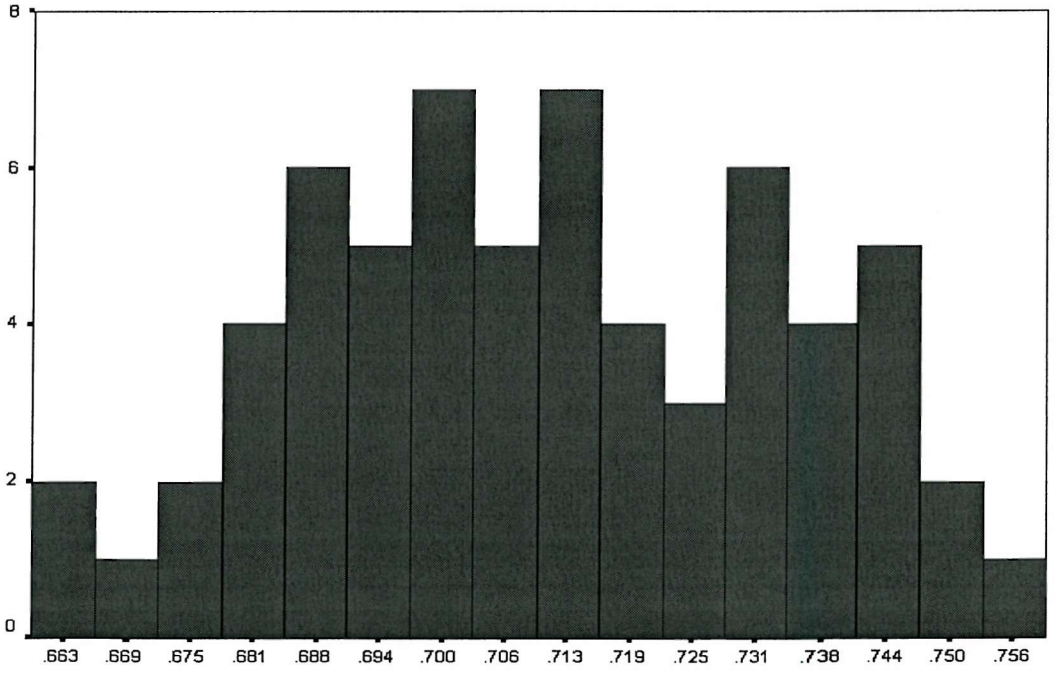


Figure D11 Distribution of ponderal index at birth (kg/m^3) in the children

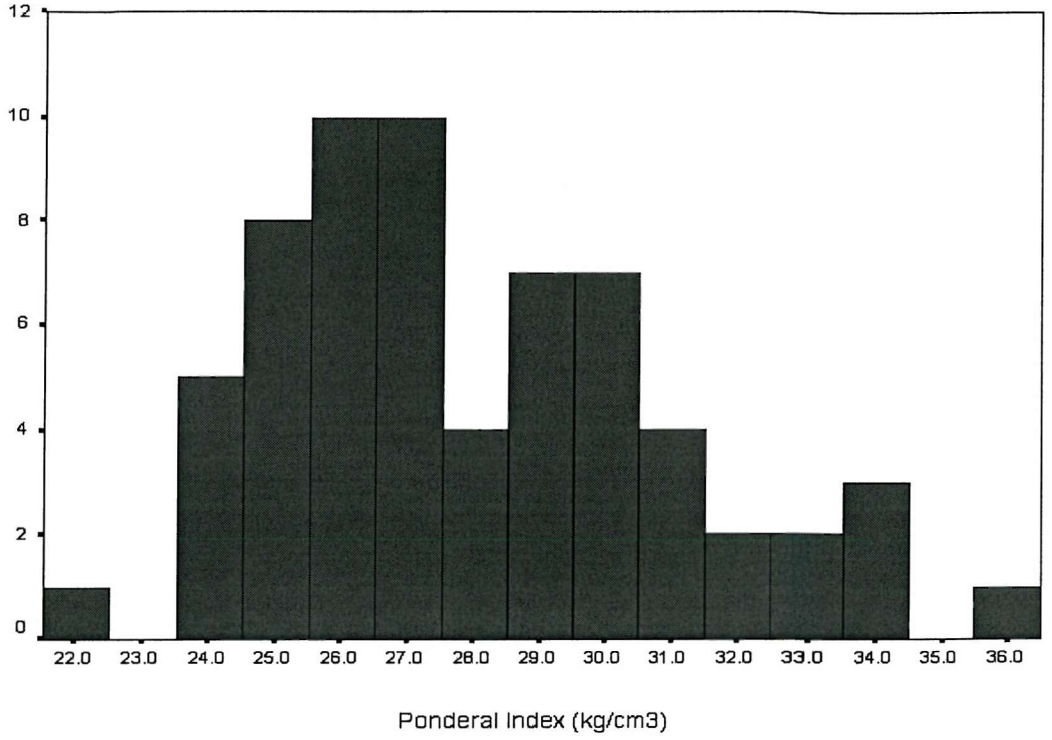


Figure D12 Distribution of placental weight at birth (g) in the children

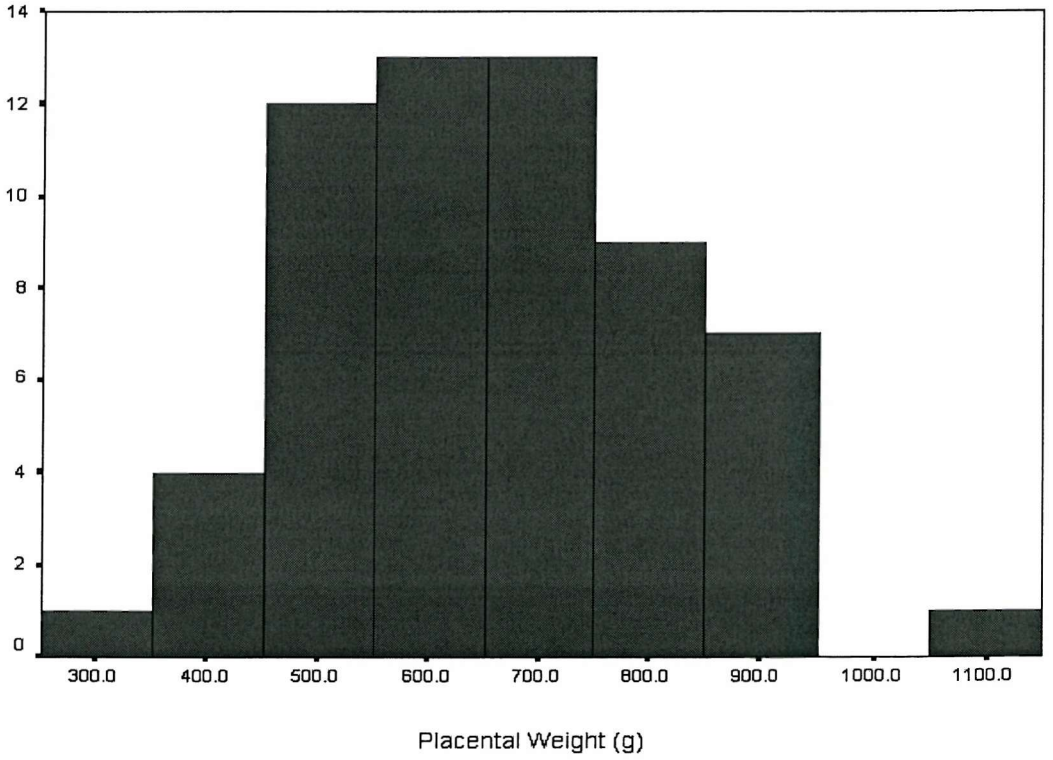


Figure D13 Distribution of placenta to birth weight ratio in the children

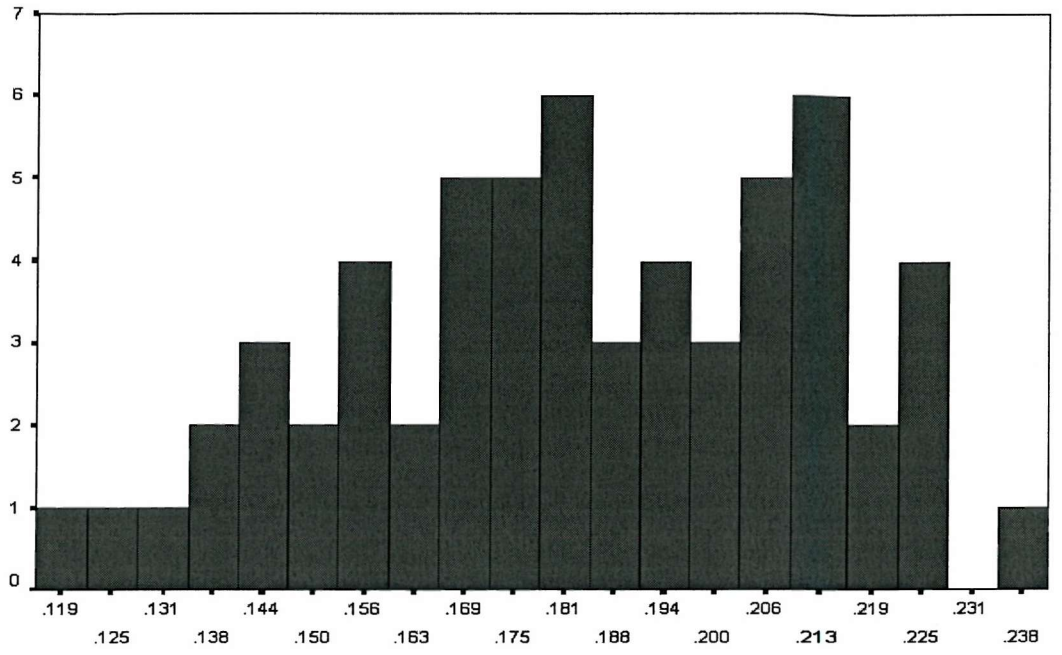


Figure D14 Distribution of gestational age at birth (days) in the children

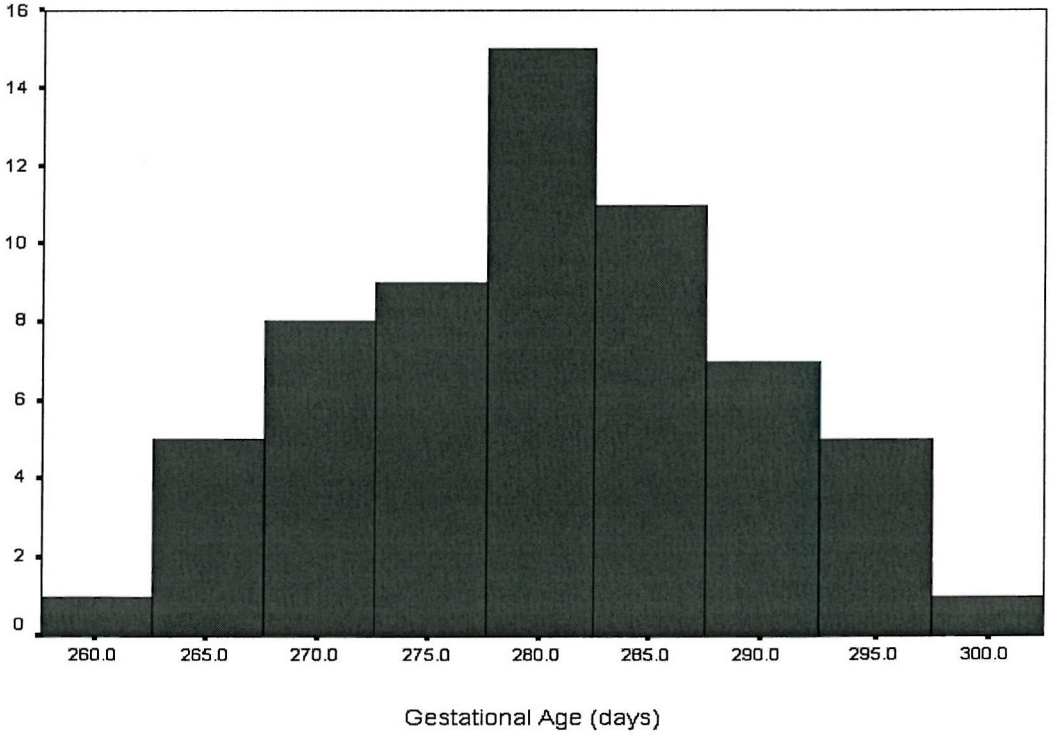


Figure D15 Distribution of systolic blood pressure (mmHg) in the children

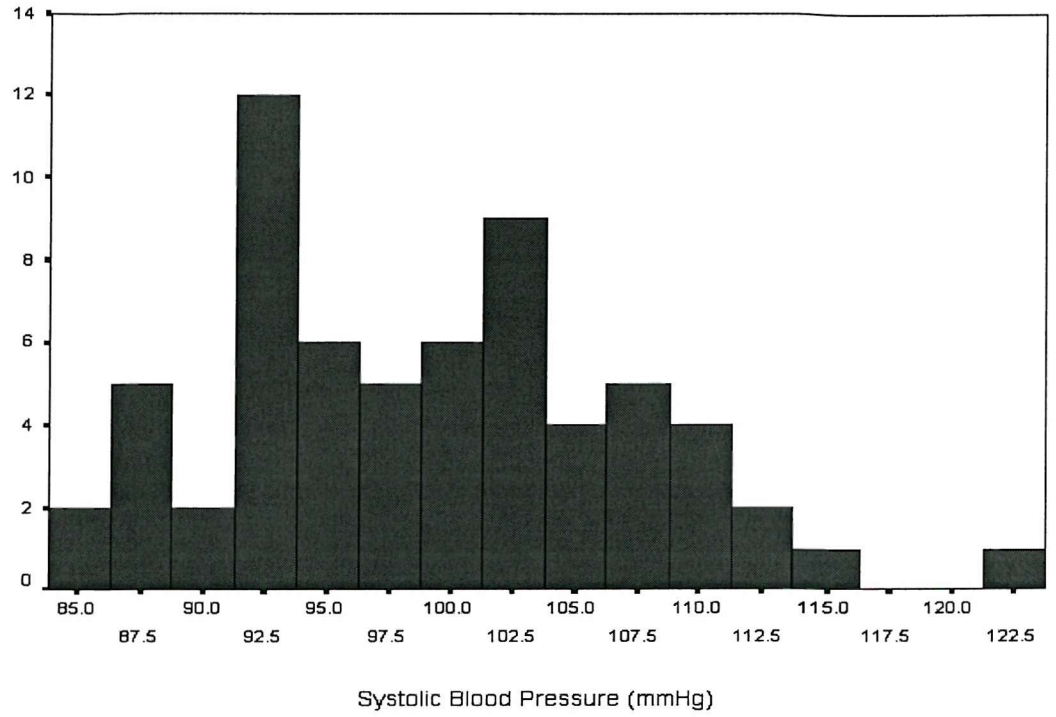


Figure D16 Distribution of diastolic blood pressure (mmHg) in the children

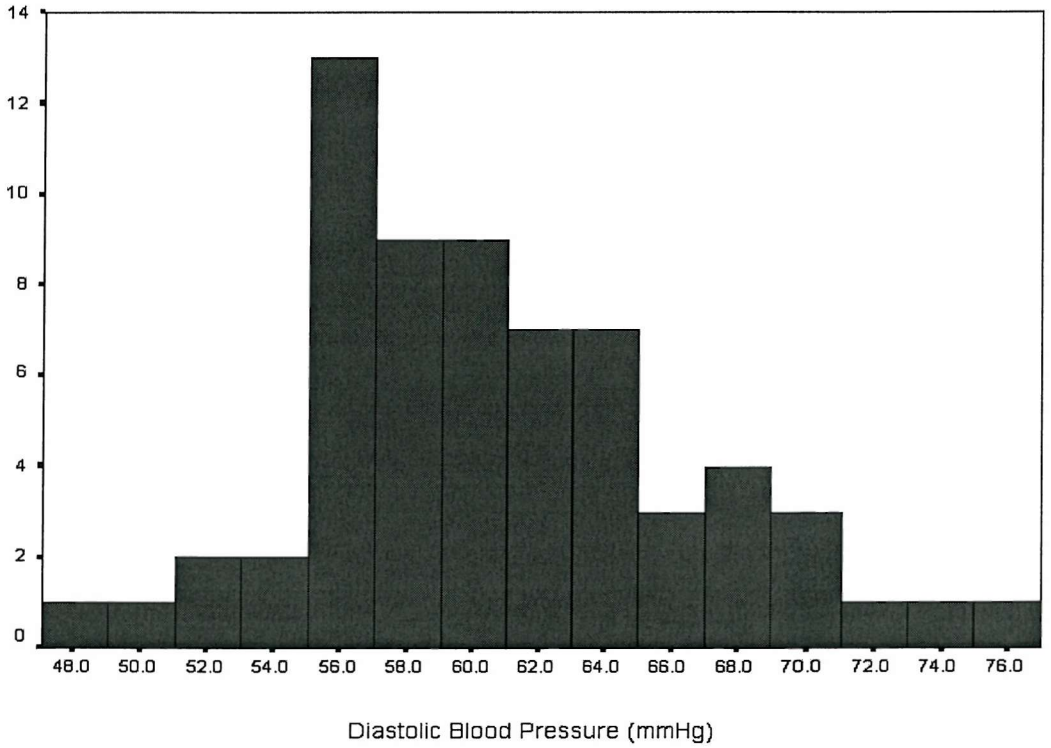


Figure D17 Distribution of aorta to femoral pulse wave velocity (m/sec) in the children

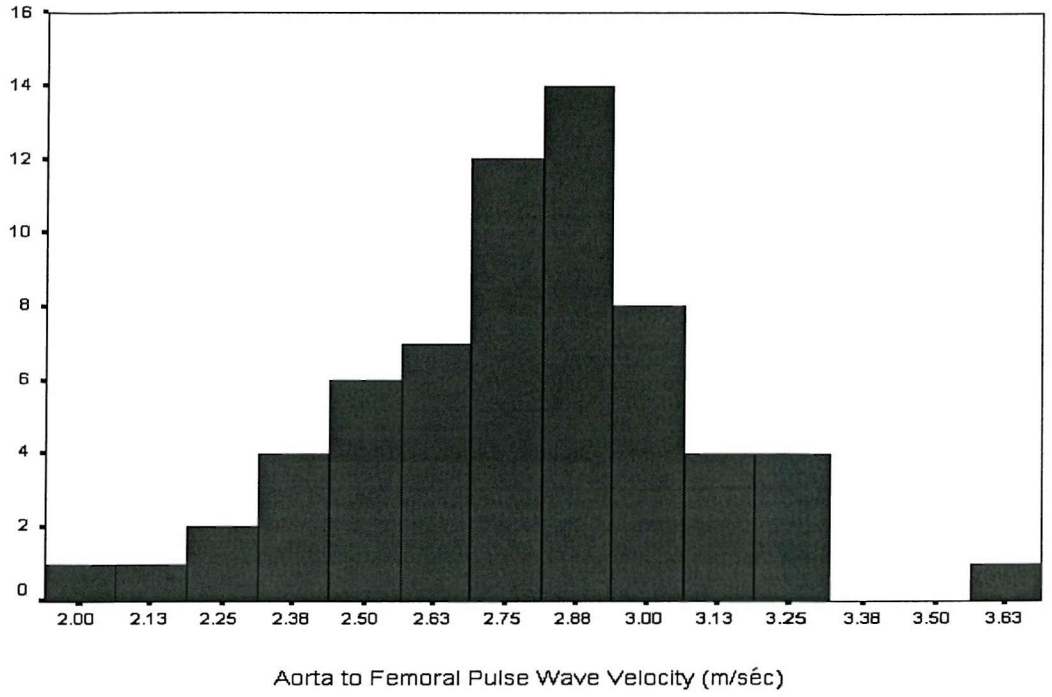


Figure D18 Distribution of aorta to radial pulse wave velocity (m/sec) in the children

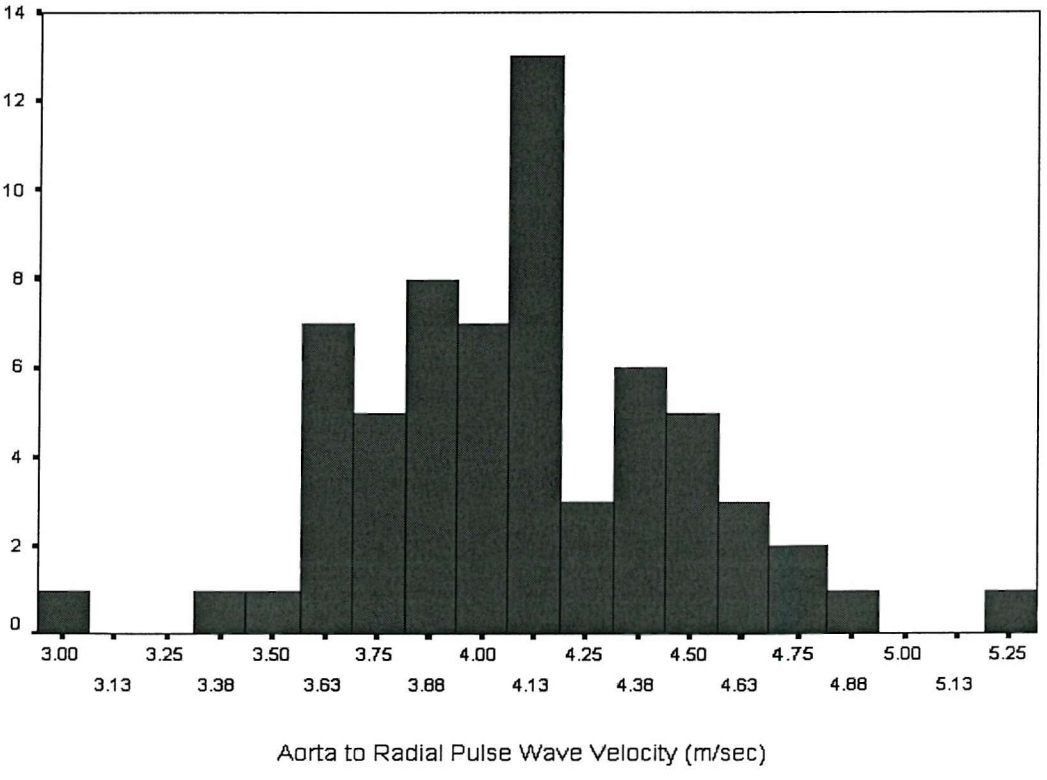


Figure D19 Distribution of aorta to foot pulse wave velocity (m/sec) in the children

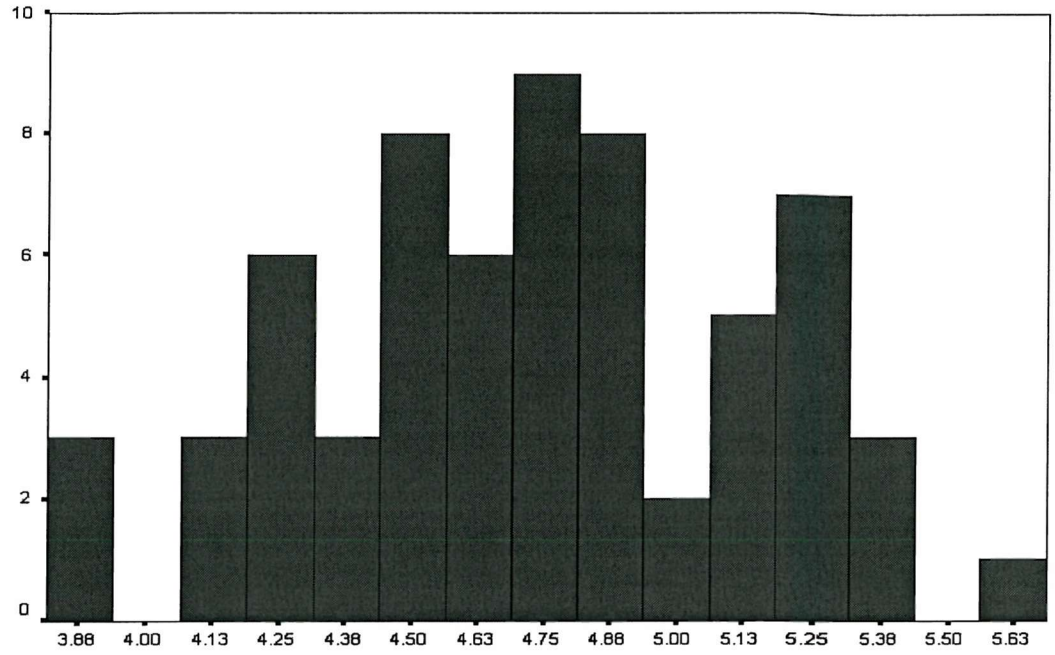
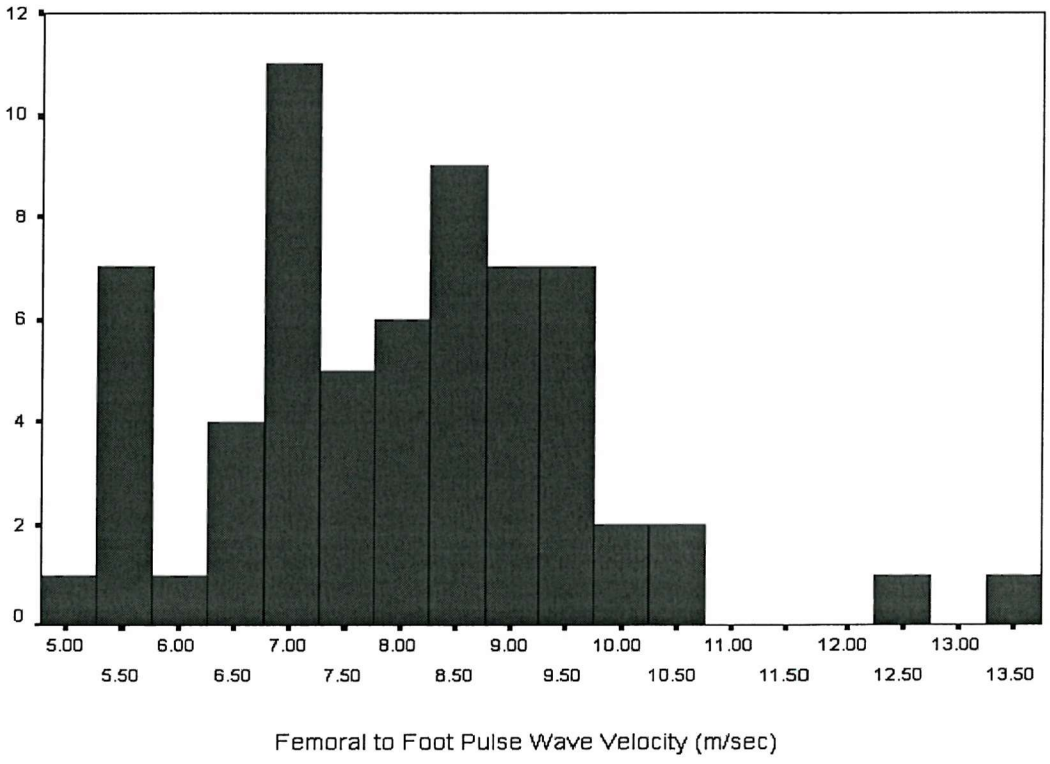


Figure D20 Distribution of femoral to foot pulse wave velocity (m/sec) in the children



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