UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Human Development and Health

Developmental Influences on Cardiovascular Structure and Function in Childhood Assessed Using Magnetic Resonance Imaging

by

Jennifer Ann Bryant

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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DEVELOPMENTAL INFLUENCES ON CARDIOVASCULAR STRUCTURE AND FUNCTION IN CHILDHOOD ASSESSED USING MAGNETIC RESONANCE IMAGING

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Cardiovascular disease (CVD) is the leading cause of death worldwide. The incidence of CVD often cannot be explained by adult lifestyle factors; epidemiological research suggests a link between the early developmental environment, and the risk of CVD in later life.

The aim of this research was to assess the influence of the early developmental environment on childhood cardiovascular structure and function measured at the age of 9 years. MRI measures of left ventricular cardiac volumes and mass, and aortic stiffness (aortic root distensibility and aortic pulse wave velocity), a recognised marker of cardiovascular risk, were developed and data acquired on subjects in a mother-offspring cohort.

Lower maternal oily fish consumption and lower maternal vitamin D status in late pregnancy were associated with increased child's arterial stiffness. Lower maternal educational attainment, poor self-reported maternal health, and higher levels of self-perceived maternal stress were associated with smaller child's left ventricular volumes and mass.

The findings suggest an effect of maternal nutrition on vascular development in utero and on arterial structure in the offspring. The findings also suggest that maternal health and wellbeing has an effect on cardiac structural development. The effect sizes were modest, but even small favourable changes to childhood cardiovascular structure and function may have substantial beneficial consequences for cardiovascular risk later in the life course. Lifestyle interventions to improve educational attainment and nutrition literacy in young women may reduce cardiovascular risk in the next generation.

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DECLARATION OF AUTHORSHIP

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and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
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- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published as:

Papers

Bryant J, Hanson M, Peebles C, Davies L, Inskip H, Robinson S, Calder PC, Cooper C & Godfrey KM: Higher oily fish consumption in late pregnancy is associated with reduced aortic stiffness in the child at age 9 years. *Circ. Res* 2015; 116:1202-1205

Black DE, Bryant J, Peebles C, Godfrey KM, Hanson M & Vettukattil JJ: Tissue motion annular displacement of the mitral valve using two-dimensional speckle tracking echocardiography predicts the left ventricular ejection fraction in normal children. *Cardiol Young* 2014 Aug; 24(4): 640-8

Black D, Bryant J, Peebles C, Davies L, Inskip H, Godfrey K, Vettukattil J & Hanson M: Increased regional deformation of the left ventricle in normal children with increased body mass index: implications for future cardiovascular health. *Paediatr Cardiol.* 2014 Feb; 35(2): 315-22

Presentation abstracts

Bryant J, Godfrey KM, Hanson MA & Peebles C: Significant incidental findings in 9-year old children undergoing cardiovascular magnetic resonance imaging for research. *Journal of Cardiovascular Magnetic Resonance* 2014, 16(Suppl 1): T8

Bryant J, Godfrey KM, Hanson MA, Davies L, Fletcher A & Peebles C: Transaxial or short axis right ventricular volume measurements – which method correlates more closely with main pulmonary artery flow values in children aged 9 years? *Journal of Cardiovascular Magnetic Resonance* 2013 15(Suppl 1): T10

Bryant J, Godfrey KM, Hanson MA, Davies L, Fletcher A & Peebles C: Steady state free precession cardiovascular magnetic resonance: accuracy of left and right ventricular assessment in children. *Journal of Cardiovascular Magnetic Resonance* 2013 15(Suppl 1): T9

Bryant J, Peebles C, Hanson MA, Crozier S, Inskip HM, Robinson S, Calder PC, Cooper C & Godfrey KM: Magnetic resonance imaging of pulse wave velocity in children aged 9 years to assess maternal influences on aortic stiffness in the offspring. *Journal of Cardiovascular Magnetic Resonance* 2012 14(Suppl 1): T8

Bryant J, Peebles C, Hanson MA, Crozier S, Inskip HM, Robinson S, Calder PC, Cooper C & Godfrey KM: Higher maternal oily fish intake in pregnancy is associated with reduced aortic stiffness in 9 year old children assessed using magnetic resonance imaging. *Journal of Developmental Origins of Health and Disease* 2011 2(Suppl 1):O-2ii-3

Bryant J, Godfrey KM, Hanson MA, Barton S & Peebles C: Comparison of manual and semi- automated analysis tools for quantifying left ventricular volumes and mass in children. *Journal of Cardiovascular Magnetic Resonance* 2011 13(Suppl 1): P358

Bryant J, Godfrey KM, Hanson MA, Barton S & Peebles C: Cardiovascular magnetic resonance: interstudy reproducibility of measurements of left ventricular function in children. *Journal of Cardiovascular Magnetic Resonance* 2011 13(Suppl 1): P359

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Date:……

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‘It is easier to build strong children than to repair broken men’

Frederick Douglass

Definitions and Abbreviations

[25(OH) D]	Serum 25 hydroxyvitamin D
3D	Three dimensional
4D	Four dimensional
11β-OHSD2	11beta-hydroxysteroid dehydrogenase type 2
ALSPAC	Avon longitudinal study of parents and children
A_{max}	Maximum cross sectional area (of the aorta)
A_{min}	Minimum cross sectional area (of the aorta)
Ao	Aorta
BMI	Body mass index – weight in kilograms divided by the square of height in metres (kg/m^2)
bpm	Beats per minute
BP	Blood pressure
BSA	Body surface area – calculated using the Dubois formula
CHD	Coronary heart disease
cfPWV	Carotid femoral pulse wave velocity
CMR	Cardiovascular magnetic resonance
cm/s	Centimetres per second
CO	Cardiac output
CVs	Coefficients of variation
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DDA	Distal descending aorta

DEXA	Dual-energy X-Ray absorbtiometry
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
ECG	Electrocardiograph
ED	End-diastolic
EDM	End-diastolic mass
EDV	End-diastolic volume
EF	Ejection fraction
EHA	Eicosapentaenoic acid
EPDS	Edinburgh postnatal depression score
ES	End-systolic
ESM	End-systolic mass
ESV	End-systolic volume
GC	Glucocorticoid
GHQ	General health questionnaire
GRAPPA	GeneRalized Auto-calibrating Partially Parallel Acquisitions
HASTE	Half acquisition single shot turbo spin echo
HSE	Health Survey for England
IMT	Intima media thickness
JCMR	Journal of Cardiovascular Magnetic Resonance
LC-PUFA	Long chain polyunsaturated fatty acids
LREC	Local research ethics committee
LV	Left ventricle
LVCO	Left ventricular cardiac output

LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
LVmass	Left ventricular mass
LVSV	Left ventricular stroke volume
LVOT	Left ventricular outflow tract
MAP	Mean arterial pressure
mm	Millimetre
mmHg	Millimetre of mercury
MPA	Main pulmonary artery
MRC LEU	Medical Research Council Lifecourse Epidemiology Unit
MRI	Magnetic resonance imaging
ms	Milliseconds
m/s	Metres per second
NRES	National Research Ethics Service
PA	Pulmonary artery
PC	Phase contrast
PDA	Proximal descending aorta
PP	Pulse pressure
PWV	Pulse wave velocity
Risk factor	Any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury (WHO)
ROI	Region of interest
RV	Right ventricle
RVOT	Right ventricular outflow tract

RVSV	Right ventricular stroke volume
SAX	Short axis
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
SNR	Signal-to-noise ratio
SSFP	Steady state free precession
SV	Stroke volume
SWS	Southampton women's survey
TE	Echo time
TR	Repetition time
Trans	Transverse
TrueFISP	True fast imaging with steady state precession
VENC	Velocity encoding
WHO	World Health Organisation

1. Introduction & Background

1.1 Cardiovascular Disease

Cardiovascular disease (CVD) is a major global health problem and the leading cause of death worldwide (WHO, 2008). Over 17.3 million deaths per year are caused by CVD with a predicted increase to over 25 million deaths by the year 2030 (WHO, 2012). Whilst partly attributable to established behavioural and metabolic risk factors, including unhealthy diet, physical inactivity and smoking, the occurrence of CVD is not fully explained by current lifestyle influences. CVD primarily manifests later in life, however epidemiological research over the last few decades has found that the risk of developing cardiovascular disease is not influenced solely by genetic background and adult lifestyle factors but also by environmental factors during early development.

Normal biological variations in early growth and development may have long-term effects on cardiovascular health, and even far reaching effects on the next generation. Developmental influences may alter vascular development in utero, changing arterial structure with long-term consequences for cardiovascular risk in later life (Martin et al., 2000, Leeson et al., 2001b, Halvorsen et al., 2006). Altered cardiovascular structure and function is now thought to precede the onset of CVD through associated impairment of ventricular function and it has come to light that factors predisposing individuals to CVD are in fact already present in childhood (Berenson, 2002, Zieske et al., 2002).

Epidemiological studies have related birth size to an altered risk in later life of CVD (Barker, 1998, Barker and Osmond, 1986), and type 2 diabetes (Hales et al., 1991). However, whilst birth weight is one marker of the intrauterine environment it is now believed that the true developmental influence on increased risk of adult disease is not mediated solely through birth weight. Birth weight is a single, not very specific, proxy for prenatal growth. It is well recognised that the prenatal environment can induce long-term cardiovascular effects without necessarily affecting size at birth (Hanson and Gluckman, 2005). In fact, differing trajectories of fetal growth in response to challenges at

various stages of gestation can nonetheless result in similar birth weights (Bloomfield et al., 2006).

It has been suggested that nutrition in utero, while not necessarily affecting birth size, and in early childhood is associated with cardiovascular health in later life (Barker, 1995). Nutritional interventions applied at critical periods in early life both before and during pregnancy therefore have the potential to remotely influence or 'program' future CVD risk (Ayer et al., 2009) and new interventions have the potential to improve cardiovascular health in the offspring.

Further insight into early life determinants of cardiovascular risk is necessary in order to facilitate early intervention with the aim of improving the developmental environment and further preventing CVD in adulthood. If individuals with an increased risk of later disease could be identified early in the life course, childhood interventions could be targeted to individuals at risk. Further, if determinants of increased cardiovascular risk in early life could be identified it may be possible to implement appropriate lifestyle, educational and nutrition interventions even earlier in the life course. Realising these possibilities requires unequivocal information about the factors in prenatal life and infancy that lead to impaired cardiovascular structure and function among children.

1.2 The Developmental Origins of Health and Disease (DOHaD) Hypothesis

A Norwegian study by Forsdahl (1977) was one of the first to suggest a relationship between poor living conditions in early life and the incidence of coronary heart disease (CHD) in later life. Infant mortality rates, indicative of standard of living, were highly correlated with later mortality rates from arteriosclerotic disease, suggesting that those who experienced poverty or poor living conditions in childhood and adolescence were more vulnerable to developing arteriosclerotic disease in later life, often despite improved living conditions.

Later studies in the UK were the first to extend the theory, suggesting that an individual's susceptibility to developing CVD in later life was influenced not

only by poor living conditions in early life but was also linked to the quality of the early developmental environment (Barker and Osmond, 1986, Barker et al., 1989). Birth cohorts were identified from which detailed records of birth weight and early growth were linked to mortality rates from CVD in later life (Osmond et al., 1993).

Subsequent epidemiological and animal studies have shown that small changes in the developmental environment can induce phenotypic changes affecting an individual's response to the later environment – altering the risk of chronic disease such as metabolic syndrome or CVD. Primary markers of metabolic syndrome are central obesity, insulin resistance and hypertension (Symonds et al., 2009), which are recognised contributors to the cardiovascular risk profile. Furthermore, profound augmentation of effects can occur if there is a mismatch between the early developmental environment and the subsequent environment in childhood and adult life (Gluckman and Hanson, 2006), manifesting as altered disease risk.

Developmental plasticity is 'the ability of a single genotype to produce more than one alternative form of structure, physiological state, or behaviour in response to environmental conditions' (Barker, 2004). Developmental plasticity utilizes epigenetic processes to 'tune' gene expression, based on cues from the intrauterine environment, to produce a phenotype best suited to meet challenges of the predicted later environment thus permitting improved survival and reproduction (Gluckman and Hanson, 2004b). From the standpoint of evolutionary biology, it is thought that such processes evolved in many species, including humans, to promote Darwinian fitness. After the plastic phase some epigenetic marks may become permanent. An individual is likely to remain healthy when their resulting phenotype is matched to their environment, as they are able to mount appropriate responses to everyday challenges. However, when the later environment differs from the prediction and is mismatched with the early environment, developmental plasticity may have a detrimental effect on survival (Bateson et al., 2004).

Mismatch can arise as a result of either a suboptimal early environmental influence (altered maternal body composition or unbalanced/low energy diet), or through the influence of an obesogenic lifestyle in the later environment (Figure 1.1). When the prediction is inaccurate and the adult nutrient

environment does not match that of the developmental period this mismatch confers a greater susceptibility to develop metabolic disease in later life (Godfrey, 1998). The relationship between the predicted and actual environment thus determines the magnitude of disease risk (Gluckman and Hanson, 2004a, Gluckman and Hanson, 2004b). Environmental mismatch is common in the developing world. However, in developed countries the increase in disease risk due to mismatch is becoming a more pronounced problem. Individuals who experienced normal conditions in the early environment are at greater risk of disease in later life as a result of increasingly affluent and obesogenic lifestyles (Gluckman and Hanson, 2004b).

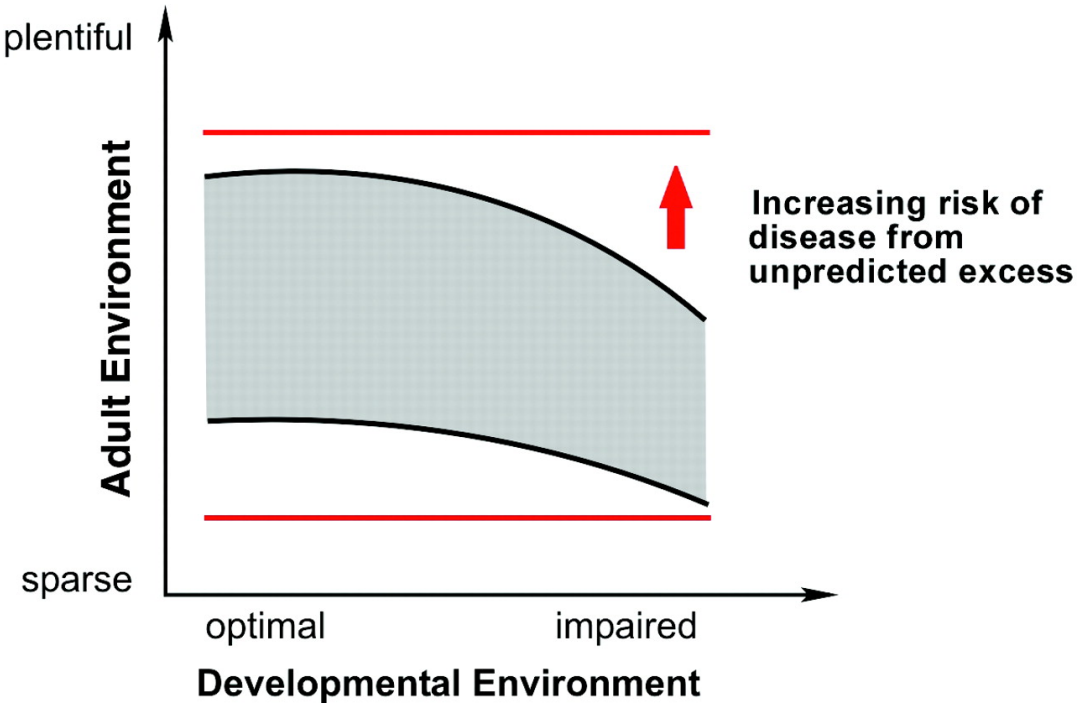

Figure 1.1 The mismatch concept.

The shaded area depicts the zone of maximal fitness where the early life prediction of the adult environment was accurate. Mismatch leads to an increased risk of disease in adulthood when phenotype is unsuited to the adult environment due to inaccurate developmental prediction of the later environment. The red lines represent the upper and lower limits of environmental range to which mature organisms could be exposed (for example nutrition). Reproduced from Gluckman and Hanson (2004b); Living with the past: evolution, development and patterns of disease, *Science,* 305, 1733-6, with permission from AAAS.

The magnitude of phenotypic changes in response to developmental influences is dependent on both the intrauterine stressor and the gestational timing of the stress (Louey and Thornburg, 2005). Developing organs are most sensitive to such stress during periods of rapid growth or maturation (Louey and Thornburg, 2005); thus there are organ-dependent critical periods of development throughout gestation and early postnatal life (Gluckman et al., 2008). For example, there are two phases of prenatal growth during which the heart is most vulnerable to stress: in the embryo patterns of gene expression in the wall of the heart are altered by haemodynamic forces, and later during fetal life when cardiomyocytes undergo a maturational phase (Louey and Thornburg, 2005).

Developmental influences may also alter vascular development in utero, changing arterial structure with long-term consequences for cardiovascular risk in later life. Recent studies have found that children who had a lower birth weight have reduced arterial compliance (Martin et al., 2000), and impaired endothelial function (Leeson et al., 2001b, Halvorsen et al., 2006) indicating early vascular structural and functional changes, which may predispose to later cardiovascular disease. Arterial stiffness, a well-established cardiovascular risk marker, is determined by the structure of the arterial wall. Collagen and elastin fibres are the principal elastic constituents of the arterial wall and development of the elastic lamellae begins early in fetal life (see section 1.9). The rate of elastin synthesis in the vascular wall is highest in the perinatal period and as there is no appreciable synthesis of mature elastin in the adult aorta (Martyn and Greenwald, 1997) failure to synthesise adequate amounts of elastin during early development has a permanent effect on arterial stiffness, with important implications for developing CVD in later life.

‘Arteriosclerosis’ and ‘atherosclerosis’ describe stiffening of the arteries. Arteriosclerosis occurs predominantly with age as a result of loss of elastin within the vessel walls while atherosclerosis describes vessel stiffening as a result of fatty degeneration. Pathological changes in arterial structure and function are evident in childhood and early adulthood decades before the clinical manifestation of atherosclerosis (Singhal, 2009). Fatty streaks in the intimal layer of the arterial wall are the first visible manifestation of atherosclerosis (Crowther, 2005) and have been identified in early life (Berenson et al., 2005). In fact, maternal hypercholesterolemia has been

associated with an increased presence of fatty streaks in the fetal aorta (Napoli et al., 1997). Fatty streaks have the potential to evolve into fibrous plaques; inflammatory stimuli, smooth muscle cell proliferation and lipid accumulation all contribute to the development of atherosclerotic lesions (Crowther, 2005), which narrow the arterial lumen.

People who were small at birth and during the first two years of life have an increased risk of CHD, particularly if their impaired growth is followed by increased childhood weight gain (Barker et al., 2005). Animal studies have shown impaired cardiovascular structure and function arising from a mismatch between pre- and postnatal environments (Khan et al., 2005, Cleal et al., 2007, Khan et al., 2004). In humans, studies have linked a mismatch between constrained prenatal and unconstrained childhood environments with later cardiovascular risk (Eriksson et al., 2001, Barker et al., 2005, Bhargava et al., 2004), but no study to date has had the data required to define the cardiovascular consequences of mismatches in the trajectories of growth and nutrient intake from early pregnancy onwards. Early pregnancy is the period of greatest growth and rapid development of major physiological systems and the placenta. As previously mentioned, developing organs are most sensitive to stressors during periods of rapid growth making early pregnancy a particularly sensitive developmental phase.

1.3 Maternal Influences

The fetus is singularly reliant on the intrauterine environment to meet nutritional requirements required to sustain growth and development. A woman's health and diet during pregnancy therefore play a crucial role in influencing fetal health and development. Maternal diet and body composition, both before and during pregnancy, may influence the child's metabolism and cardiovascular structure, and function, and therefore determine future cardiovascular health.

Population studies of the long-term effects of famine suggest that an adverse early nutritional environment is associated with an increased risk of CVD in later life. The effects of famine in early life appear to be largely dependent on the timing of exposure. Li et al. (2011) found that adults exposed to the Chinese famine in fetal and early life had a higher risk of metabolic syndrome

than those exposed in mid and late childhood. Interestingly, the association was stronger in those with an obesogenic lifestyle in adulthood, suggesting an influence of the 'mismatch' phenomenon. Further, there was an increased prevalence of high blood pressure (BP) in those exposed to famine in the first 2-3 years of life but no association with those exposed in utero or infancy (Huang et al., 2010a). Fetal-infant and early childhood exposure to famine in Biafra during the Nigerian civil war was associated with increased BP in adulthood (Hult et al., 2010), however the study did not distinguish between fetal and infant associations. Following the Leningrad siege, malnutrition in utero was associated with endothelial dysfunction in adulthood; the concentration of von Willebrand factor, a marker of endothelial damage, was raised in subjects exposed to intrauterine malnutrition (Stanner et al., 1997) but there was no reported association with hypertension or CVD.

The 'Dutch Hunger Winter' (Lumey et al., 2007) offers further insight into the importance of timing of the exposure during gestation. The Dutch Hunger Winter lasted for a defined period of time, prior to and following which food supplies were close to providing normal requirements. As a result it was possible to clearly define the impact of malnutrition during specific stages of gestation on later health outcomes. Early gestation was identified as a particularly vulnerable period – exposure to famine during this period was associated with an increased risk of CHD (independent of birth weight), reduced glucose tolerance, higher insulin concentrations, and increased obesity in adult life (Roseboom et al., 2006). Malnutrition in the third trimester was associated with lower birth weight, but there was no association between malnutrition during early to mid gestation and child's weight at birth, suggesting that measures of child's size at birth (weight, length and head circumference) are a poor indicator of maternal nutrition in pregnancy (Stein et al., 2004).

Whilst these studies describe the effects of an adverse nutritional environment in utero and in early childhood, as a result of severe famine, **there is little information on the effects of normal variations in maternal nutrition on cardiovascular outcome measures in the offspring**. Further, there are limited studies where maternal nutrition has been characterised in detail during pregnancy.

Early nutrition in infancy may have a long-term influence on the risk of developing CVD in later life. Nutrient levels in breast milk and therefore in the child are representative of maternal levels. Breastfeeding has been associated with a reduction in BP measured in later life in both children (Martin et al., 2004), and adults (Owen et al., 2003). Although there are conflicting data in the literature regarding the association between breastfeeding and cardiovascular risk in later life, the majority of studies report that breastfeeding confers a protective effect in terms of cardiovascular risk (Ravelli et al., 2000). A systematic review showed that breastfeeding is associated with a reduction in both systolic and diastolic BP later in life (Martin et al., 2005).

Few studies have reported on the influence of breastfeeding on arterial structure and function, again with contradicting findings. Although there was no association found in women, adult men who were breastfed in the Young Finns study had better endothelial function than those who were formula fed (Jarvisalo et al., 2009). Leeson et al. (2001a) reported controversial findings that longer breast feeding duration was related to reduced arterial function in the adult.

Obesity rates are rising in both developed and developing countries. Trend data from the Health Survey for England (HSE) shows an increase in the prevalence of obesity (defined as body mass index (BMI) >30kg/m^2) in women of childbearing age (16-44 years) from 1993 to 2013 (HSE, 2014). 18% of Southampton women have a body mass index >30kg/m^2 before pregnancy and more than half were overweight or obese (Duggleby et al., 2006).

Evidence from both human and animal studies suggests that maternal obesity has an adverse effect on the cardiovascular health of offspring. In the Helsinki cohort study, offspring of women of below average height, due to constrained early development, and increased BMI due to an unconstrained later environment had higher rates of CHD (Forsen et al., 1997). Maternal obesity prior to and during pregnancy has been associated with impaired cardiometabolic health in the offspring, in both childhood and in young adults, increasing the risk of CVD in later life. In the 'Generation R Study' higher maternal pre-pregnancy BMI was associated with higher systolic BP, higher BMI and higher insulin levels in offspring at the age of 6 years (Gaillard et al., 2014). Similarly, Hochner et al. (2012) found that greater pre-pregnancy

maternal BMI was significantly associated with higher BMI, systolic and diastolic BP, insulin and tiglycerides in young adult offspring, suggesting that these effects track throughout life. Animal studies have demonstrated structural changes in both the heart and vasculature of offspring following maternal overnutrition (Samuelsson et al., 2008); following maternal diet induced obesity offspring of obese dams had higher systolic BP, exhibited poorer arterial endothelial function and had higher fasting insulin and glucose levels when compared to control offspring.

Whilst maternal obesity has been linked with hypertension, endothelial dysfunction, dyslipidaemia, insulin resistance and greater adiposity in the offspring, the underlying physiological mechanisms are not clear. Taylor and Poston (2007) suggest this may be explained by an increased delivery of glucose, amino acids and free fatty acids across the placenta to the fetus.

Maternal thinness has also has been linked with offspring insulin resistance, dyslipidaemia and CHD (Godfrey, 2006, Mi et al., 2000, Stein et al., 1996). Unbalanced or low maternal dietary intake has been linked with raised BP, glucose intolerance, altered stress response and CHD in the offspring (Godfrey, 2006, Reynolds et al., 2997, Painter et al., 2006, Shiell et al., 2001, Herrick et al., 2003, Painter et al., 2005). Both reduced fetal nutrient supply and impaired early development have been linked with an increased risk of cardiovascular disease in adulthood. Gale et al. (2006) found that carotid artery intima-media thickness (IMT), a strong predictor of vascular events in adults, measured in 216 children at the age of 9 years was greater in children whose mothers had energy intake in the lowest quartile in pregnancy when compared with those whose mothers had energy intake in the highest quartile. Rat studies have shown that changes in maternal fatty acid intake in pregnancy are associated with increased arterial stiffness in the offspring (Armitage et al., 2005a). **Unbalanced maternal diet may adversely affect cardiovascular structure and function in the child – measurable by the age of 9 years.**

Maternal weight and body composition has been linked with CHD in the offspring. The relationship of maternal BMI to the offspring's size at birth is well documented but there is a paucity of data on the specific effects of maternal fat and lean mass on cardiovascular structure and function. BMI, based on measures of height and weight, can be misleading, often providing

an inaccurate measure of body fat. BMI does not take into account muscle mass, bone density, ethnic or sex differences, and does not distinguish contributions of fat and lean mass to overall body composition. Whilst BMI gives an indication of body composition, alternative anthropometric methods such as measures of arm muscle area or skin fold thickness are essential to provide a more reliable assessment.

Maternal stress during pregnancy has been associated with an increase in emotional or cognitive disorders including hyperactivity, anxiety and language delay in the offspring (Talge et al., 2007). Maternal health and wellbeing during pregnancy may also be associated with cardiovascular structure and function in the offspring. Maternal distress is associated with increased fetal cortisol exposure (Taal et al., 2013); animal studies have shown that transient exposures to glucocorticoids during development influence vascular outcomes in the offspring and have been associated with an increase in BP (Cottrell and Seckl, 2009). Taal et al. (2013) found that maternal psychological distress measured at 20 weeks of gestation was associated with smaller measures of LV mass in the offspring at age 5-7 years but reported no association with childhood BP or pulse wave velocity (PWV). Interestingly paternal distress was measured in the same study and showed similar associations suggesting that the findings could be attributed to other shared environmental factors. However, evidence from a study by Rondo et al. (2010) found that maternal salivary cortisol levels measured during pregnancy were associated with childhood systemic vascular resistance in the offspring at age 5-7 years, but found no association with BP. **A poor developmental environment in terms of maternal health and wellbeing, and maternal nutritional status may adversely affect cardiovascular structure and function in the child – measurable by the age of 9 years.** Few studies assess the influence of maternal psychological stress during pregnancy and none have reported on the influence of on-going 'chronic' levels of stress established prior to conception on the health of the offspring.

1.4 Childhood Influences

It has become apparent that in addition to the prenatal developmental environment, factors during infancy and early childhood may also influence the risk of developing cardiovascular disease in later life. **Further, a mismatch**

between the pre and post-natal environments may lead to altered cardiovascular structure and function measurements at the age of 9 years.

The prevalence of obesity in childhood is rapidly increasing and has become a serious global public health challenge (WHO, 2014). Obesity has been shown to track through childhood and is linked to overweight and obesity in adulthood (Berenson, 2002), and an increased risk of CVD (Srinivasan et al., 1996). Overweight and obesity in childhood and adolescence has been consistently linked with an increased risk of diabetes, hypertension, ischaemic heart disease and stroke in adult life (Reilly and Kelly, 2011).

Several childhood lifestyle factors have been identified as risk factors for obesity. In the 'Avon longitudinal study of parents and children' (ALSPAC) study, Reilly et al. (2005) found that birth weight, parental obesity, and sleep duration and sedentary behaviour at age 3, were all independently linked to the prevalence of obesity at age 7. Children who slept for 10.9 hours or less per night at 30 months of age were more likely to be obese at the age of 7 than those who slept for more than 12 hours. There was a linear relationship between the number of hours of television viewing per week at age 3 and obesity at 7 years. Sleep duration of less than 12 hours per day during infancy has been associated with an increased risk of obesity in preschool children, independent of television viewing, daily activity, maternal education and socioeconomic status (Taveras et al., 2008). A strong predictor of childhood obesity is parental BMI; children are more likely to be overweight or obese if both parents are obese (Reilly et al., 2005). Poor quality diet in infancy and early childhood has been linked to increased adiposity measured by BMI and dual-energy X-Ray absorptiometry (DEXA) at the age of 6 years (Okubo et al., 2015).

The above studies suggest that early implementation of strategies to improve sleep duration, reduce sedentary behaviour, increase activity levels and improve quality of diet in infancy with the aim of preventing childhood obesity could reduce the risk of cardiovascular disease in later life.

1.5 Mother-offspring Cohorts: the Opportunity for this Research

In order to explore associations of early life exposures with measures of cardiovascular structure and function, and measures of cardiovascular risk, in childhood there is a need for prospective cohort studies in which there is detailed information about the mother's health, diet, lifestyle and the early developmental environment.

Many such pregnancy and birth cohorts have been established within Europe over the last few decades, offering the opportunity to examine the influence of early life exposures on childhood health and development (Larsen et al., 2013).

The Southampton Women's Survey (SWS) (Inskip et al., 2006) is unique amongst these in that enrolment onto the study was pre-pregnancy and provides the opportunity for this research. Extensive information is available on the women's health, diet and lifestyle prior to conception, measures during pregnancy of maternal nutritional status and body habitus, and fetal, infant and early childhood measures (outlined in section 2.1).

1.6 Cardiovascular Risk

The World Health Organisation defines a risk factor as 'any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury'.

Much of CVD management has focussed on established behavioural and metabolic risk factors in adulthood with raised BP historically recognised as the leading risk factor. 13% of global deaths due to CVD are attributed to hypertension, followed by smoking (9%), hyperglycaemia (6%), physical inactivity (6%), and obesity (5%) (WHO, 2011). However it has become evident that cardiovascular risk factors identifiable in childhood are predictive of future risk. The Bogalusa heart study, for instance, has shown that BP tracks from childhood, and fatty streaks appear in the aorta from infancy (Berenson, 2002, Berenson et al., 2005). Further, observations from the Bogalusa study have shown that clinical risk factors and anatomical changes in the aorta and coronary arteries in early life are associated with the presence of both

atherosclerosis and cardiac and renal changes related to hypertension in later life (Berenson, 2002).

In order to implement intervention and prevention strategies in pregnancy and childhood we require unequivocal information about the factors in prenatal life and infancy that lead to impaired cardiovascular structure and function among children, starting with thorough characterisation of maternal health, diet and lifestyle factors prior to conception, and measures during pregnancy of maternal nutritional status and body habitus, followed by comprehensive assessment of cardiovascular structure and functional changes in early childhood.

1.7 Fetal Development of Cardiovascular System

The heart is one of the first organs to develop in the embryo, and the cardiovascular system one of the first systems to function. In the early embryo nutrient requirements are met via diffusion from the contents of the yolk sac – this process cannot sustain the nutritional demands of the growing embryo (Mitchell and Sharma, 2005). Early development of the cardiovascular system is thus necessary to meet the increasing oxygen and nutritional requirements of the rapidly developing embryo.

A critical period of development of the heart is thought to occur between days 20 and 50 after fertilisation. The heart begins to develop towards the end of the third week of gestation in humans with the first heartbeat at day 22-23 and circulation of blood throughout the embryo beginning by day 24 (Larsson, 2001). The primitive heart tube is formed early in the 4th week and in the following three weeks is transformed into the four chambers of the definitive heart through a process of looping, remodelling and septation (Larsson, 2001). The formation of the heart is complete by 10 weeks and the ventricles increase in size and volume due to myocardial growth.

Three principal cardiac cell types comprise the endocardium, myocardium and the epicardium. The endocardium forms the endothelial lining of the heart, the myocardium forms the muscular wall, and the epicardium (or the visceral pericardium), forms the outer protective layer of the heart. The epicardium is the source of cardiac fibroblasts and is responsible for coronary artery formation (both the endothelial lining and smooth muscle) (Sadler, 2012).

The myocardium expands through replication, or proliferation, of cardiomyocytes (hyperplasia) and in normal development the heart size and chamber volume increase in proportion to growth of the body (Louey and Thornburg, 2005). The rate of cardiomyocyte proliferation gradually declines during the later stages of fetal development (Li et al., 1996, Soonpaa et al., 1996). There is a transition from a proliferative hyperplastic phenotype to a hypertrophic phenotype in late pregnancy and early postnatal life with cardiomyocytes losing the ability to replicate shortly after birth. As the number of cardiomyocytes is established from birth, damage to heart muscle later in life is not simply reparable by cell regeneration (Li et al., 1996, Porrello et al., 2008). An individual with a reduced number of cardiomyocytes is therefore more vulnerable when exposed to increases in cardiac demand (workload) and is more susceptible to left ventricular hypertrophy and ischaemic heart disease in adulthood (Porrello et al., 2008).

The early developmental environment influences the rate of binucleation of cardiomyocytes and thus the transition to terminal differentiation of cardiomyocytes. Porrello et al. (2008) suggest that exposure to poor nutrition, hypoxia and endocrine stress during the perinatal period limits the number of cardiomyocytes available to maintain the myocardial growth trajectory, resulting in increased hypertrophy. Myocardial hypertrophy may occur later in life in response to increased load from physical activity or exercise training, or in response to pathological stimuli such as hypertension, increased peripheral vascular resistance or increased cardiac afterload following myocardial infarction (McMullen and Jennings, 2007).

The coronary vascular system begins to form in week three over the epicardial surface of the myocardium (Bernanke and Velkey, 2002). The coronary arteries, originating from the ascending aorta, are located within the epicardium and are responsible for myocardial blood supply. Normal cardiac development and later function are dependent on the coronary circulation. The coronary sinus returns venous blood from the myocardium to the right atrium.

1.8 Cardiac Structure and Function

Standard BP measurements give an indication of cardiac afterload (systolic), and the amount of peripheral resistance encountered (diastolic). More specific measures of cardiac structure and function are outlined below:

- Left ventricular end-diastolic volume (LVEDV): the maximum volume of the left ventricle (LV) (in end-diastole).
- Left ventricular end-systolic volume (LVESV): the minimum volume of the LV (in end-systole)
- Left ventricular stroke volume (LVSV): the volume of blood ejected from the LV during systole. A measure of systolic function calculated by subtracting left ventricular end-systolic volume from left ventricular end-diastolic volume.
- Left ventricular ejection fraction (LVEF): the ratio of left ventricular stroke volume to left ventricular end-diastolic volume expressed as a percentage. A measure of cardiac efficiency.
- Left ventricular cardiac output (LVCO): the volume of blood ejected from the LV to the aorta per minute. LVCO is a measure of systolic function and is calculated by multiplying the stroke volume by the heart rate in beats per minute.
- Left ventricular mass (LVmass): 'an independent risk factor for the prediction of cardiovascular events' (Anderson et al., 2012), LV mass is calculated by subtracting endocardial volume measures from epicardial volume measures in systole and multiplying by the density of myocardium (1.05g/cm^3).

1.9 Arterial Structure and Development

The arterial wall is comprised of three concentric layers comprising the tunica intima, media, and adventitia. The tunica adventitia is a region of collagen with some elastin fibres, which merge with surrounding connective tissues. The tunica intima consists of the vascular endothelium. The mechanical properties of a vessel are largely determined by the tunica media, which forms the greatest component of the vascular wall (Nichols and O'Rourke, 1998). Arterial stiffness is determined by the structure of the arterial wall and is proportional to the ratio of elastin to collagen in the vessel wall. Vascular elastic properties

are thus dependent on the elastin content of the vessel wall (Martyn and Greenwald, 1997) and this differs between central and peripheral arteries. Elastin is the dominant component of the proximal aortic wall, comprising approximately 60% of total fibrous tissue. There is an abrupt transition to about 30% in the aorta distal to the diaphragm (Harkness et al., 1957), and in peripheral arteries collagen dominates. The reduction in elastin content is related to an increase in aortic stiffness (and PWV) distally. Increasing stiffness as arteries become smaller is responsible for the increase in PWV with distance from the heart and changes in arterial and pressure flow pulses are seen accordingly (Figure 1.2).

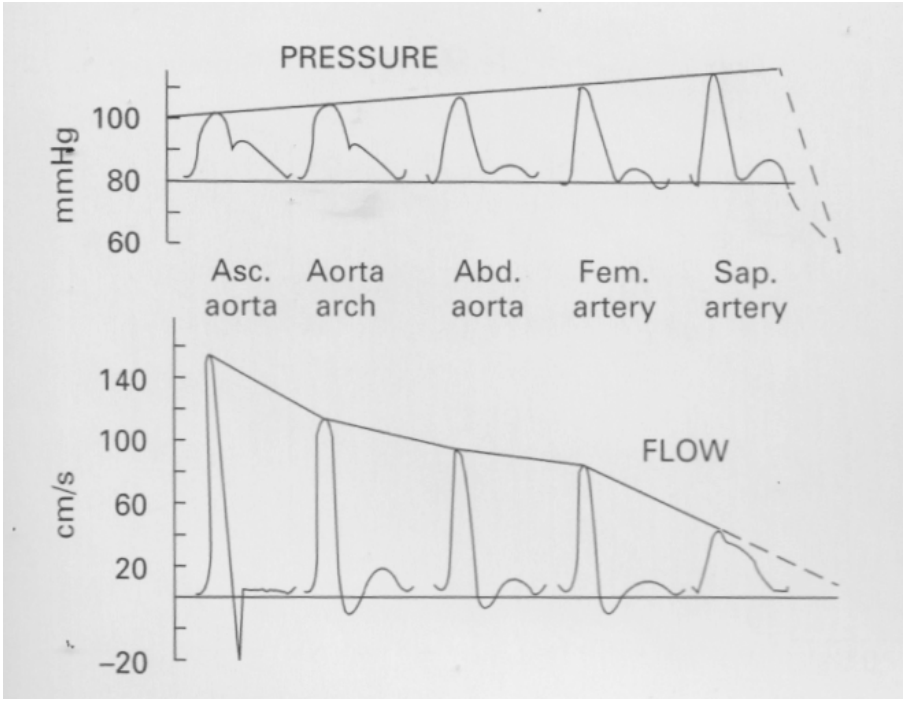

Figure 1.2 Arterial pressure and flow pulse velocity changes in arteries as they travel away from the heart (McDonald, 1960).

Collagen and elastin fibres are the principal elastic constituents of the arterial wall and form a closely meshed matrix. In the aorta, elastin is arranged in multiple concentric lamellae interspersed with smooth muscle and collagen. Development of the elastic lamellae begins early in fetal life. The rate of elastin synthesis in the vascular wall is highest in the perinatal period (Bendeck and

Langille, 1991, Davis, 1995), after which the rates fall rapidly (Berry and Looker, 1972) (Figure 1.3). Reduced growth during fetal life may compromise elastin synthesis in the arterial walls. As there is no appreciable synthesis of mature elastin in the adult aorta (Martyn and Greenwald, 1997), failure to synthesise adequate amounts of elastin during early development has a permanent effect on arterial stiffness.

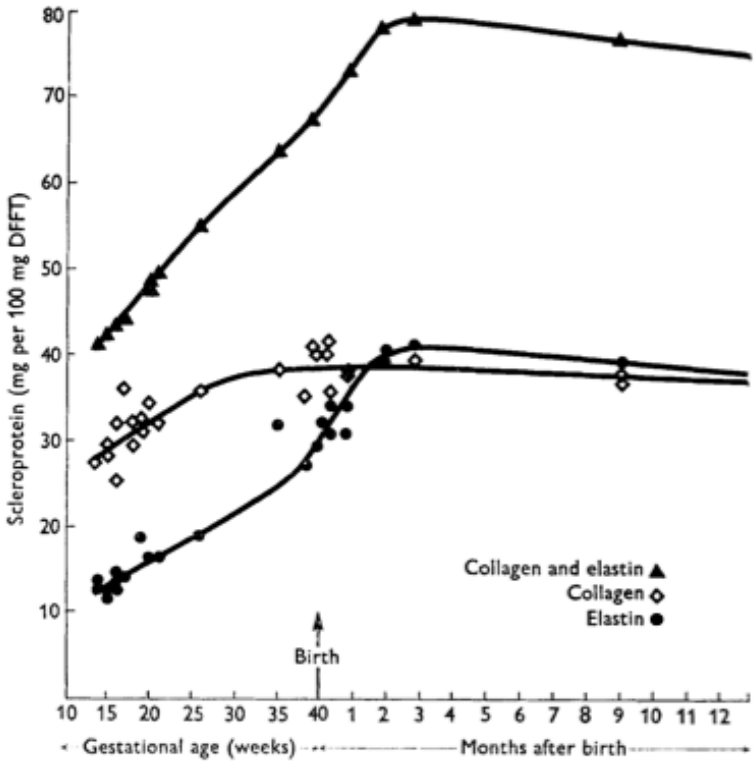

Figure 1.3 Changes in scleroprotein (collagen and elastin) content of the human aorta in fetal and early life.

The rate of elastin synthesis is greatest in the perinatal period (DFFT - dried fat free tissue). Reproduced from Berry, C.L & Looker, T. 1972 Nucleic acid and scleroprotein content of the developing human aorta *J Pathology,* 108, 265-74 with kind permission granted by John Wiley & Sons, Inc.

Thus the period during development when elastin is laid down is critical to arterial stiffness, BP, and subsequent cardiovascular risk in later life. Impairment in elastin synthesis during the critical period of blood-vessel

development may have important implications for the risk of developing CVD. Further, ageing is associated with structural and functional changes in the vessel wall, which may manifest from as early as childhood.

Impaired development of the vascular wall is a precursor to increased arterial stiffness, increased PWV, and an associated increase in pulse pressure (PP), which in turn lead to further arterial stiffness. Increased arterial stiffness is linked to increased BP, resulting in greater cardiac afterload. The means by which early developmental changes contribute to hypertension and CVD in later life could be explained by this pathophysiological feedback mechanism (Lehman, 1996). Impaired synthesis of elastin in the aorta may play a significant role in the pathogenesis of later hypertension (Martyn and Greenwald, 1997).

1.10 Vascular Stiffness

Arterial stiffness, "one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall" (Cavalcante et al., 2011), is increasingly recognized as a risk marker for cardiovascular disease (Laurent et al., 2006). Vascular stiffness is associated with:

a) An increase in systolic blood pressure (SBP), which in turn causes an increase in left ventricular workload, and subsequent left ventricular hypertrophy (Westerhof and O'Rourke, 1995, Mattace-Raso et al., 2006).
b) A decrease in diastolic blood pressure (DBP), which is associated with impaired coronary perfusion (Watanabe et al., 1993, DeLoach and Townsend, 2008).

The aorta acts as a conduit conveying blood from the LV to the peripheral circulation and is the largest contributor to the arterial buffering system (Laurent et al., 2006, Nichols and O'Rourke, 2005). The aorta functions by suppressing the pulsatile flow from left ventricular contraction during systole. The elastic buffering capacity of the aorta thus facilitates the transformation of pulsatile flow from ventricular ejection into steady blood flow in the peripheral circulation (Metafratzi et al., 2002, London and Guerin, 1999). The pressure wave generated by left ventricular contraction during systole is propagated forward until it meets resistance (strictly, impedance) peripherally at which

point the wave is reflected back towards the heart. Flow is less pulsatile downstream from the peripheral resistance. Therefore the final pressure of the arterial wave has two components; the forward wave generated by the heart, and the reflected wave which returns to the heart from peripheral sites (DeLoach and Townsend, 2008, O'Rourke and Staesson, 2002). The efficiency of this buffering process is determined by the viscoelastic properties of the arterial wall.

The influence of viscoelastic properties of vessels on cardiovascular function is well established. Viscoelastic properties of the aortic wall determine the propagation velocity of the pulse wave. With elastic arteries the reflected wave returns to the central circulation during diastole and augments coronary artery blood flow (DeLoach and Townsend, 2008), whereas stiffer arteries result in faster travel of the forward pressure wave and arterial waves are reflected from the periphery sooner and reach the heart during late systole. The associated increase in SBP and decrease in DBP results in increased cardiac workload and a reduction in coronary perfusion pressure respectively (O'Rourke and Staesson, 2002). Left ventricular load is therefore dependent on aortic elasticity, with coronary blood supply dependent on the elastic recoil of the aortic wall (DeLoach and Townsend, 2008).

In summary, vascular structural and functional changes lead to increased vascular stiffness and higher SBP, with an associated increase in left ventricular afterload, lower DBP with associated decreased coronary perfusion, and an increased risk of developing CVD.

Historically increased BP was considered a measure of cardiovascular risk. Arterial stiffness has emerged as an early marker of cardiovascular risk and an important predictor of increasing SBP and PP and decreasing DBP with age (O'Rourke and Staesson, 2002).

1.10.1 Measuring arterial stiffness

Arterial stiffness can be evaluated by several means. Regional arterial stiffness may be evaluated by measuring the PWV between two sites in the arterial tree, with higher PWV indicating stiffer arteries. Alternatively, distensibility (the relative change in arterial lumen area with change in pressure), strain, and compliance (the absolute change in arterial lumen area with change in

pressure) derived from the change in vessel lumen area across the cardiac cycle at a specific arterial site adjusting for the PP (Mattace-Raso et al., 2006) are measures of local arterial stiffness. Lower measured distensibility and compliance indicate stiffer arteries. PWV is inversely proportional to distensibility.

Measures of PWV track in pre-clinical cohorts and reliably predict future cardiovascular risk independent of SBP, age, sex and race (Willum Hansen et al., 2006). PWV has yielded prognostic value beyond and above traditional risk factors. Carotid femoral PWV, due to high accuracy and reproducibility, low cost, and relatively simple measurement has emerged as the preferred non-invasive measure of arterial stiffness (Boutouyrie and Vermeersch, 2010, Laurent et al., 2006).

1.10.1.1 Pulse wave velocity

PWV is a measure of arterial stiffness and measures the time it takes for the arterial pressure wave to travel a given distance between two sites in the arterial system in metres per second (m/s). The aorta is the largest contributor to the arterial buffering system and accounts for most global arterial stiffening. Aortic PWV is considered the gold standard measure of vascular stiffness and increased PWV is a strong predictor of CV risk.

A meta-analysis including more than 15,000 adult subjects reported that an increase in aortic PWV of 1m/s corresponded to a cardiovascular risk increase of greater than 10% (Vlachopoulos et al., 2010). Aortic PWV is a well-established independent predictor of cardiovascular disease in both patient groups (DeLoach and Townsend, 2008) and the general population (Sutton-Tyrell et al., 2005).

1.10.1.2 Aortic area, distensibility, strain and compliance

Aortic distensibility and compliance describe the ability of an artery to expand and contract in response to change in pressure (Cecelja and Chowienczyk, 2012) and are measures of arterial stiffness. Lower aortic distensibility indicates increased stiffness of the aortic wall.

Historically, measurements of aortic diameter are used in studies assessing aortic structure; however, the aorta is not completely round and vascular cross-

sectional area is a more accurate dimensional assessment. Advances in imaging technology permit the visualisation of the true cross-section of vessels. Image analysis software is able to accurately calculate cross-sectional area from such images.

Distensibility and compliance are calculated as the relative and absolute change in cross sectional area of an artery respectively for a given change in pressure. Measures of compliance are dependent on the initial volume of the vessel, with smaller volumes measuring reduced compliance for any given elasticity of the arterial wall. Distensibility is a measure of compliance relative to the initial vessel volume and relates more closely to stiffness of the vascular wall (Cecelja and Chowienczyk, 2012).

Arterial strain and distensibility (calculated with central PP), and aortic arch PWV determined using magnetic resonance imaging (MRI) were found by Redheuil et al. (2010) to be the most sensitive and specific markers of age related arterial stiffness. Further, when compared with carotid distensibility measured using ultrasound, and carotid femoral PWV (cfPWV) measured by tonometry, MRI measures better demonstrated early alteration in central arterial function in individuals free of overt cardiovascular disease (Redheuil et al., 2010). In adults, the best markers of subclinical large artery stiffening were ascending aorta distensibility in younger individuals, and aortic arch PWV in older individuals. The accuracy of distensibility measurement by MRI is limited by the PP, which is measured indirectly at a peripheral artery and used for the estimation of distensibility and compliance of the central aorta. Peripheral PP is higher than that measured in central arteries in younger individuals and the use of brachial BP can therefore induce large errors (Metafratzi et al., 2002). However many studies have reported accurate and reproducible results using MRI.

1.11 Gaps in Knowledge

The extent to which the developmental environment contributes to cardiovascular risk is unknown. However Penrose (1954) suggested that maternal and fetal genotype contribute to 38% of variation in birth weight and that 62% of variation is influenced by the intrauterine environment alone.

Further, the exacerbation by acquired risk burden in later life of the level of cardiovascular risk determined by developmental conditions is unknown.

The influence of maternal nutrition on offspring cardiovascular development has been extensively studied in animals. However maternal nutrition is associated with other factors such as socioeconomic status, education, health and lifestyle factors. The SWS mother-offspring cohort offers an excellent opportunity to investigate the impact of these factors on maternal nutrition and the influence on offspring cardiovascular structure and function.

Animal studies and famine studies following severe nutritional challenges during fetal life and infancy have shown an influence of nutrition on cardiovascular structure and function in the offspring. However few studies investigate the influence of normal variations in maternal diet on cardiovascular outcomes in the child. More specifically this research offers the opportunity to investigate the influence of specific micronutrients such as n-3 fatty acids, vitamin D and vitamin A on measures of cardiovascular structure and function in the offspring.

Historically BP has been a recognised marker of metabolic syndrome and CV risk. However more accurate measures of cardiovascular structure and function, even within normal ranges, may identify preclinical changes as an indication of future CV risk permitting the implementation of earlier intervention.

1.12 Hypotheses

1. That normal variation in maternal micronutrient status and oily fish consumption in late pregnancy affect cardiovascular structure and function in the child, measurable at age 9 years.

2. That a poor developmental environment, in terms of maternal health and wellbeing, adversely affects cardiovascular structure and function in the child, measured at age 9 years.

1.13 Aims and Objectives

The aims and objectives of this research were:

- To develop a paediatric cardiovascular magnetic resonance (CMR) imaging protocol suitable for acquiring accurate measures of cardiovascular structure and function in children in a research environment.
- To use paediatric CMR imaging techniques to examine the influence of early development on arterial structure and function, and therefore determine future cardiovascular health.
- To characterize the fetal and maternal (before and during pregnancy) influences that interact with the infant and childhood environment to underlie early cardiovascular pathology at age 9 years.
- To identify potential opportunities for intervention.

2. Methods

2.1 The Southampton Women's Survey

The Southampton Women's Survey (SWS) is a unique prospective mother-child cohort study with a wealth of detailed information on maternal health, diet and lifestyle prior to conception, and both pre- and postnatal trajectories of growth and their maternal determinants. The SWS therefore offers the opportunity to investigate the impact of the intrauterine, and subsequent postnatal environment on the cardiovascular health of the offspring and has made this research possible.

The SWS (Inskip et al., 2006) is the largest and most complete prospective study of its kind, and is the only population-based study in the developed world of a large, representative group of women who were characterized before pregnancy and had detailed longitudinal fetal, infant and childhood measurements from as early as 11 weeks in pregnancy.

Between 1998 and 2002 the SWS collected data from 12,583 non-pregnant Southampton women aged 20-34 years. Research nurses interviewed the women on diet, physical activity, social circumstances and lifestyle, as well as collecting DNA. Body composition was assessed with detailed anthropometric measurements. Body composition gave an indication of the women's nutritional status. Triceps, biceps, subscapular and supra-iliac skinfold thickness measures were taken. In order to estimate adult muscle mass measures of arm muscle area were made using triceps skin fold thickness and measures of mid arm circumference. Venous blood samples were collected in the luteal phase of their menstrual cycle.

At the initial interview the women were questioned about their general wellbeing. The women were asked to rate their general health on a scale of 1 to 5: 1= very good, 2= good, 3= fair, 4= bad, 5= very bad. They were asked to what extent they felt that the stress or pressure they have experienced in their life has affected their health: 1= none, 2= slightly, 3= moderately, 4= quite a lot, 5= extremely. They were also asked to define in general how much stress or pressure they had experienced in their daily living in the last 4 weeks: 1= none, 2= just a little, 3= a good bit, 4= quite a lot, 5= a great deal.

The women were asked to complete the General Health Questionnaire (GHQ-12), a short screening questionnaire used to detect depression and anxiety disorders (Goldberg and Williams, 1988), in order to identify preconception psychological distress. The questionnaire consists of 12 questions scored on a 4 point Likert scale. Significant psychological distress was indicated in women with a score >13.

3,160 of the women were subsequently followed through pregnancy. At 11, 19, and 34 weeks gestation detailed fetal scans were performed and further information on body composition and diet was collected (Inskip et al., 2006). In early and late pregnancy a food frequency questionnaire was administered. At 34 weeks gestation blood serum samples were taken and serum 25-hydroxyvitamin D [25(OH) D] concentrations were analysed by Diasorin radioimmunoassay. At birth, infant anthropometry was performed and samples of umbilical cord, cord blood and placenta were collected.

At 6 months postnatal the women were asked to complete the Edinburgh Postnatal Depression Scale (EPDS), a 10 item self-report questionnaire designed to screen women for symptoms of emotional distress, routinely used as a means of measuring the severity of postnatal depression (Cox et al., 1987). Answers were scored on a Likert scale from 1 to 4 and a score of >11 indicated the presence of symptoms of distress.

The growth and development of the children was characterised. Dietary information was collected and detailed anthropometry performed at 6 months, 1, 2, 3 years of age. At age 2 years detailed information on social circumstances was collected. BP measurements were made at 3 years. Growth was re-measured at ages 4 and 6 years.

Cardiovascular structure and function was evaluated using CMR in a sub cohort of children as part of the SWS 8-9 year cardiovascular phenotyping assessment. Variations in cardiovascular structure and function in childhood were related to data collected in the SWS, specifically evaluating the influence of maternal and childhood factors.

2.2 Cardiovascular Magnetic Resonance Imaging

Cardiovascular magnetic resonance imaging (CMR) is recognised as the gold standard imaging modality for cardiac structural and functional assessment (Pennell, 2003). CMR is a well-tolerated, non-invasive imaging modality with no known side effects and involves no exposure to ionising radiation. CMR real time cine acquisitions, with excellent inherent tissue contrast and high spatial and temporal resolution imaging capabilities, permit the acquisition of functional and structural datasets for the assessment of both right ventricular (RV) and left ventricular (LV) function, and measurement of LV myocardial mass.

CMR is an accurate and reproducible non-invasive imaging technique for the analysis of left ventricular volumes in adults but is less well validated in children. Compliance with breath hold requirements for the acquisition of accurate cine images is essential. The imaging protocol was optimised in order to minimise breath hold duration. As outlined in Chapter 3, image segmentation techniques and tools were assessed in order to determine the most appropriate method and tool for use in children. LV cine image acquisition was repeated in 10 children in order to determine inter-study reproducibility. Image analysis of these datasets was performed using both manual and semi-automated segmentation tools. Two methods of segmentation were compared; inclusion, and exclusion, of trabeculae and papillary muscles from the blood pool. Inter-observer variability was assessed on 5 datasets. Accuracy of the method was determined by comparing short axis (SAX) cine LV stroke volumes with aortic flow derived flow volumes.

In addition to the assessment of ventricular structure and function, high resolution imaging capabilities of MRI permit the accurate assessment and quantification of blood flow velocity and aortic geometry. Assessment of regional arterial stiffness is possible using velocity flow mapping techniques in conjunction with steady state free precession (SSFP) sequences which permit precise measurement of the true path length of the pulse wave along the aortic length (Dogui et al., 2011). High resolution SSFP cine acquisitions allow precise quantification of change in cross sectional area of the aorta throughout the cardiac cycle, permitting direct estimation of localized aortic strain and accurate assessment of local arterial stiffness.

2.3 Image Acquisition

Following the development of appropriate imaging methodology, 355 healthy 9-year-old children were invited to attend for CMR assessment of cardiovascular structure and function as part of the SWS 9 year cardiovascular phenotyping cohort.

Ethical approval was obtained from a local research ethics committee (LREC) (NRES Committee South Central – Southampton A, LREC no. 08/H0502/95, approval granted 5/9/2008). The participants and parent/guardian provided informed assent and consent respectively.

MRI compatibility of the child and parent/guardian was determined as per departmental local procedures and the completion of a safety questionnaire. Clothing with metal was removed and/or the child changed into a hospital gown as appropriate. The child's height (to the nearest mm) and weight (to the nearest 0.1kg) were measured immediately prior to the scan using a Leicester height measure and medical digital scales (Seca 778). The parent/guardian accompanied the child into the scan room.

All scans were performed on a 1.5 Tesla dedicated cardiac MRI scanner (Avanto, Siemens Medical Systems, Erlangen, Germany), optimised for paediatric cardiac MRI, using a phased array spine coil in combination with a 5 channel torso array coil. The child was positioned supine on the scan table. Ear defenders and an emergency buzzer were provided.

A paediatric BP cuff was positioned over the right brachial artery and BP measurements taken using an MRI compatible monitor (Invivo 3155MVS). Three ECG electrodes were positioned on the chest and all imaging retrospectively vector gated. Standard three plane orthogonal localiser images were acquired. All breath hold sequences were acquired on arrested inspiration. Although it has been shown that breath hold position is more reproducible in end expiration rather than elsewhere in the respiratory cycle (Taylor et al., 1997), it was more achievable for the children to stop breathing consistently on inspiration. SSFP cine imaging was accelerated using a parallel imaging technique GRAPPA (generalized auto-calibrating partially parallel acquisitions) in order to minimise acquisition time and therefore reduce breath hold duration.

2.3.1 Vascular structure and function

Measures of aortic PWV and aortic root distensibility were acquired using the following methods.

2.3.1.1 Pulse wave velocity

TrueFISP (true fast imaging with steady state precession) coronal views were acquired along the plane of the aorta, and sagittal oblique SSFP cine views acquired of the aortic arch and descending aorta in order to facilitate positioning of flow sequences. A free breathing, retrospectively gated phase contrast (PC) flow-mapping sequence was acquired at three locations in the aorta in order to evaluate aortic PWV. Flow sequences were positioned perpendicular to the long axis of the aorta at the level of the pulmonary artery in the ascending aorta, the proximal descending aorta (PDA), and a third positioned above the aortic bifurcation in the distal descending (abdominal) aorta (DDA) (Figure 2.1).

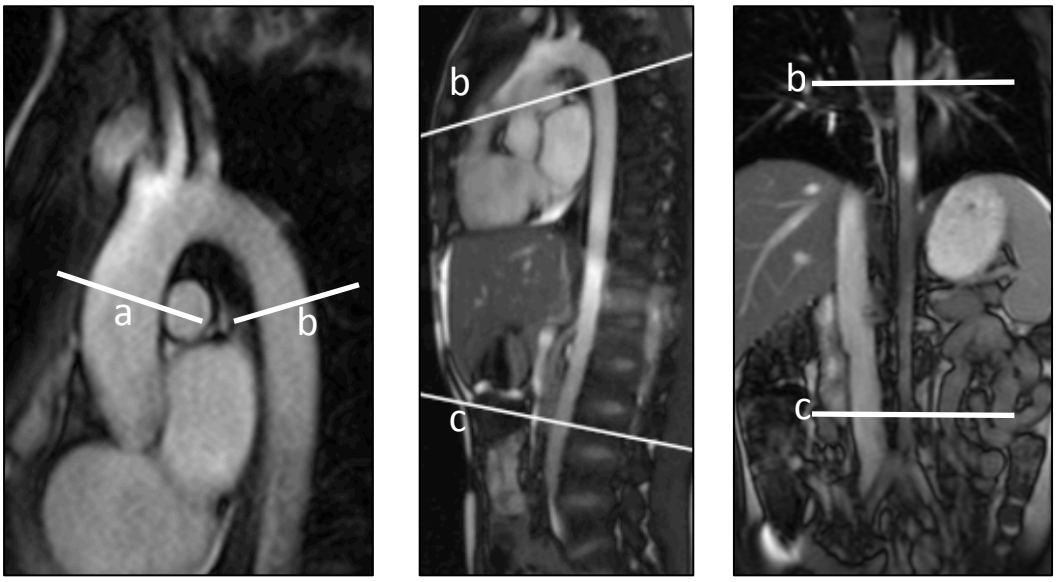

Figure 2.1 SSFP cine images (from left to right) sagittal oblique view of the aortic arch, sagittal descending aorta, and coronal TrueFISP view of the descending aorta demonstrating slice positioning of flow sequences; (a) at the level of the pulmonary bifurcation in the ascending aorta (b) in the proximal descending aorta, and (c) above the aortic bifurcation in the distal descending aorta.

A velocity encoding gradient was applied in the through plane direction. Sequence parameters as follows: field of view (FOV) 280mm, Voxel size 1.1x1.1x5mm, TR/TE 27.2/3.18ms, velocity encoding (VENC) 150-200 cm/s, slice thickness 5mm, flip angle 30º. Magnitude and phase images were acquired at 30 phases throughout the cardiac cycle. Brachial BP was recorded immediately following acquisition of the flow sequences.

The flow sequence acquired in the ascending aorta also serves as a means of assessing accuracy of the SAX SSFP LV functional data. This sequence, with a sagittal oblique SSFP cine sequence of the aortic arch, and the PDA and DDA flow data, permits the assessment of PWV across the aortic arch, in the descending aorta, and of the entire aorta.

2.3.1.2 Aortic area, distensibility, strain, and compliance

A single slice high-resolution breath hold SSFP cine acquisition, planned from long axis 3 chamber and left ventricular outflow tract (LVOT) views, was applied across the aortic root. The slice was positioned perpendicular to the long axis of the ascending aorta at the level of the pulmonary trunk, 2-4 cm above the aortic valve to avoid distortion from aortic valve motion (Dogui et al., 2011) (Figure 2.2).

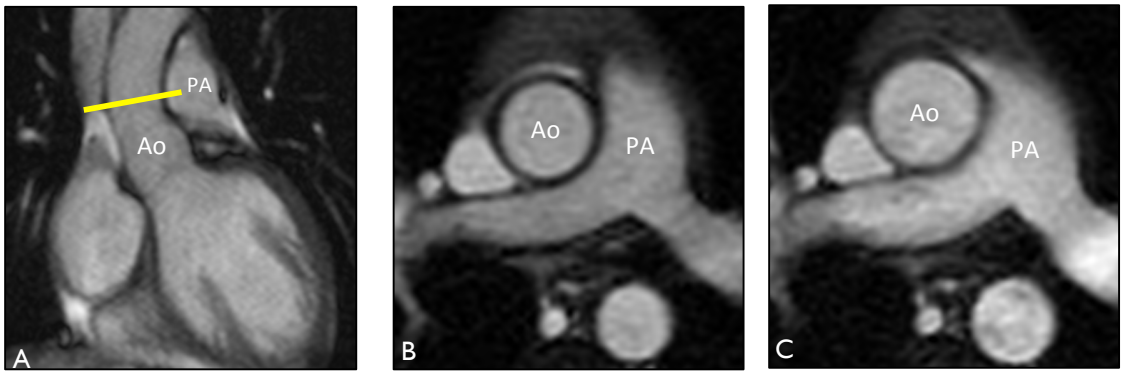

Figure 2.2 Left ventricular outflow tract image (A) showing aortic root SSFP cine slice position (yellow line) at the level of the pulmonary arteries (PA). Corresponding cross-sectional cine images of the aortic root (Ao) at minimum volume in diastole (C) and maximum volume in systole (D).

Sequence parameters: repetition time / echo time (TR/TE) 41.8/1.4ms, flip angle 52º, views per segment 13, FOV 280mm, acquisition matrix 192x236, slice thickness 6mm, pixel size 1.09x1.09mm. Images were acquired at 25 phases across the cardiac cycle. Brachial BP was recorded immediately following the acquisition.

2.3.2 Cardiovascular structure and function

2.3.2.1 Left ventricular function

A SAX stack of contiguous SSFP cine images was planned from an end-diastolic long axis 4-chamber cine image. The slices were positioned perpendicular to the long axis of the inter-ventricular septum. The basal SAX slice was positioned immediately on the myocardial side of the atrio-ventricular junction. The stack was positioned to cover from the mitral valve plane through to include the apex of the LV. Scan Parameters: TR/TE 43.65/1.24, field of view 280mm, flip angle 70º, slice thickness 7mm, imaging matrix 192x100.

Output values for both LV and RV in the SAX plane: End-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), stroke volume (SV), and cardiac output (CO), all normalised to body surface area (BSA), and LV mass in systole and diastole.

2.3.2.2 Right ventricular function

A transaxial stack of contiguous 7mm thick SSFP cine slices (scan parameters as per SAX acquisition) was positioned on the sagittal and coronal scout images to cover the entire RV from the pulmonary valve superiorly to the diaphragmatic surface of the heart inferiorly. Images were acquired on arrested inspiration as per SAX cine imaging.

Output values: RV EDV, ESV, EF, SV, and CO, all normalised to body surface area.

A phase contrast velocity flow mapping sequence was acquired through the main pulmonary artery, positioned perpendicular to the long axis of the vessel on SSFP cine views of the RVOT and MPA, as a means of assessing the accuracy of RVSV derived from the SSFP cine stack acquisitions.

2.4 Image Analysis

All image analysis was performed on a Mac Book Pro laptop computer using open source imaging software Osirix (Rosset et al., 2004), and/or Matlab software (The Mathworks, Inc, Natick, MA, USA, R2010a).

2.4.1 Vascular structure and function

2.4.1.1 Pulse wave velocity

Regions of interest (ROI) were traced around the aortic lumen across 30 phases of the cardiac cycle on the magnitude images of each flow dataset and propagated to the phase contrast images. Velocity flow curves were generated for flow acquisitions acquired at each aortic level.

Distance between the flow acquisitions was measured on a sagittal oblique cine image of the aortic arch, and on a long axis sagittal cine view of the descending aorta. Distance was measured along the midline of the aorta. Measurements were made across the aortic arch between the flow sequences acquired in the ascending and proximal descending aorta, and in the descending aorta between the proximal and distal descending aortic flow locations (Figure 2.3). Each measurement was made three times and the mean used for PWV analysis. Total aortic distance (between the ascending aortic flow position and the distal descending aortic flow position) was calculated from the two measurements.

Using Matlab software (The Mathworks, Inc, Natick, MA, USA, R2010a) PWV was calculated using the transit time method (Ibrahim et al., 2010), as a ratio of the distance between the flow measurements (in metres) to the transit time (in seconds) of the velocity pulse wave between the two sites. Pulse transit time was measured between the points of maximal systolic upstroke of the waveforms at each measurement site (Figure 2.4).

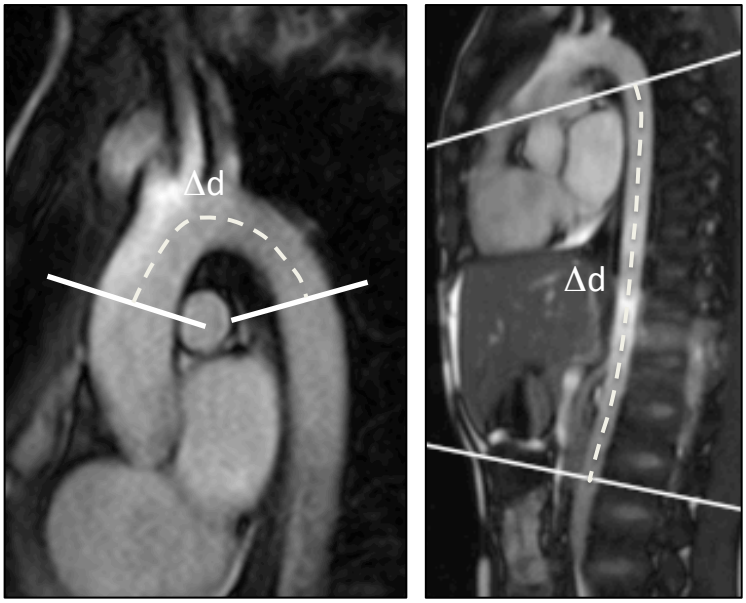

Figure 2.3 Sagittal views of the aortic arch (left) and descending aorta (right) demonstrating distance measurements for PWV calculation; distance (Δd) (dashed line) measured across the aortic arch between the ascending and proximal descending flow positions, and along the descending aorta between the proximal and distal descending aortic flow positions.

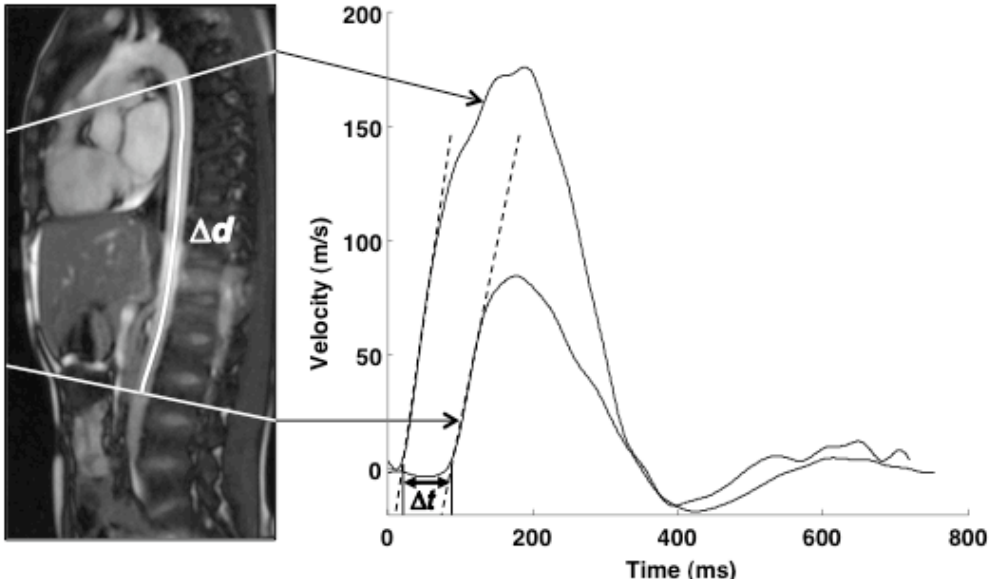

Figure 2.4 Sagittal TrueFisp image showing position of flow sequences in the descending aorta and resultant flow curves. PWV was calculated as distance between the flow acquisitions (Δd) / time difference between the arrival of the wave front (Δt). (Circulation Research: February 2015 – Volume 116 – p1203 Wolters Kluwer Health Lippincott Williams & Wilkins©).

2.4.1.2 Aortic area, distensibility, strain, and compliance

Distensibility, strain and compliance were measured across the aortic root using a high resolution SSFP cine acquisition. SSFP MRI sequences have inherently good contrast between bright blood signal and that of surrounding tissue. Change in vessel lumen area across the cardiac cycle was measured on the bright blood SSFP cine images using an automated segmentation method described and validated by Jackson et al. (2009), and implemented under Matlab (The Mathworks, Inc, Natick, MA). The segmentation method is outlined in Figure 2.5 and requires minimal user input.

Following user selection of the vessel of interest on the cine images (ascending or descending aorta), the data from the selected ROI was first interpolated to radial co-ordinates so that the vessel wall contour is represented by a straight line. This endeavours to minimise underestimation of vessel area caused by the smoothing along circular boundaries. Edge detection then produces a gradient image based on differences in signal intensity between the vessel and surrounding structures. Tracing the peaks of the gradient creates an objective outline of the vessel wall. Conversion back to Cartesian co-ordinates gives a contour of the vessel.

Vessel lumen area was measured at each of 25 phases across the cardiac cycle (Figure 2.6). Area measurement was repeated 20 times at each phase. Compliance, arterial strain, and distensibility values were calculated from mean values for Maximum vessel area (A_{max}) and minimum vessel area (A_{min}), corresponding to end-systole and end-diastole respectively, and recorded pulse pressure (PP) (difference between systolic and diastolic BP).

- Compliance (mm^2 / mmHg) = A_{max} - A_{min} / PP
- Arterial strain (arbitrary units) = A_{max} - A_{min} / A_{min}
- Distensibility ($10^{-3}mmHg^{-1}$) = arterial strain / PP

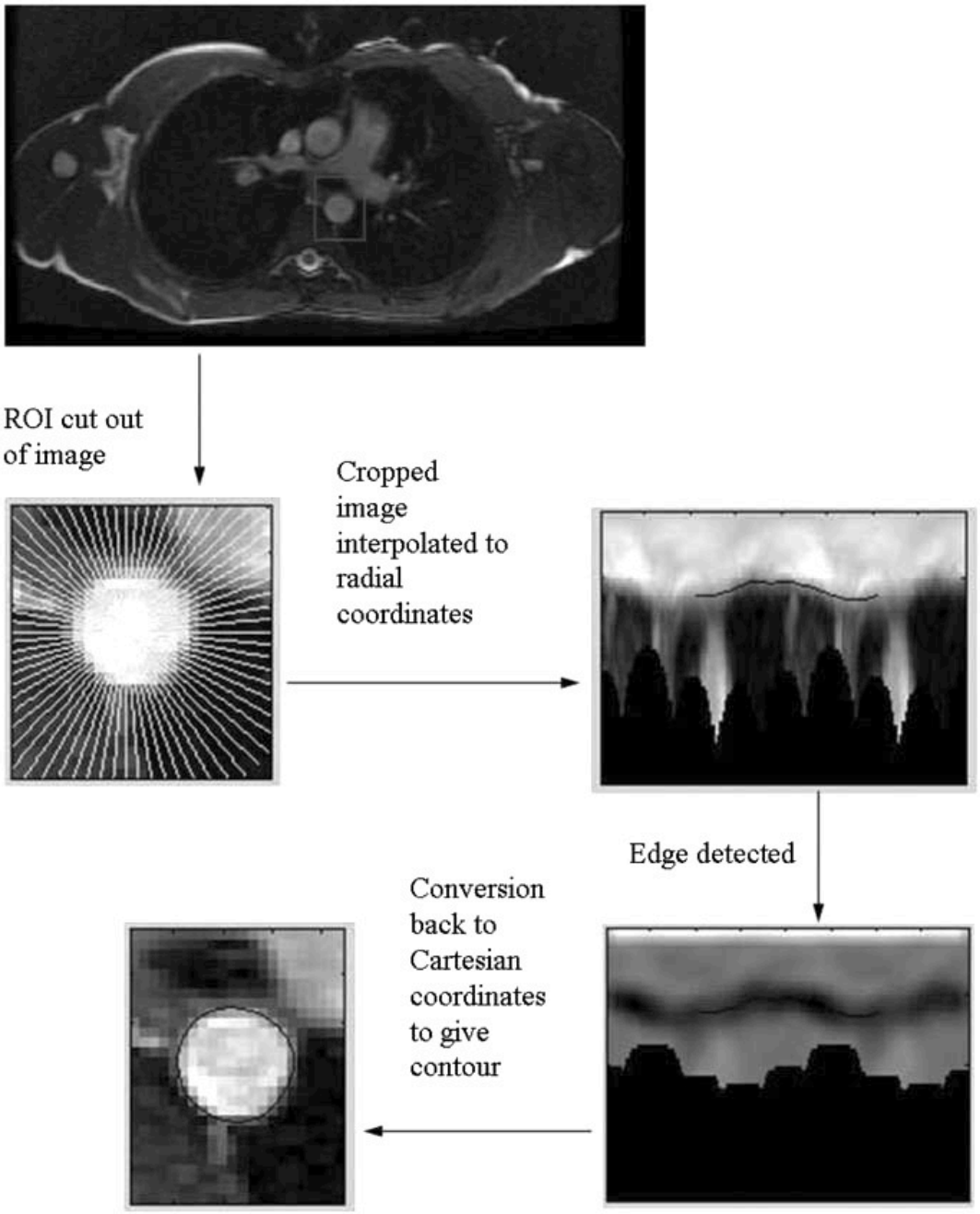

Figure 2.5 Automated segmentation method for assessing vessel lumen area – the processing sequence.

Reproduced from Jackson CE, Shirodaria CC, Lee JM, Francis JM, Choudhury RP, Channon KM, Noble JA, Neubauer S & Robson MD 2009. Reproducibility and accuracy of automated measurement for dynamic arterial lumen by cardiovascular magnetic resonance. *Int J Cardiovasc Imaging,* 25, 797-808 © Springer Science + Business Media, B.V, 2009, with kind permission from Springer Science and Business Media.

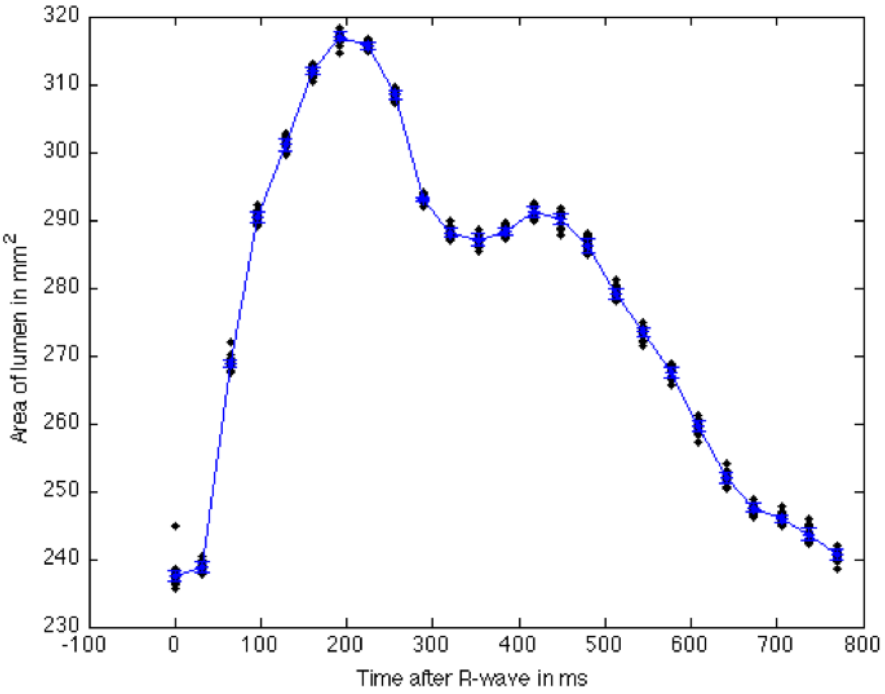

Figure 2.6 Change in aortic lumen area (mm^2) across 25 phases of the cardiac cycle. Minimum and maximum areas defined and used in conjunction with pulse pressure to derive strain, compliance and distensibility measures.

2.4.2 Cardiovascular structure and function

Analysis of right and left ventricular structure and function was performed using open source image analysis software (Osirix).

2.4.2.1 Left ventricular structure and function

The end-diastolic (ED) phase showing the largest cavity area, and the end-systolic (ES) phase showing the smallest cavity area were identified for the SAX images at each slice position. The most basal images were defined as the images at ED and ES with myocardium extending over at least 50% of the myocardial circumference (Papavassiliu et al., 2005).

Epicardial and endocardial borders at end-systole and end-diastole for each slice position were manually traced. The papillary muscles were included in the mass and excluded from the blood volume. LV volumes were calculated using Simpson's rule where the volume of a cavity is estimated by the sum of the cross-sectional area of multiple single slices multiplied by the slice thickness. Values were derived for EDV, ESV, EF, SV, CO, and myocardial mass (in systole

and diastole), and normalized to body surface area (BSA) (calculated using the Du Bois formula).

Stroke volume = end-diastolic volume – end-systolic volume

Cardiac output = stroke volume x heart rate

Ejection fraction = [(end-diastolic volume – end-systolic volume) / end-diastolic volume] x 100.

Myocardial mass = myocardial volume (difference between epicardial and endocardial volumes) x the specific density of myocardium (1.05g/ml).

2.4.2.2 Right ventricular structure and function

RV volumes were calculated from both the SAX and the transaxial datasets. Using Osirix image analysis software the endocardial borders of the RV were defined at end-systole and end-diastole on the SAX images as for the LV, and on the transaxial images at each slice position. Papillary muscles were include in the mass and excluded from the blood volume. Where the moderator band was prominent it was excluded from the RV volume and assigned to the myocardial mass.

2.5 Statistical Analysis

All statistical analyses were performed under the supervision and guidance of MRC LEU statisticians.

A generic power calculation was performed in order to determine the sample size required for MRI - statistical power for analyses of 250 children is set out below.

Exposures	**No.**	**Power, at p<0.05**
Continuous variables	250	80% power to detect a correlation coefficient of 0.18
Interactions	250	80% power to detect a difference in correlation between exposure & outcome of 0.18 per SD change in interaction variable

The SWS MRI cohort was a sub-cohort of the SWS cohort. In order to determine whether the initial MRI cohort was representative of the SWS cohort the MRI cohort was compared with a group represented by the SWS cohort of children born up to the end of 2003 (still births, neonatal deaths and intrauterine deaths were excluded). Similarly, the cardiac MRI cohort was compared with the total SWS birth cohort (still births, neonatal deaths and intrauterine deaths were excluded). For variables that were normally distributed a *t* test was used to compare the groups, a *Wilcoxon-Mann-Whitney* test was used for variables not normally distributed. Categorical variables were compared with a *Chi-square* test.

Outcome measures were changes in cardiovascular structure and function that reflect early cardiovascular pathology and CVD risk factors; specifically impaired vascular structure and function (greater PWV and reduced arterial distensibility), and altered cardiovascular structure (lower LV mass), and altered cardiovascular function (LV stroke volume, ejection fraction, end-diastolic and cardiac output). Measures of cardiovascular structure and function were normalised to body surface area to assess the influence of child's current size on findings.

Cardiovascular structure and function was related to maternal size and body composition, diet before and during pregnancy, measures of maternal health and stress, and child's size and body composition at birth and at 9 years of age.

Statistical analysis of the scan data was performed using STATA version 13.1 (STATA Statistical Software, STATA Corporation, College Station, Texas, USA). Descriptive statistics (measures of central tendency and variability, and/or frequency) were used to describe the characteristics of the study population. Histograms of outcome variables were checked to note normal or skewed distribution. Univariate linear regression analyses were performed to model the relationship between maternal and childhood characteristics and CV outcomes measured by MRI. Analyses were adjusted for child's sex. Additional adjustments were made for potential confounding factors.

A 'p' value of 0.05 or less was considered statistically significant.

3. Methods Work-up & Evaluation

3.1 Cardiac Structure and Function

CMR is an accurate and reproducible non-invasive imaging technique for the analysis of left ventricular volumes in adults but is less well validated in children. Imaging with MRI requires a constant trade-off between spatial resolution, signal-to-noise ratio (SNR) and image acquisition time, with any improvements to one factor resulting in a compromise of one or both of the other factors. Cardiac MRI has the additional challenge of being required to image a moving structure. Compliance with breath holding requirements is essential for the acquisition of high quality images and the subsequent derivation of accurate structural and functional measures.

There are limited data on CMR reproducibility in children - compliance with breath hold instructions and motion control can be significantly reduced compared with that in adults often resulting in motion artefact and respiratory misregistration with detrimental consequences for the accuracy of analysis. Respiratory misregistration is more frequently encountered when scanning children with breath hold sequences and can prove challenging when processing images for analysis. Further, various CMR analysis tools and segmentation methods are available but it is not clear which are most appropriate for use in children.

3.1.1 Image analysis tools and segmentation technique

Conventional manual segmentation tools require time-consuming contour tracing but allow some compensation for image misregistration. Semi-automated analysis packages for assessment of ventricular volumes have proven reliable in adults (Garciade-Pablo et al., 2005, Messali et al., 2009), but there are limited data on accuracy in children.

The semi-automated tool (Argus 4D, Siemens Healthcare) employs a heart model based algorithm with reported significantly reduced analysis times, however correction for the image misregistration more frequently seen in children is more difficult. The software generates a four-dimensional (4D) heart model following the identification of several anatomic landmarks on short and

long axis cine images of the heart: the central axis of the LV, the base plane, and the transition from the left to the right ventricle (RV) (Figure 3.1).

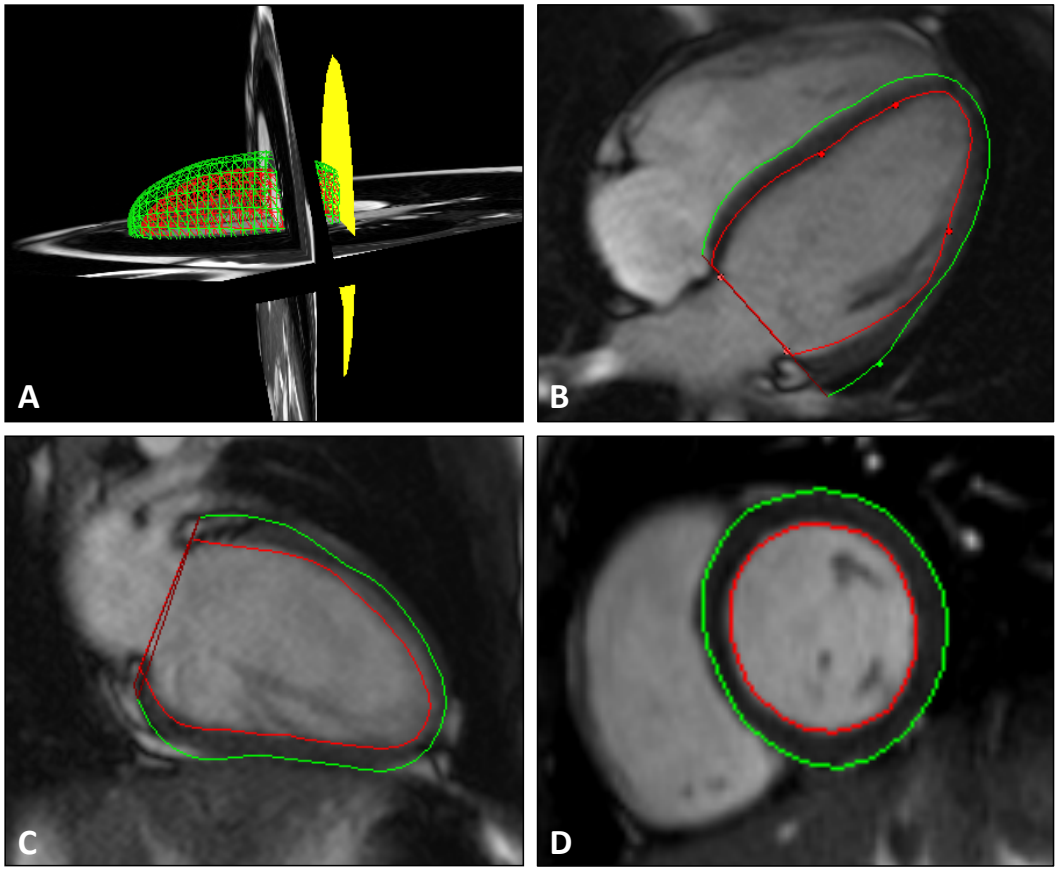

Figure 3.1 LV volumetric images produced with Argus 4 D following identification of key anatomical landmarks. 3 Dimensional map of LV mass and volume including the mitral valve plane (A), 2 dimensional images in 4-chamber (B), 2 chamber (C) and short axis (D) views.

LV analysis using manual segmentation methods involves tracing the epicardial and endocardial borders on end-diastolic and end-systolic SAX cine images at each slice position. Papillary muscles and trabeculae may be either included in the blood pool, or excluded from the blood pool and included in the myocardial volume (Figure 3.2).

Endocardial trabeculae significantly affect LV volume and mass quantification with the cumulative effect of papillary muscles and trabeculae on EF calculations reported at approximately 10% (Pednekar et al., 2006). Consistent

analysis in both diastole and systole can reduce the extent of overestimation. Papavassiliu et al. (2005) consider that the superior reproducibility of LV measurements with inclusion of endocardial trabeculae in the cavity warrants this method for clinical use. However, animal studies have shown close correlation between necropsy measurements and LV mass calculated with papillary muscles and trabeculae included in the myocardium – to within 2.1% (Fieno et al., 2002) and 1.2% of necropsy weights (Francois et al., 2004). Inclusion of the papillary muscles and trabeculae in the blood pool is reportedly more reproducible while exclusion from the blood pool is considered a more accurate segmentation method.

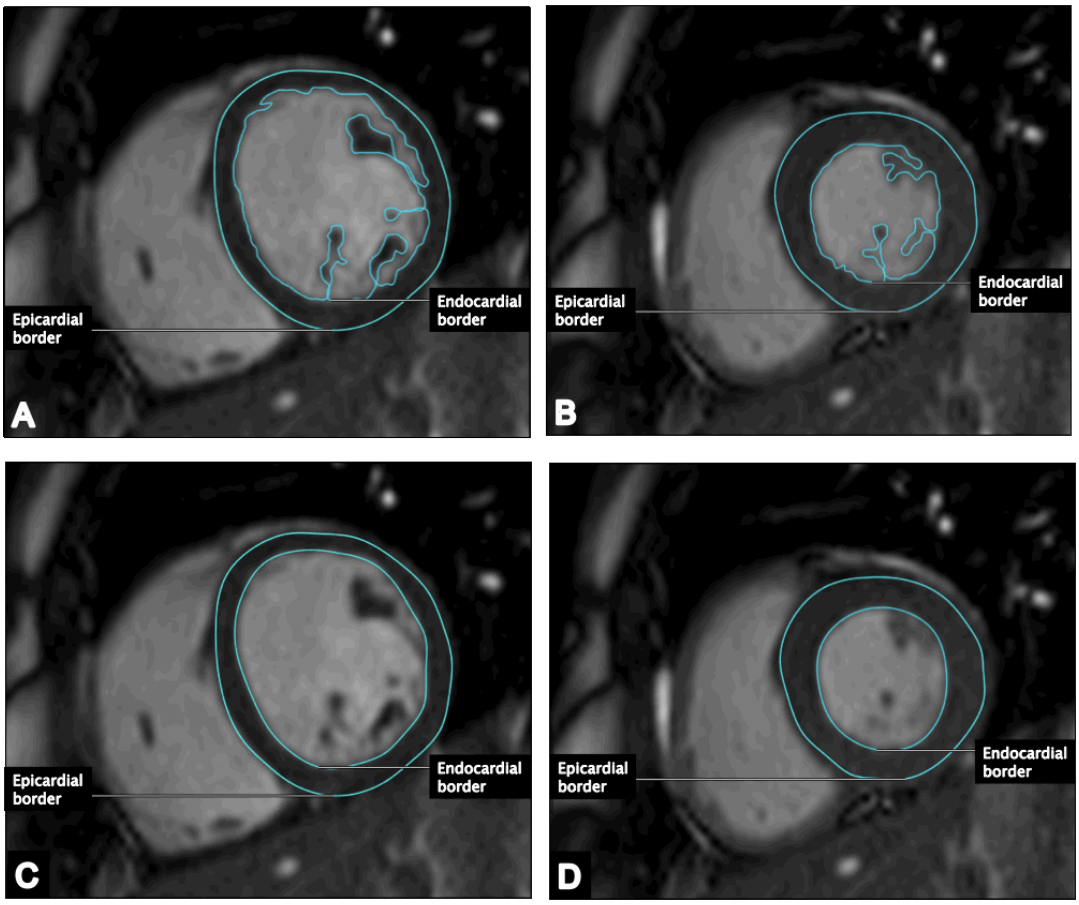

Figure 3.2 Short axis and mid ventricular SSFP images at end-diastole (A, C) and end-systole (B, D). Delineation of endocardial borders with papillary muscles and trabeculae excluded from the blood pool (A, B), included in the blood pool (C, D).

3.1.2 Inter-study reproducibility and segmentation techniques

In order to determine the inter-study reproducibility of CMR LV measurements in children and examine the influence of analysis tools and techniques, LV cine images were repeated in 10 SWS participants. LV SAX cine images were acquired twice within the same examination with a short interval between.

3.1.2.1 Inter-study variability and segmentation technique

Inter-study variability and segmentation technique were assessed on the datasets analysed using Osirix software with a manual post processing technique. Each dataset was analysed with the papillary muscles a) included, and b) excluded from the blood pool (Figure 3.2). Intra- and inter-observer reproducibility was assessed on datasets from 5 of the children.

Inter-study coefficients of variation (CVs) were similar for measurements including or excluding the papillary muscles and trabeculae from the blood pool, though marginally smaller for the latter (Table 3.1). CVs for measurements excluding the papillary muscles and trabeculae from the blood pool were generally higher than those reported from studies of adults (CVs for EDV, ESV, SV, EF, CO, ESM (end-systolic mass), and EDM (end diastolic mass) were 8%, 10.9%, 9.7%, 4.4%, 10.5%, 10.7%, and 8.6% respectively).

CMR measurements tend to be less reproducible in children than in adults, most likely due to difficulty in following breath hold instructions. However, for all parameters (end-diastolic volume, end-systolic volume, stroke volume, ejection fraction, cardiac output, end-systolic mass and end-diastolic mass) the variability between subjects was greater than the inter-study and inter-observer variability combined. For example, looking at cardiac output - standard deviations were 1.83 for between subject variability and 0.42 for inter-study variability (Table 3.1), indicating that useful measurements can be obtained for research studies. The intra- and inter-observer coefficient of variation was in the range 2% - 19% for EDV, ESV, EDM and ESM.

Table 3.1 Standard deviation and coefficients of variation for left ventricular parameters within child and between children as determined by image analysis excluding and including papillary muscles and trabeculae.

ANOVA		**Excluding Papillary Muscles**		**Including Papillary Muscles**	
		Standard Deviation	Interstudy coefficient of variation	Standard Deviation	Interstudy coefficient of variation
LVEDV	Between children	7.07	10.2%	6.83	8.8%
	Within child	5.54	8.0%	6.36	8.2%
LVESV	Between children	6.17	26.8%	7.09	24.7%
	Within child	2.51	10.9%	3.33	11.6%
LVSV	Between children	11.10	24.1%	11.50	23.6%
	Within child	4.45	9.7%	5.16	10.6%
LVEF	Between children	10.53	15.8%	10.80	17.2%
	Within child	2.91	4.4%	3.49	5.6%
LVCO	Between children	1.83	46.3%	2.21	52.7%
	Within child	0.42	10.5%	0.45	10.8%
LVESM	Between children	12.63	23.9%	9.72	21.5%
	Within child	5.64	10.7%	5.12	11.3%
LVEDM	Between children	8.09	14.8%	5.88	12.6%
	Within child	4.69	8.6%	5.54	11.9%

3.1.2.2 Analysis tool and segmentation technique

LV volume and LV mass analysis were repeated on all datasets using the semi-automated Argus 4D technique, which included the papillary muscles and trabeculae in the blood pool. This technique was compared with the manual Osirix method (including papillary muscles and trabeculae in the blood pool to permit an accurate comparison). LV stroke volumes from each technique were

compared with aortic flow data derived from aortic root phase contrast velocity flow mapping sequences (analysed with Siemens Argus software).

Using the Bland Altman method (Bland and Altman, 1986), the mean difference in SVs for Osirix and Argus 4D was 7ml, with Argus 4D generally measuring larger values. The estimated coefficient of variation for SV measurements calculated using Osirix was lower than that using Argus 4D (10.6% vs. 13.1%). Mean differences between the Osirix and Argus 4D SVs, and aortic flow SV measurements were 1.4ml and 5.7ml respectively (Figure 3.3).

Based on these results, LV analysis was performed in this study using the Osirix manual technique with the papillary muscles and trabeculae excluded from the blood pool (as the more accurate technique). SV measurements were more reproducible using the Osirix manual technique when compared with the semi-automated tool. Osirix derived SVs were more accurate than Argus 4D derived SVs when compared with the aortic phase contrast derived flow data. Although both manual-tracing techniques showed similar repeatability, it was prudent to adopt the reportedly more accurate technique of excluding the papillary muscles and trabeculae from the blood pool for LV and RV analysis in this study.

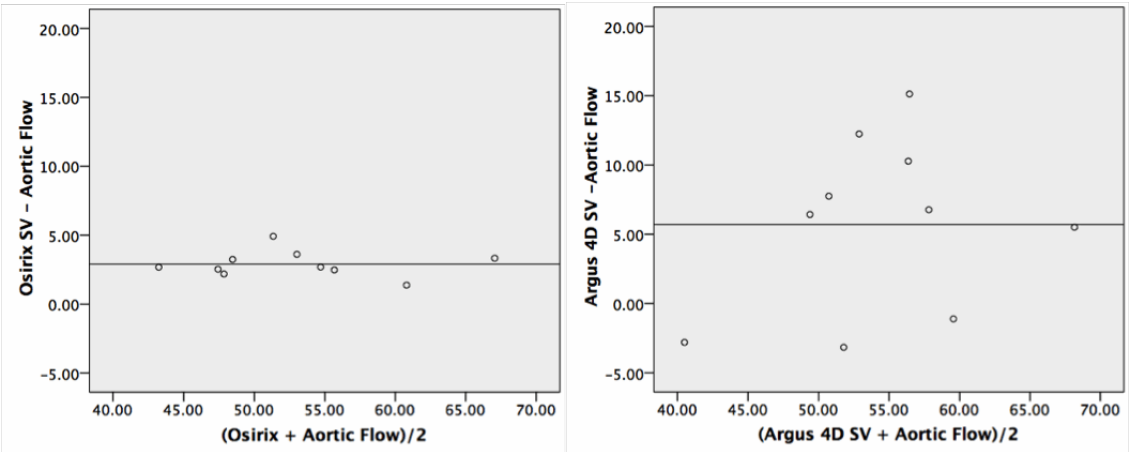

Figure 3.3 Bland-Altman plots showing comparisons of manual Osirix (left) and semi automated Argus 4D (right) derived left ventricular stroke volumes (SV), to Argus flow measurements.

3.1.3 Accuracy of LV and RV functional assessment

A manual segmentation technique with exclusion of papillary muscles and trabeculae from the blood pool was determined the most appropriate method of image analysis for this study, with good intra and inter-observer agreement.

As previously mentioned, image quality in children is reliant on compliance with motion control and breath hold instructions. Inability to maintain repeated breath holds in a consistent phase of respiration can result in respiratory misregistration errors. CMR in children is prone to image misregistration due to less consistent breath holding which may be more problematic when imaging in the transaxial plane. In adults right ventricular (RV) measurements from datasets acquired in the transaxial orientation have been found to be more accurate and reproducible than in the SAX plane.

Sequences in this study were optimised to minimise scan acquisition times and required breath hold duration. As previously mentioned this resulted in compromised image quality. As there are limited data on the accuracy of CMR in children it was necessary to assess the accuracy of LV and RV functional assessments from multi-slice SSFP cine imaging. In adults quantification of RV function from images acquired in the transaxial plane is more accurate and reliable than from SAX imaging. Imaging in children is more susceptible to respiratory misregistration, more problematic when acquiring sequential images in the transaxial plane. It was important to determine the most accurate method for RV volume evaluation in children.

3.1.3.1 Accuracy of LV and RV function

In order to determine the accuracy of CMR measurements, LV and RV SVs measured from SSFP images acquired in the SAX plane were compared with flow data derived from the phase contrast velocity flow mapping sequences through the aortic root and the main pulmonary artery respectively.

There were good correlations between aortic flow and LVSV ($r=0.69$, $p<0.0001$, $n=198$), and between pulmonary flow volume and RVSV ($r=0.64$, $p<0.0001$, $n=189$). Using the Bland Altman method (Bland and Altman, 1986) the mean difference between LVSV and aortic flow was 10ml (95%CI 9 to 11ml). Mean difference between RVSV and MPA flow was 12ml (95%CI 10.8 to 13.2ml). There was a strong correlation between aortic and pulmonary flow volumes

(r=0.87, p<0.0001, n=198). The mean difference between aortic and MPA flow volumes was 3.4ml.

3.1.3.2 Accuracy of RV volume evaluation

Contiguous steady state free precession breath hold cine images were obtained to cover the RV in both SAX and transaxial planes. In 189 children RV stroke volumes were compared with flow data derived from phase contrast velocity flow mapping sequences through the main pulmonary artery (MPA).

Mean (SD) SAX RVSV and transaxial RVSV were 48.1ml (10.16) and 51.1ml (10.23) respectively. There was a good correlation between SAX RVSV and MPA Flow volumes (r=0.64, p<0.0001, n=189), but a less strong correlation between transaxial RVSV and MPA flow volumes (r=0.53, p<0.0001, n=189) (Figure 3.4). Using a Bland Altman analysis the mean difference between SAXRVSV and MPA flow volumes was 12ml (95%CI 10.8 to 13.2). Mean difference between transaxial RVSV and MPA flow volumes was 8.72ml (95%CI 6.73 to 9.82). Measured transaxial RVSV was on average 3.76ml (95%CI -5.1 to -2.3ml) greater than SAX RVSV. There was a good correlation between SAX and transaxial RVSV (r=0.63, p<0.0001, n=216).

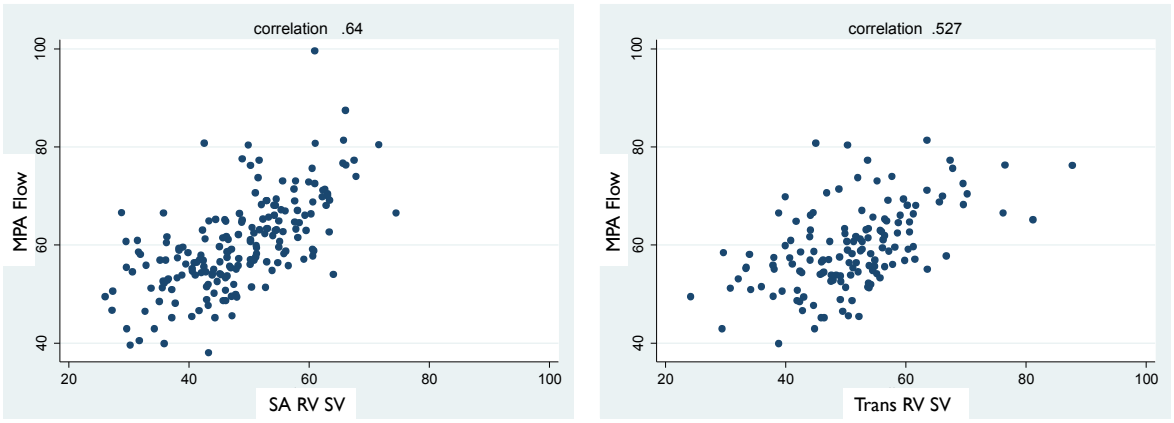

Figure 3.4 Good correlation between short axis derived right ventricular stroke volumes (SA RVSV) and MPA flow volumes (left). A weaker correlation between transverse derived right ventricular stroke volumes (Trans RVSV) and MPA flow volumes (right).

3.2 Vascular Structure and Function

Measures of vascular stiffness (PWV, arterial distensibility, compliance and strain) were performed using magnetic resonance imaging. These techniques are well-established non-invasive measures of arterial stiffness and are well validated against ultrasound and gold standard invasive pressure measurements acquired in the ascending, proximal descending, and abdominal aorta (Grotenhuis et al., 2009, Leeson et al., 2006). PWV is the most validated of CMR methods used to quantify arterial stiffness (Kawel-Boehm et al., 2015). MRI permits a direct and accurate measurement of aortic length whereas tonometry and ultrasound, well-established non-invasive methods of quantifying global vascular function, measure distance on the body surface so provide an *estimation* of aortic PWV (Dogui et al., 2011). MRI quantification of PWV is well validated against both intra-aortic pressure measurements (Grotenhuis et al., 2009) and ultrasound. Leeson et al. (2006) found MRI PWV to be the most reproducible measurement over time when compared with ultrasound assessment of PWV.

3.2.1 Distensibility - aortic root phase contrast flow acquisition

An alternative method for assessing aortic root distensibility was considered using data from the aortic root phase contrast flow sequence acquired for the PWV measurements outlined in section 2.3.1.1. The free breathing flow sequence was not reliant on breath hold compliance whereas the high resolution SSFP cine acquisition was susceptible to respiratory motion.

Flow data were assessed using Osirix imaging software. The vessel lumen was traced manually on magnitude images across the cardiac cycle and ROIs propagated to the phase images. Flow velocity curves were generated. Aortic resistance and compliance were calculated (using an Osirix plugin) from the flow data and systolic, diastolic and mean arterial pressures measured immediately following the flow acquisition.

Unfortunately, mean arterial pressure (MAP) was not recorded for the first 138 children scanned. MAP has been estimated from the SBP, and DBP for all 196 children, and measured for those following the 138th child.

Estimation of MAP was determined as: MAP = (SBP/3) + 2x(DBP/3)

The estimation of MAP, and associated assumptions about aortic compliance, brings into question the validity of this technique. However, a Bland Altman analysis (Bland and Altman, 1986) showed good agreement between MAP(E) and MAP(M) with a mean difference of 2.47ml, with MAP(M) generally measuring larger values (Figure 3.5).

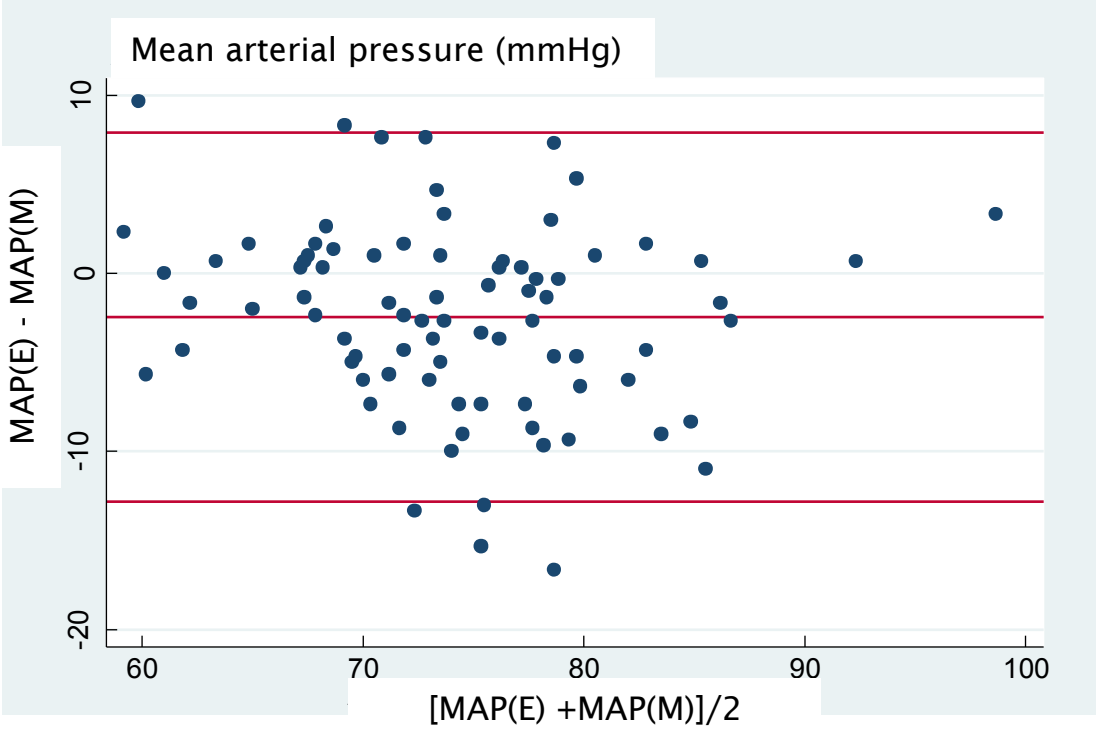

Figure 3.5 Bland Altman plot showing comparison of measured [MAP(M)] and estimated [MAP(E)] mean arterial pressure. The mean difference was 2.47ml with MAP(M) measuring higher. 95% limits of agreement (-12.8, 7.9).

When compared to the high resolution breath hold SSFP cine imaging acquired for assessment of distensibility, the free-breathing flow imaging acquired over approximately 2 minutes is of much lower spatial and temporal resolution, and whist not dependent on arrested respiration was more susceptible to motion artifact, and subject to manual tracing error.

Due to the poor spatial resolution of these images measurement of the vessel boundaries was deemed inaccurate and measurement of distensibility was therefore performed using the aforementioned automated edge definition analysis technique (section 2.3.1.2).

3.2.1.1 Pulse wave velocity

As detailed in methods section 2.3.1.1 phase contrast flow mapping sequence were acquired at three sites; ascending aorta, proximal and distal descending aorta with the intention of measuring PWV across three lengths of the aorta:

- The aortic arch
- The descending aorta
- The entire aorta (from aortic root to just above the bifurcation)

As previously mentioned accurate measure of distance between sites is crucial to the accuracy of derived PWV measures. The ability to directly measure distance between flow measurement sites with MRI is a distinct advantage of MRI over ultrasound PWV assessment where the distance between the two points is estimated.

It proved difficult to reliably measure the distance across the aortic arch (between the ascending aorta and descending aortic flow positions) due to the mobility of the proximal aorta throughout the cardiac cycle. Accuracy of PWV values is dependent on both the algorithm employed for the detection of the 'foot of the wave', and accurate path length measurement.

Regional PWV measured in the descending aorta has been shown to correlate more strongly with gold standard invasive PWV measurements than both measurements across the arch, or global measurement along the total distance of the aorta (Grotenhuis et al., 2009, Leeson et al., 2006). This may be as a result of the difficulty in obtaining accurate distance measurements across the mobile aortic arch with MRI. Fatty streaks – precursor to atheromatous changes are more likely to appear in the abdominal aorta (Lewandowski et al., 2011). Measures of PWV acquired across the descending aorta were therefore investigated in this study.

3.2.2 Coronary artery imaging

The aim of imaging the coronary arteries was to obtain a high-resolution isotropic three-dimensional (3D) image dataset, which could be manipulated following acquisition to produce cross sectional images of the coronary artery origins from which measurements could be made.

3.2.2.1 Image acquisition

In order to determine the time period of minimal coronary artery motion during the cardiac cycle, a real time 20-second free breathing sequence was acquired in the coronal plane positioned through the aortic root on an axial HASTE (half acquisition single shot turbo spin echo) image. Sequence parameters: TR/TE 57.5/1.15ms, FOV 250mm, flip angle 69º, slice thickness 7mm, voxel size 1.0x1.0x0.8mm.

A free breathing ECG triggered respiratory navigator-gated 3D whole heart TrueFISP sequence was employed for coronary artery imaging. Two vertical two-dimensional selective real-time navigator beams were positioned through the dome of the right hemi-diaphragm on coronal and axial scout views with a gating window at end expiration of 5mm. The 3D slab was angled perpendicular to the aortic root and positioned to cover the coronary artery origins. The period of minimal cardiac motion was determined (usually in late diastole) and the trigger window adjusted accordingly in order to acquire data during this time period only (when synchronised with expiration). The child was instructed to remain still and to breathe normally.

Coronary artery imaging has proven unsuccessful. Performed towards the end of the imaging protocol after approximately 45 minutes in the scanner, the sequence was dependent on regular respiration, and the child remaining completely still. The rapid heart rates of the children resulted in long scan times required to obtain the full coronary data sets, and difficulty determining period of minimal cardiac motion.

Coronary data were acquired on the first 39 children to complete the imaging protocol. On subjective assessment, optimal images were obtained in 27 out of the 39 coronary acquisitions (12 were non-diagnostic due to poor image quality; 11 due to motion degradation, and one as a result of poor positioning of the volume slab due to movement of the child). However, due to the small

size of the coronary arteries in the children the images were not of sufficiently high resolution to permit the accurate measurement of coronary artery dimensions. Following 3D reconstruction the coronary artery diameter covered only 2-4 pixels. Accurate vessel lumen determination and subsequent measures of coronary dimensions were therefore not possible.

Due to the small calibre of the coronary vessels, difficulties in suppressing cardiac and respiratory motion, limitations on spatial resolution on the 1.5T magnet, and physical motion of the child, coronary artery imaging was discontinued.

3.3 Conclusion – Study Technique

3.3.1 Cardiovascular structure and function

An achievable protocol was developed for the assessment of left and right ventricular structure and function in children and was demonstrated to be both repeatable and reliable. Although CMR measurements of cardiac structure tend to be less reproducible in children than in adults, between-subject variability in the SWS cohort was greater than inter-study and inter-observer variability, indicating that useful measurements could be obtained for this study.

A manual segmentation technique using open source image analysis software Osirix was found to be more reliable than semi-automated techniques.

Left and right ventricular stroke volumes were compared with aortic and main pulmonary artery flow derived stroke volumes respectively. Left ventricular stroke volumes derived using the manual segmentation technique were most accurate when compared with aortic phase contrast derived flow data.

In children RVSV analysis in the SAX plane correlates more closely with MPA flow derived SVs than those from the transaxial plane. This is likely to be due to greater respiratory misregistration error in the transaxial plane. One limitation of the study is that the MPA flow was obtained immediately after the SAX stack, whereas the transaxial stack was acquired much later in the protocol. RVSV measurements derived from the transaxial stack were potentially influenced by changes in heart rate, and motion and respiratory artefact as a result of reduced compliance with consistent breath holding due

to fatigue. Both RV and LV measurements can be obtained from a single short SAX stack, reducing the total imaging time for children of this age for research studies.

Children at the age of 9 years are able to comply with multiple breath hold requirements, allowing accurate assessment of cardiovascular function using steady state free precession CMR. Although less accurate than in adults, there was a good correlation between flow derived SVs and those derived from the SAX cine stack. Therefore useful measurements can be obtained at this age for research studies.

4. Results – Vascular Structure & Function

4.1 Background

MRI measures of arterial stiffness; aortic PWV and aortic distensibility were acquired using the techniques outlined in Chapters 2 and 3. The relationship between developmental influences (maternal, and childhood) and arterial stiffness (PWV measured in the descending aorta, and aortic root distensibility) at 9 years of age are presented in this chapter.

255 SWS children (127 female, 128 male) attended for MRI scans between May 2009 and February 2012. Four children declined to have the MRI scan at the appointment; seven proved claustrophobic and were unable to tolerate either the noise or the confined environment, as a result of which no imaging was performed beyond the basic scout views. In total 244 children completed all, or part of, the vascular imaging protocol (dependent on tolerance, attention span, ability to keep still, and level of compliance with breath holding requirements).

Table 4.1 shows the descriptive statistics of the MRI cohort for maternal and infant characteristics. A formal comparison was made between the MRI sub cohort and the SWS cohort of children born up to the end of 2003 (still births, neonatal deaths and intrauterine deaths have been excluded). For variables that were normally distributed (birth weight and placental weight) a *t* test was used to compare the two groups. Otherwise a *Wilcoxon-Mann-Whitney* test was used. The two cohorts were very similar in terms of maternal educational attainment, social class, age, diet and smoking status, and child's birth weight, although the mothers in the MRI cohort were more likely to be nulliparous. Characteristics of the study population at MRI are presented in Table 4.2. Haemodynamic measurements acquired following acquisition of PWV and distensibility data are presented in Table 4.3.

Characteristic	MRI Cohort		SWS Birth Cohort		p-value
	n	Median (IQR) Mean (SD)*	n	Median (IQR) Mean (SD)*	
Pre-pregnancy smoking status, n (%)					
Smoker	70 (28)		485 (28.1)		0.97
Non-smoker	180 (72)		1239 (71.9)		
Parity, n (%)					
0	130 (52)		752 (43.6)		0.01
1+	120 (48)		972 (56.4)		
Age at child birth (years)	250	30.1(3.7)*	1723	30.1(3.8)*	0.96
Infant at birth					
Birth weight (kg)	247	3.4(0.6)*	1707	3.5(0.6)*	0.47
Placental weight (g)	229	484.2(117)*	1558	479.8(110.4)*	0.58

Table 4.1 Characteristics of the MRI cohort study population and the SWS birth cohort (maternal, and infant at birth). Formal comparison of MRI sub cohort with that of SWS birth cohort of children born up to the end of 2003 (still births, neonatal deaths and intrauterine deaths excluded).

Characteristic	MRI Cohort		SWS Birth Cohort		p-value
	n	Median (IQR) Mean (SD)*	n	Median (IQR) Mean (SD)*	
Maternal					
BMI (kg/m^2)	250	24.2(22.3,27.7)	1709	24.3(22.0, 27.7)	0.64
Early pregnancy: oily fish (portions/week)	205	0.5(0.1, 1.5)	1338	0.5(0.1, 1.5)	0.99
Late pregnancy: oily fish (portions/week)	240	0.5(0.3,1.5)	1647	0.5(0.0, 1.5)	0.11
Ethnic origin, n (%)					
White	241 (96.4)		1626 (94.3)		0.16
Other	9 (3.6)		99 (5.7)		
Social class, n (%)					
Professional/Management/Technical	110 (44.9)		643 (38.6)		0.12
Skilled non-manual/manual	107 (43.7)		779 (46.7)		
Partly skilled/unskilled	28 (11.4)		246 (14.8)		
Qualification level, n (%)					
None/CSE	30 (12)		247 (14.4)		0.28
Secondary education	140 (56)		999 (58.1)		
HND or degree	80 (32)		474 (27.6)		

Table 4.2 Characteristics of the study population: child at 9 year follow up.

Characteristic	n	Median (IQR) / *Mean (SD)
Oily fish (portions/week)	235	0.5 (0,1)
Age (years)	250	9.4 (9.3,9.6)
Height (cm)	235	136.3 (6.3)*
Weight (kg)	235	32.3 (29.2,37.4)
BMI (kg/m^2)	235	17.3 (15.9,19.2)
Pulse wave velocity (m/s)	234	3.5 (0.51)*
Maximum aortic area (mm^2)	207	331.0 (291.9,373.1)
Minimum aortic area (mm^2)	207	228.2 (201.2,259.2)
Distensibility ($10^{-3}mmHg^{-1}$)	207	11.1 (8.5,13.8)
Compliance ($mm^2/mmHg$)	207	2.46 (2.0,3.2)
Strain (Arbitrary Units)	207	0.43 (0.4,0.5)

Table 4.3 Haemodynamic measurements at time of PWV and distensibility measurement by MRI.

	Pulse wave velocity (m/s)		**Aortic distensibility ($10^{-3}mmHg^{-1}$)**	
Characteristic	n	Median (IQR) *Mean (SD)	n	Median (IQR) *Mean (SD)
Systolic blood pressure (mmHg)	233	99.4 (9.2)*	221	98.5 (8.7)*
Diastolic blood pressure (mmHg)	233	59.1 (7.5)*	221	58.4 (7.2)*
Pulse pressure (mmHg)	233	40.3 (8.6)*	210	40.2 (9.2)*
Mean arterial pressure (measured) (mmHg)	90	76.9 (9.0)*	87	75.3 (8.1)*
Mean arterial pressure (estimated) (mmHg)	233	72.0 (68.0,76.0)	213	71.3 (67.3,76.3)
Heart rate (bpm)	234	81.2 (74.8,88.0)	212	79.0 (71.2,85.1)

4.2 Pulse Wave Velocity

PWV was measured on 235 (117 male and 118 female) of the 244 children who were imaged. Nine children either did not complete the imaging protocol or image quality was substandard due to motion, and accurate PWV analysis was not possible. One extreme outlier, attributed to measurement error, was subsequently eliminated from the analysis. Mean PWV was 3.5 m/s (standard deviation 0.51 m/s). PWV values were within ranges previously reported for children in this age group using cfPWV applanation tonometry (Reusz et al., 2010). Univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to PWV (adjusted for sex) are presented in Table 4.4.

Table 4.4 Univariate regression analyses of childhood and maternal characteristics in relation to pulse wave velocity (m/s) at age 9 years (adjusted for sex of child).

Exposure	n	β	95%CI	p-value
Maternal characteristics				
Early pregnancy: oily fish (portions per week)	191	-0.062	-0.124, -0.001	0.046
Late pregnancy: oily fish (portions per week)	226	-0.084	-0.138, -0.031	0.002
Social class*	229	0.109	0.011, 0.207	0.03
Educational attainment*	234	-0.175	-0.278, -0.072	0.001
Parity (primiparous vs. multiparous)	234	-0.026	-0.158, 0.107	0.7
Smoking (non-smoker vs. current smoker)	234	0.057	-0.092, 0.206	0.5
Infancy characteristics				
Birth weight (kg)	231	0.031	-0.09, 0.152	0.6
Breastfeeding duration (months)	226	-0.015	-0.027, -0.003	0.015
Child's characteristics				
Height (cm)	234	0.007	-0.004, 0.017	0.2
Weight (kg)	234	0.008	-0.002, 0.018	0.1
BMI (kg/m^2)	234	0.014	-0.010, 0.039	0.3
Oily fish consumption (portions/week)	234	-0.037	-0.111, 0.037	0.3
Child's cardiovascular measures				
Systolic BP (mmHg)	232	0.011	0.004, 0.019	0.002
Diastolic BP (mmHg)	232	0.018	0.009, 0.026	<0.001
Pulse pressure (mmHg)	232	-0.0003	-0.008, 0.007	0.9
Mean arterial pressure (mmHg)	232	0.020	0.011, 0.029	<0.001
Heart rate (beats/minute)	234	0.015	0.009, 0.021	<0.001

* 3 groups high-low

4.2.1 Maternal influences

Taking account of child's sex, higher maternal oily fish consumption in late pregnancy was associated with lower MRI aortic PWV (lower aortic stiffness) in childhood (β = -0.084 (m/s)/ (portions/week), 95% confidence interval -0.137 to -0.031, p = 0.002) (Figure 4.1).

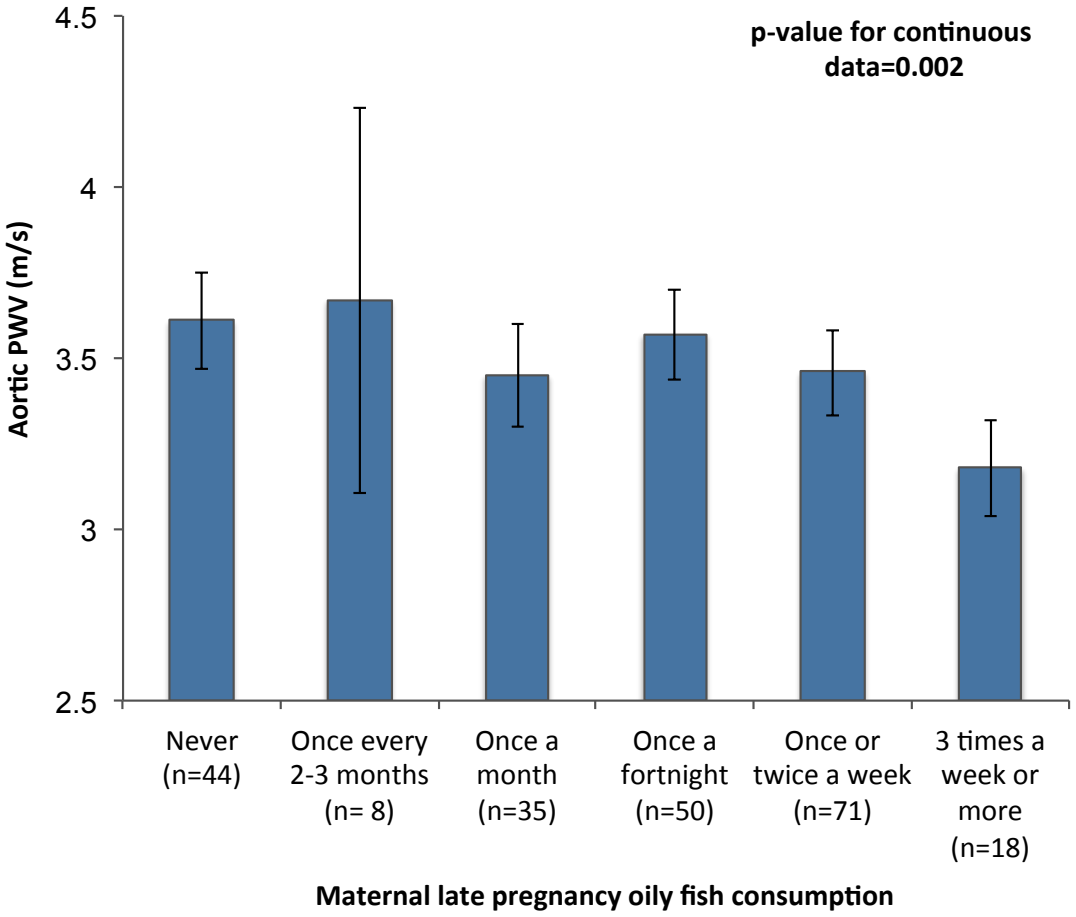

Figure 4.1 Maternal late pregnancy oily fish consumption in relation to child's pulse wave velocity (PWV) at age 9 years (taking account of child's sex). Values are means and SEM (Circulation Research: February 2015 – Volume 116 – p1203 Wolters Kluwer Health Lippincott Williams & Wilkins©).

There was a similar but weaker association with early pregnancy oily fish consumption (β = -0.062 (m/s) / (portions/week), 95% confidence interval -0.124 to -0.001, p = 0.046). There was no association with the child's current oily fish consumption. Relating childhood PWV to maternal factors there were

no associations with maternal smoking, age, height, or fatness. Increased duration of breastfeeding was associated with lower PWV (lower aortic stiffness) (β = -0.015 (m/s) / month, 95% confidence interval -0.028 to -0-002, p= 0.026).

Lower educational attainment was associated with higher PWV (increased aortic stiffness) (β = -0.075 (m/s) / level, 95% confidence interval -0.122 to -0.028, p=0.002) (Figure 4.2). The strength of the association between late pregnancy maternal oily fish consumption and PWV remained after a multivariate analysis of mother's qualifications and late pregnancy oily fish consumption versus PWV (oily fish p<0.002, mother's qualifications p=0.019) after inclusion of both social class and educational attainment in the regression model (Table 4.5).

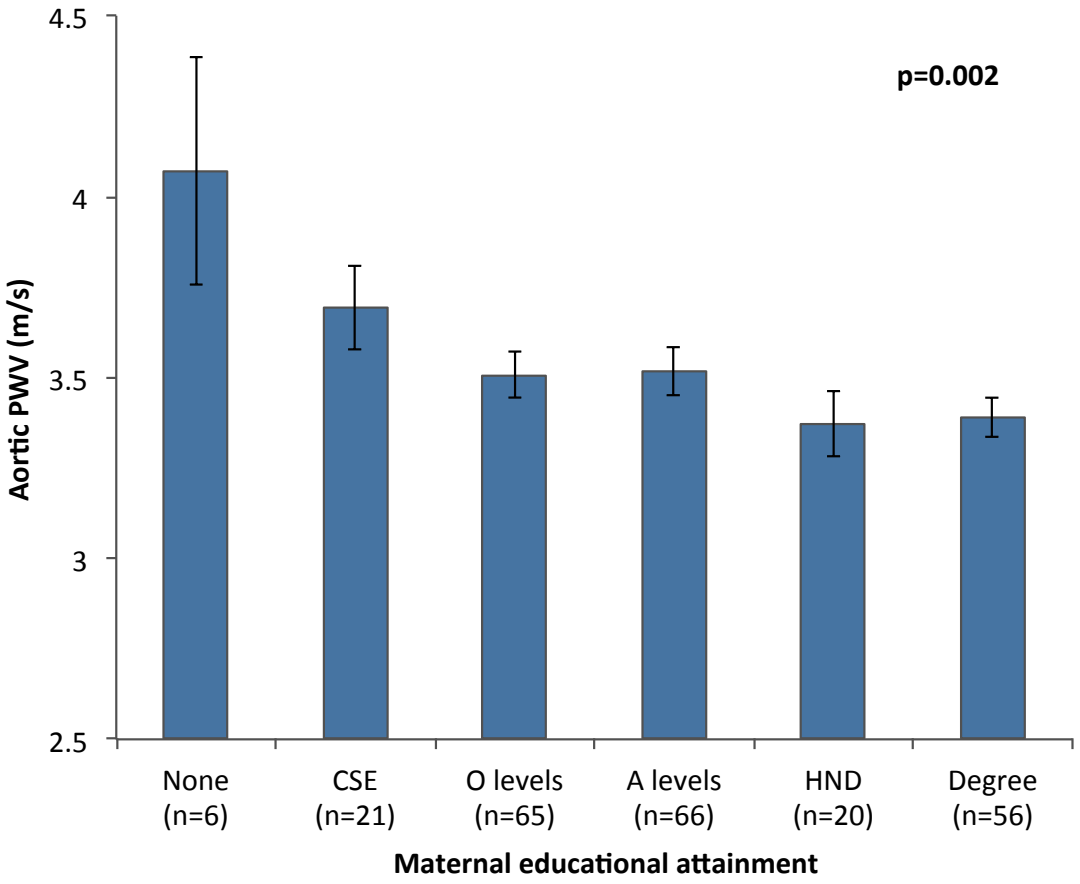

Figure 4.2 Maternal educational attainment in relation to child's pulse wave velocity (PWV) measured at 9 years. Values are means and SEM.

Table 4.5 Multivariate analyses of maternal, neonatal and childhood characteristics in relation to PWV (m/s) at age 9 years (n=214).

	Coefficient	95% CI	p-value
Maternal characteristics			
Late pregnancy: oily fish (portions/week)	-0.083	-0.136, -0.030	0.002
Social class*	-0.031	-0.150, 0.088	0.611
Educational attainment*	-0.155	-0.285, -0.026	0.019
BMI (kg/m^2)	-0.009	-0.023, 0.004	0.179
Infancy characteristics			
Breastfeeding duration (months)	-0.008	-0.021, 0.006	0.258
Child's characteristics			
Sex (reference category, male)	-0.066	-0.196, 0.063	0.314

*3 groups high-low

4.2.2 Childhood – cardiovascular measures

Although PWV was significantly associated with childhood systolic and diastolic BP, MAP, heart rate, duration of breastfeeding and mother's social class (Table 4.4), maternal oily fish consumption was not associated with childhood heart rate or BP when adjusted for sex of the child and mother's qualification level (Table 4.6).

4.2.3 Childhood – size and body composition

There were no statistically significant associations between size at birth or at 9 years and childhood PWV (p=0.62 and p=0.25, respectively).

Table 4.6 Regression analyses of mother's oily fish consumption in relation to childhood haemodynamic measures at time of MRI.

	n	Coefficient	95% CI	p-value
Heart rate (beats per minute)*	227	-0.644	-1.59, 0.3	0.179
Systolic blood pressure (mmHg)*	225	-0.466	-1.52, 0.59	0.385
Diastolic blood pressure (mmHg)*	225	-0.251	-0.95, 0.45	0.481
Mean arterial pressure (mmHg)*	225	-0.323	-1.01, 0.37	0.357
Heart rate (beats per minute)**	227	-0.584	-1.53, 0.36	0.224
Systolic blood pressure (mmHg)**	225	-0.405	-1.45, 0.64	0.446
Diastolic blood pressure (mmHg)**	225	-0.187	-0.86, 0.48	0.583
Mean arterial pressure (mmHg)**	225	-0.259	-0.92, 0.40	0.442

*Adjusted for sex of child

** Adjusted for mother's qualification level

4.3 Aortic area, distensibility, strain and compliance

Arterial distensibility, strain and compliance were measured across the aortic root in 207 of the 244 children who were imaged. 37 children either did not complete the imaging protocol to this stage or image quality was substandard (due to motion or inability to maintain the breath hold required to obtain the high resolution scan) rendering accurate measurement of lumen area impossible. One child declined BP measurement therefore distensibility and compliance calculations were not possible. Values for aortic area and distensibility were similar to those previously reported for children at this age measured using MRI (Voges et al., 2012). Measures of aortic area, distensibility, strain and compliance at the level of the aortic root are presented in Table 4.2.

Univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to aortic root distensibility (adjusted for sex) are presented in Table 4.7.

Table 4.7 Univariate regression analyses of childhood and maternal characteristics in relation to aortic distensibility (10^{-3}mmHg^{-1}) at age 9 years.

	n	Coefficient	95% CI	p-value
Maternal Characteristics				
Late pregnancy: oily fish (portions/week)	200	0.011	-0.028, 0.051	0.568
Late pregnancy serum Retinol (Vitamin A) (umol/l)	164	0.1931	0.057, 0.329	0.006
Late pregnancy serum Vitamin D (nmol/l)	196	0.002	0.0004, 0.004	0.015
Late pregnancy serum Vitamin E (nmol/l)	164	0.002	-0.005, 0.008	0.583
Qualification level**	207	-0.011	-0.046, 0.024	0.546
Currently Smoking	202	0.058	-0.082, 0.199	0.414
Height (cm)	206	-0.004	-0.011, 0.004	0.346
Infancy characteristics				
Birth weight (g)*	205	-0.1	-0.0002, -0.00004	0.005
Placental weight (g)*	190	-0.5	-0.001, -0.0001	0.021
Ponderal index at birth (kg/m^3)*	200	-0.015	-0.033, 0.003	0.093
Child's characteristics				
Sex	207	0.18	0.088, 0.273	0.0002
Height at MRI (cm)*	205	-0.011	-0.018, -0.004	0.002
Weight at MRI (kg)*	205	-0.004	-0.012, 0.003	0.248
BMI (kg/m^2)*	205	0.004	-0.015, 0.023	0.671
SBP (mmHg)*	207	-0.013	-0.019, -0.008	<0.001
DBP (mmHg)*	207	0.012	0.006, 0.018	<0.001
Pulse Pressure (mmHg)*	207	-0.019	-0.025, -0-013	<0.001
Mean Arterial Pressure (mmHg)*	207	0.001	-0.005, 0.008	0.656
Heart Rate (beats per minute)*	205	-0.006	-0.010, -0.002	0.005

*Adjusted for sex of child

**3 levels low-high

4.3.1 Childhood – size and body composition

Univariate analyses of child's size at birth and at MRI, and aortic root size (adjusted for sex) are presented in Table 4.8. Aortic cross sectional areas were significantly associated with placental weight and birth weight, and child's height, weight and BMI at MRI. Arterial compliance was significantly associated with child's weight and BMI at MRI, and SBP, DBP and PP, and heart rate. Arterial strain was significantly related with placental weight, birth weight, child's height at MRI and PP. Distensibility was significantly associated with placental weight, child's height at MRI, BP measures (SBP, DBP and PP), and heart rate.

Table 4.8 Univariate regression analyses of child's size at birth and at MRI in relation to aortic cross sectional area (mm^2) at end-diastole (A_{min}) and end-systole (A_{max}) at age 9 years.

		A_{min} (mm^2)			A_{max} (mm^2)		
	n	β	95%CI	p-value	β	95%CI	p-value
Placental Weight (g)	190	0.004	0.0001, 0.001	0.006	0.0003	0.00004, 0.001	0.025
Birth Weight (g)	205	0.0001	0.0001, 0.0002	<0.001	0.0001	0.001, 0.0002	<0.001
Height at MRI (cm)	205	0.016	0.012, 0.020	<0.001	0.014	0.010, 0.018	<0.001
Weight at MRI (kg)	205	0.014	0.010, 0.019	<0.001	0.014	0.010, 0.018	<0.001
BMI at MRI (kg/m^2)	205	0.020	0.009, 0.031	<0.001	0.023	0.013, 0.033	<0.001

Values adjusted for sex of child

Heavier birth weight and greater placental weight were associated with greater A_{max} (β= 0.0001 mm^2/g, [95%CI 0.0001 to 0.0002], p<0.001, n=205) and (β= 0.0003 mm^2/g, [95%CI 0.00004 to 0.001], p= 0.025, n=190) respectively, taking account of child's sex. Higher birth weight and placental weight had similar associations with A_{min}. Taller height, heavier weight and higher BMI at

time of MRI were associated with greater maximum aortic cross-sectional area (β= 0.014 mm^2/cm, [95%CI 0.010 to 0.018], p<0.001, n=205), (β= 0.014 mm^2/kg, [95%CI0.010 to 0.018], p<0.001, n=205) and (β= 0.023 $mm^2/(kg/m^2)$, [95%CI 0.013 to 0.033], p<0.0001, n=205) respectively, taking account of child's sex. Taller height, heavier weight and higher BMI had similar associations with greater A_{min}.

Aortic distensibility was significantly related to child's sex with greater distensibility found in females (Figure 4.3). Larger placental weight was associated with lower aortic distensibility (β= -0.006 $10^{-3}mmHg^{-1}$/g, [-0.011 to -0.001] p=0.016, n=190), and strain (β= -0.0002 AU / g, [-0.0003 to 0], p=0.047, n=190) taking account of child's sex. Higher birth weight had similar associations with lower aortic distensibility and strain. Child's height at time of MRI was inversely associated with aortic distensibility (Figure 4.4) and strain (β= -0.011 $10^{-3}mmHg^{-1}$ / cm, [-0.018 to -0.004], p=0.002, n=205), and (β= -0.006 AU/cm, [-0.013 to -0.002], p=0.045, n=205) respectively.

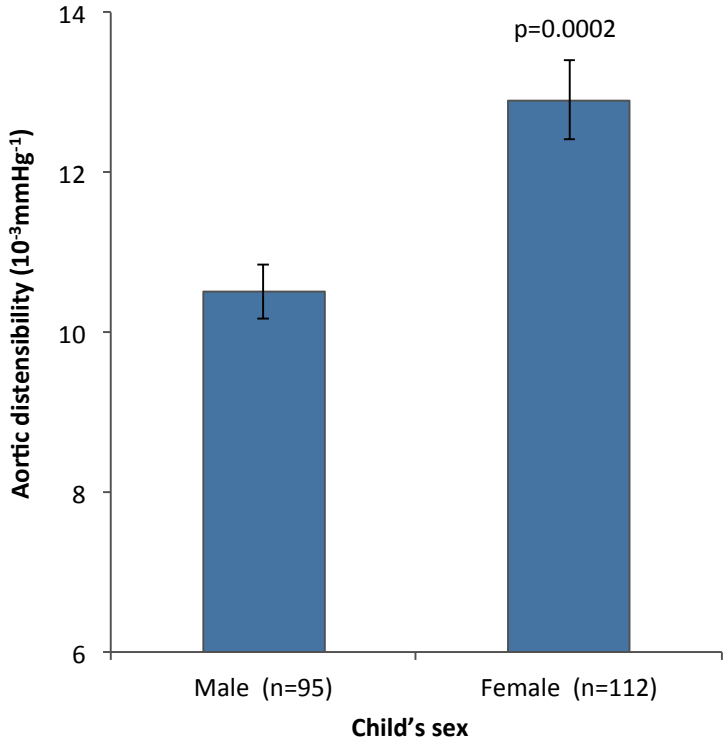

Figure 4.3 Relationship between child's sex and aortic distensibility at age 9 years. Values are means and SEM.

Measures of compliance showed no association with birth weight or placental weight. Lower childhood DBP and higher SBP were associated with lower aortic distensibility and compliance. Higher heart rate and PP were associated with lower aortic distensibility and strain. Both placental weight and birth weight are independent of diastolic and systolic BP. Faster heart rates were associated with lower aortic distensibility, strain and compliance (β= -0.006 10^{-3}mmHg^{-1} / bpm, [-0.010 to -0.002], p=0.005, n=205), (β= -0.004 AU/bpm, [-0.008 to -0.0004], p=0.031, n=205), and (β= -0.004 (mm^2/mmHg)/bpm, [-0.008 to -0.0004], p=0.029, n=205) respectively.

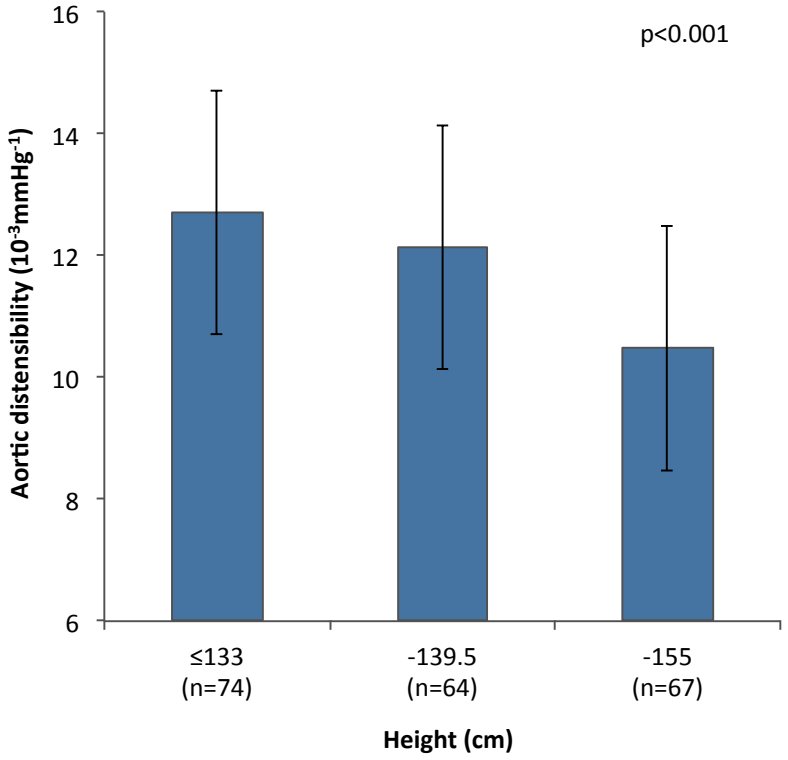

Figure 4.4 Relationship between child's height and aortic distensibility at age 9 years taking account of child's sex. Values are means and SEM.

4.3.2 Relations to maternal characteristics

There was no association between maternal smoking, weight, social class, parity or level of educational attainment and aortic dimension, strain, compliance or distensibility. Taller maternal height was associated with greater A_{min} and A_{max} (β= 0.006 mm^2/cm, [0.0008 to 0.010], p=0.022, n=206) and (β=

0.005 mm^2/cm, [0.0005 to 0.009], p=0.031, n=206) respectively, taking account of child's sex but there was no association with strain, distensibility or compliance.

Lower late pregnancy maternal vitamin D concentration was associated with reduced distensibility (β= 0.002 $10^{-3}mmHg^{-1}$/nmol/l, [0.0004 to 0.004], p=0.015, n=196) (Figure 4.5), and compliance (β= 0.002 (mm^2/mmHg)/nmol/l, [0.0004 to 0.004], p=0.014, n=196).

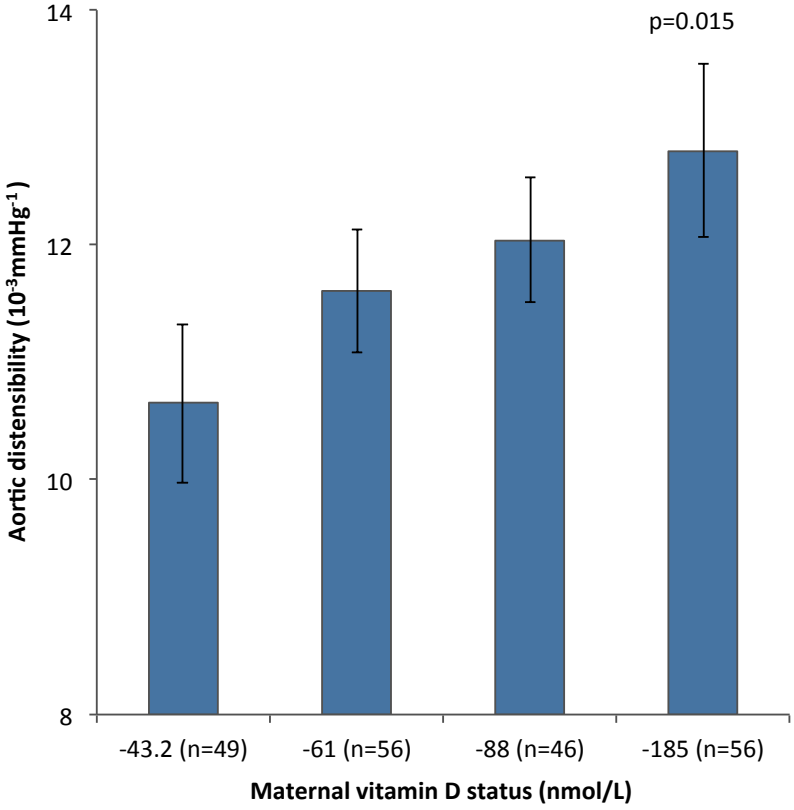

Figure 4.5 Relationship between late pregnancy maternal vitamin D status and child's aortic distensibility measured at MRI at age 9 years. Values are means and SEM.

Adjustment for child's sex and height had little effect on the findings with regard to the relationship between maternal vitamin D status and child's aortic distensibility. Additional inclusion of maternal BMI, educational attainment, social class and smoking status in the regression model also had little effect on the findings (Table 4.9).

Table 4.9 Multivariate analyses of maternal and childhood characteristics in relation to arterial distensibility (10^{-3}mmHg^{-1}) measured in the child at the age of 9 years (n=183).

	Coefficient	95% CI	p-value
Maternal			
Late pregnancy: vitamin D (nmol/l)	0.005	0.002, 0.009	0.04
Social class*	0.051	-0.19, 0.29	0.68
Educational attainment*	0.017	-0.246, 0.028	0.89
BMI (kg/m^2)	-0.016	-0.044, 0.011	0.25
Smoking status	-0.31	-0.711, 0.099	0.14
Child			
Sex (reference category, male)	0.36	0.089, 0.629	0.009
Height (cm)	-0.03	-0.049, -0.007	0.01

* 3 levels low-high

Lower late pregnancy maternal serum vitamin A concentration was associated with lower aortic distensibility (Figure 4.6), strain, and compliance (β= 0.19 10^{-3}mmHg^{-1}/umol/l, [0.06 to 0.33], p=0.006, n=164), (β= 0.17 AU/umol/l, [0.04 to 0.29], p=0.008, n=164), and (β= 0.2 (mm^2/mmHg)/umol/l, [0.06 to 0.34], p=0.006, n=164) respectively.

Following adjustment for child's sex and height, and inclusion of maternal social class, educational attainment, BMI and smoking status in the model the association between maternal vitamin A concentration in late pregnancy and arterial distensibility remained significant (β= 0.46 10^{-3}mmHg^{-1}/umol/l, [0.05 to 0.87], p=0.028, n=153).

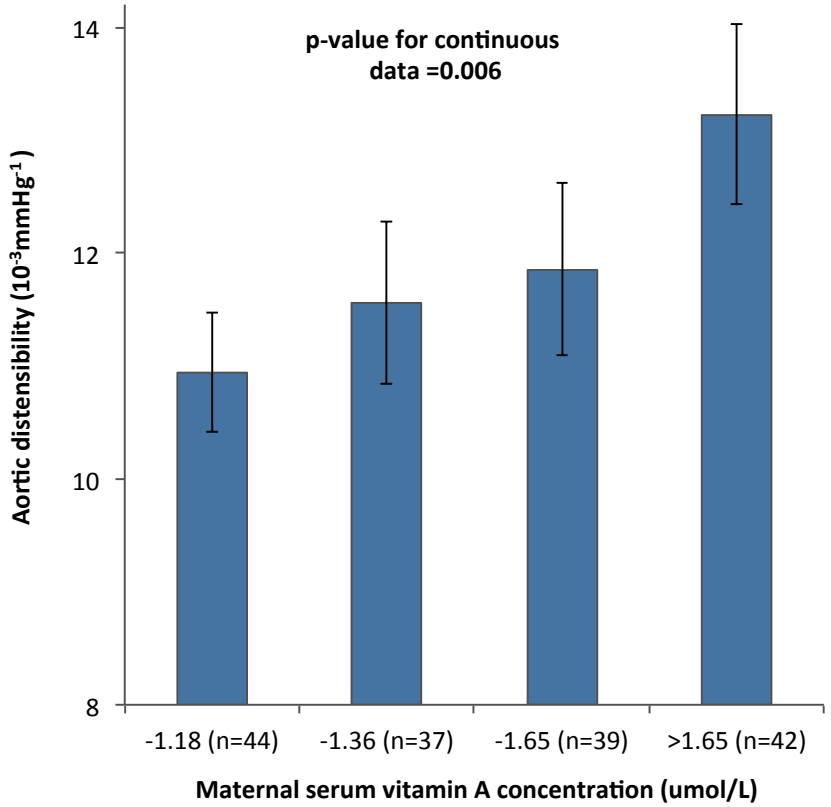

Figure 4.6 Relationship between late pregnancy maternal serum vitamin A concentration and child's aortic distensibility measured at MRI at age 9 years. Values are means and SEM.

4.4 Discussion

4.4.1 Summary of findings

This study investigated the associations between developmental influences (maternal, infant and childhood characteristics) and measures of vascular stiffness, assessed using CMR. Main findings of maternal, infant and childhood characteristics in relation to arterial stiffness; aortic PWV and aortic distensibility; measured at 9 years of age are summarised below:

- Higher late pregnancy oily fish consumption was associated with lower PWV and lower arterial stiffness.
- Lower educational attainment was associated with greater arterial stiffness in the children (higher PWV).
- Longer duration of breastfeeding was associated with lower PWV.

- Larger infant measures of birth weight and placental weight, and childhood measures of height, weight and BMI were associated with larger cross sectional aortic area.
- Distensibility was significantly associated with sex; measures were greater in females than in males.
- Adjusting for sex, distensibility was significantly associated with the child's height; taller children had lower distensibility and increased arterial stiffness.
- Adjusting for sex, higher late pregnancy maternal vitamin D status was associated with higher distensibility.
- Adjusting for sex, higher late pregnancy maternal vitamin A status was associated with higher distensibility.

4.4.2 Regional aortic stiffness - aortic pulse wave velocity

MRI measurements of childhood aortic PWV suggest that normal variations in maternal oily fish consumption in pregnancy may alter vascular development in utero - changing arterial structure and function with long-term consequences for cardiovascular risk in later life.

Findings of this study show that maternal oily fish consumption in late pregnancy is related to aortic PWV in the offspring at the age of 9 years, with an increase in maternal oily fish consumption in late pregnancy associated with less stiff arteries. There was a similar but weaker association with oily fish consumption in early pregnancy. The association with late pregnancy oily fish consumption persisted when adjusted for maternal education and social status and was independent of the child's sex.

Associated with a reduced risk of cardiovascular disease, long chain *n*-3 (omega-3) polyunsaturated fatty acids (LC PUFAs) found in fatty fish and fish oils have anti-thrombotic, anti-arrhythmic and anti-inflammatory properties, and are known to lower BP, increase endothelial relaxation and vascular compliance and increase heart rate variability (Calder, 2004). Studies have shown that omega-3 fish oil supplementation in adults reduces arterial stiffness (Pase et al., 2011). Rat studies have shown that changes in maternal fatty acid consumption in pregnancy are associated with increased arterial stiffness in the offspring (Armitage et al., 2005a).

LC PUFAs in the fetal circulation increase during the third trimester of pregnancy, when DHA fetal fatty acids correlate more strongly to maternal levels (Cetin et al., 2009), which could explain the stronger association with late pregnancy maternal oily fish consumption in this study. Oily fish is rich in several micronutrients so n-3 fatty acids may not be the only possible explanation for the association with vascular stiffness. Oily fish is rich in vitamin D, however there was no association between maternal vitamin D status in late pregnancy and PWV in the child at 9 years.

There was no effect of child's current oily fish consumption on measures of vascular stiffness. Any association between maternal oily fish consumption and child's aortic PWV was independent of child's oily fish consumption. In keeping with this, LC PUFA supplementation during childhood appears to offer little significant medium to long-term cardiovascular benefits. Studies investigating the effect of LC PUFA supplementation in neonates and early childhood found no lasting influence on cardiovascular measures (such as SBP, DBP, heart rate, or arterial distensibility) when assessed several years after the supplementation period (Ulbak et al., 2004, Ayer et al., 2009, de Jong et al., 2011). Cardiovascular measures of infants *during* a period of supplementation of fish oil, or formula supplemented with Docosahexaenoic acid (DHA) or LC PUFA showed favourable changes in both heart rate and BP (Damsgaard et al., 2006, Colombo et al., 2011, Pivik et al., 2009).

These studies, together with the data in this research implying a programming effect of early nutrition, suggest a need to 'get in earlier' with nutritional intervention. Maternal nutrition, in particular oily fish consumption, plays a vital role in the development of vascular structure and function. The effect appears to manifest during development, with important consequences for cardiovascular risk in later life.

The quality of women's diets is strongly dependent on their level of educational attainment and social class (Robinson et al., 2004). Frequency of oily fish consumption is related to maternal educational attainment and social class. Maternal educational attainment and social class had an independent and additive effect on childhood vascular stiffness in this study indicating that there may be a role for improving the diets of young women, and subsequent offspring cardiovascular health through better education. Even normal

variation in maternal diet can induce changes in vascular structure with long-term consequences for cardiovascular risk in the offspring.

Arterial compliance measured by PWV was strongly linked to the child's SBP with increased aortic stiffness associated with increased SBP, however there was no association between maternal oily fish consumption and SBP. Impaired development of the vascular wall is a precursor to increased arterial stiffness and arterial stiffening is the principal cause of both increased cardiac load and increasing SBP with advancing years (O'Rourke, 1990). Structural changes in the vascular endothelium during development influence arterial stiffness in childhood, with implications for hypertension and cardiovascular load in later life.

4.4.3 Local arterial stiffness - aortic distensibility, strain & compliance

This study has shown that maternal vitamin D status in late pregnancy is related to measures of child's aortic root distensibility, with increasing vitamin D concentrations associated with less stiff arteries in the offspring. The association was independent of the child's sex and height. MRI measurements of aortic root distensibility in children at the age of 9 years indicate that normal variations in maternal vitamin D status in late pregnancy may alter vascular development in utero with potential implications for cardiovascular risk in later life.

The role of vitamin D in both skeletal health and neurological development is well established. Recent evidence however has linked vitamin D with cardiovascular measures in both healthy and patient populations. A cross sectional study of over 1200 healthy participants found that higher vitamin D levels were associated with lower measures of arterial stiffness by PWV (Giallauria et al., 2012). This association was irrespective of traditional risk factor burden. Randomised controlled trials in both healthy adolescents (Dong et al., 2010), and diabetic patients (Breslavsky et al., 2013), found that daily vitamin D supplementation of 16 weeks and 12 months duration respectively, favourably improved measures of arterial stiffness.

Rat studies have shown that maternal consumption of a diet low in Vitamin D retards metabolic and contractile development in the neonatal rat heart (Morris et al., 1995), and is associated with endothelial vasodilator dysfunction in the

offspring (Tare et al., 2011). Few studies have investigated the influence of maternal vitamin D status in pregnancy on childhood cardiovascular outcomes. Gale et al. (2008) found no association between maternal 25-(OH) vitamin D concentrations in late pregnancy and arterial stiffness measured by cfPWV in children at the age of 9 years. However, carotid femoral, and aorta to foot PWV was measured by ultrasound, and aortic diameter by echocardiography.

The accuracy of PWV measured by ultrasound is limited by the estimation of path length along the body surface. Although in this research MRI measures of descending aortic PWV were also not associated with maternal vitamin D status in late pregnancy, MRI measures of local arterial stiffness in the aortic root may provide a more accurate measure of early subtle changes in arterial stiffness in children of this age. Subtle changes in arterial stiffness are likely to be more evident in more elastic vessels as opposed to more muscular distal arteries, which may explain the findings in this age group. MRI cine imaging affords direct imaging of the aortic root permitting highly accurate measurement, and aortic area visualised directly throughout the cardiac cycle is likely to be a more accurate measurement than aortic diameter at undetermined cardiac phase.

The findings suggest a modest effect of vitamin D status during pregnancy on vascular development of the fetus in utero, changing arterial structure in the offspring. Vitamin D insufficiency is a common problem in the UK and in this study 35% of the SWS mothers were considered to be vitamin D insufficient with serum vitamin D concentrations of less than 50nmol/L.

There are several plausible mechanisms by which vitamin D may reduce arterial stiffness – vitamin D is known to suppress proliferation of vascular smooth muscle cells, improve endothelial cell-dependent vasodilation, regulate the renin-angiotensin system, and inhibit anticoagulant activity and myocardial cell hypertrophy (Li et al., 2002, O'Connell et al., 1997).

Similar to the findings with maternal late pregnancy vitamin D concentrations, this study has shown that maternal vitamin A concentration in late pregnancy is related to measures of child's aortic root distensibility, with increasing vitamin A concentrations associated with less stiff arteries in the offspring.

Vitamin A is known to play an important role in both fetal renal and cardiovascular development; however, there is little in the literature investigating the effects of maternal vitamin A levels on cardiovascular risk factors in the offspring. Offspring of women who had received 7000 μg retinol equivalents of preformed vitamin A before and during pregnancy in a large cluster-randomised double blind study in Nepal showed no difference in measured cardiovascular risk factors compared to control groups (Stewart et al., 2010). The study measured systolic and diastolic BP, fasting glucose, triglycerides and cholesterol in children aged 9-13 years but did not assess measures of arterial stiffness.

Retinoic acid, the active form of vitamin A, plays a role in cell growth and differentiation and is an important regulator of embryonic development (Zile, 1998). Rat studies have shown changes in lung morphology in fetuses of vitamin A deficient dams, associated with a reduction in elastin deposition (Antipatis et al., 1998) suggestive that maternal vitamin A levels in pregnancy may regulate elastin development in utero which could explain the association seen in this study with aortic distensibility. However, it is important to note that interpretation of serum retinol concentrations is notoriously complex (Tanumihardjo et al., 2014) and these findings must thus be considered with caution.

Aortic root size (both minimum and maximum cross sectional area) was larger in taller and heavier children, and significantly smaller in children who weighed less at birth. Aortic root size was associated with mother's height, with greater maternal height associated with an increased size of aortic area. These findings support those of a previous study suggesting that constrained early growth leads to structural changes in coronary circulation and major arteries (Jiang et al., 2006).

Increased heart rate was associated with increased arterial stiffness (lower distensibility). It has been established in animals that arterial distensibility is a function of heart rate (Mangoni et al., 1996). Heart rate and distensibility are both measures of cardiovascular health and inherently related.

4.4.4 Strengths of study

The participants are part of a large prospective longitudinal cohort study covering a wide socioeconomic background, reducing recall bias. The women were characterised before conception providing a wealth of information on diet and lifestyle factors. The SWS provides detailed information on both pre- and post-natal growth trajectories of the children. The SWS has data on assessment of growth trajectories in utero, at birth, throughout childhood to date, and data recorded of maternal exposures, whereas adult studies are reliant on recall and the associated bias.

The vascular MRI cohort was broadly representative of the initial SWS birth cohort (those born up to the end of 2003). The mothers in the initial SWS birth cohort were more likely to be parous than mothers in the vascular MRI cohort but other characteristics were similar.

The children in this study were assessed at the age of 9 years; old enough to tolerate the MRI environment and comply with breath hold requirements, before the onset of puberty, and before the acquisition of significant risk burden, ensuring minimal influence of other lifestyle risk factors. Results are less likely to be confounded by the influence of other CV risk factors than in adult studies. The children were all the same age at MRI enabling direct comparison of outcomes.

MRI is considered the gold standard imaging modality for assessing cardiac structure and function - it is non-invasive and involves no ionising radiation.

PWV assessment by MRI is a well-validated, accurate and reproducible means of determining arterial stiffness. MRI permits the accurate automated measurement of aortic area across the cardiac cycle providing accurate measures of minimum and maximum area. Previous studies have measured aortic diameter with ultrasound – the aorta is not necessarily a perfect circle, which questions the accuracy of the measurement of diameter and manual measurement is subject to operator error. It is also often not reported which cardiac phase the measurements are acquired in.

4.4.5 Limitations of study

The accuracy of MRI aortic distensibility measures is limited by the use of brachial BP, which can differ significantly from central aortic BP measures. However, invasive BP measures were not appropriate for this study and brachial BP has been considered an acceptable substitute in many studies and is unlikely to have significantly influenced the findings.

The extent of progression of childhood measures of aortic stiffness throughout the life course is unknown; however, it is reasonable to speculate that the effect size found in this study may be amplified by acquired risk burden in later life.

CMR measurements are not as reliable or accurate in children compared to those in adults due to reduced ability to comply with breath hold requirements in children. However, PWV values obtained in this study are similar to those reported using cfPWV applanation tonometry measurements in children (Reusz et al., 2010). There are limited data on normal childhood measures of distensibility; however, measures in this study were within MRI measures reported in children by Voges et al. (2012).

Maternal intake of fish oil supplements was not considered in the analysis. Measures of the child's vitamin D status were not available but season at time of MRI was considered as a proxy of child's vitamin D status and there was no association found with measures of arterial stiffness.

The children were not fasted or asked to abstain from fatty foods or caffeinated drinks prior to the MRI. Environmental factors are known to effect measures of arterial stiffness however it has been shown that the contribution of factors such as time of day, room temperature, and consumption of fried foods or caffeine to variation in measurements is minor, and unlikely to confound findings (Donald et al., 2010).

4.4.6 Clinical significance of findings

The effect sizes seen in this study are modest compared to those seen in disease processes and both PWV and distensibility measures fell within normal ranges previously reported for children. However modest adverse changes may

set the child on a higher trajectory of cardiovascular risk with detrimental effects later in life.

In adults a 1m/s increase in PWV relates to a 14% increase in total CV events (Vlachopoulos et al., 2010). In this study children of mothers who had no oily fish had a mean PWV of 3.61m/s compared with 3.18m/s in children whose mothers consumed 3 or more portions of oily fish per week. A study by Wiesmann et al. (2004) found a similar increase in MRI measured PWV of 19% in smokers compared to non-smokers.

There are limited data on the progression of measures of arterial stiffness throughout the life course and the predictive value of higher PWV is uncertain however baseline measures of PWV do predict later aortic dilation in children with tetralogy of Fallot (Seki et al., 2014). BP is known to track from childhood to adulthood (Bao et al., 1995). In this study measures of both PWV and aortic distensibility were strongly associated with BP, so it is reasonable to assume that childhood measures of arterial stiffness will track into later life. The Bogalusa Heart Study (Li et al., 2004) has shown systolic BP in childhood to be a consistent and independent predictor of PWV in adults, suggesting that BP in childhood plays a role in arterial stiffening, but did not report on measures of arterial stiffness in childhood. Bao et al. (1995) found that elevated BP progresses in childhood long before hypertension and hypertensive disease is clinically evident, stressing the importance of establishing preventative measures early in childhood. Whilst the changes seen in this study are subclinical, it is likely that acquired risk burden and life style influences may confer greater magnitude in PWV changes across the life course.

5. Results - Cardiac Structure and Function

5.1 Background

Standard MRI measures of left ventricular structure and function were acquired as per JCMR guidelines (Schulz-Menger et al., 2013), using the imaging methods outlined in sections 2.3.2 and 2.4.1.

The original SWS cohort included 12,583 women from whom there were 3,160 births. A sample of these children underwent cardiovascular phenotyping at the age of 8-9 years. Measurements of cardiac structure and function were acquired on the MRI vascular cohort described in Chapter 4. An additional 100 children were invited to attend for scan; in total 355 children accepted an invitation to undergo MRI. Diagnostic cardiac images were obtained on 338 of these children.

Table 5.1 displays a formal comparison between the MRI cardiac cohort and the SWS birth cohort (still births, neonatal deaths and intrauterine deaths excluded). Variables were checked for normal distribution; for categorical variables a Chi^2 test was used, for variables that were normally distributed a *t*-test was used, and for non-normally distributed variables a Wilcoxon-Mann-Whitney test was used. The two cohorts were very similar with regard to child's size at birth and mother's parity, age at childbirth, smoking status, ethnicity and BMI, although the mothers in the MRI cohort were more likely to be of a higher social class and have higher levels of educational attainment.

Of the 355 children who attended for scan, five declined to have the MRI at appointment, and twelve proved claustrophobic – unable to tolerate either the scanner noise or the confined environment and no imaging was performed beyond the initial localiser views. 338 children completed all or part of the imaging protocol. Of these, 29 did not complete the scans required for LV analysis, or the imaging was not of sufficient quality for accurate analysis due to degradation from breathing or motion artefact. Measures of ventricular volumes and mass were achieved on 309 children, 147 boys and 162 girls (47.6% and 52.4%, respectively).

	MRI Cohort		SWS Birth Cohort		
Characteristic	n	Median (IQR) Mean (SD)*	n	Median (IQR) Mean (SD)*	p-value
Age at child birth	338	30.4(3.7)*	2810	30.7(3.9)*	0.27
Infant at birth					
Birth weight (g)	335	3482.7(545.3)*	2779	3427.3(560.8)*	0.09
Placental weight (g)	312	485.2(114)*	2419	476.8(111.4)*	0.21
Total lean mass at birth (kg)	154	3.0(0.3)*	848	2.9(0.3)*	0.15
Total fat mass at birth (kg)	154	0.6(0.2)*	848	0.5(0.2)*	0.11

p-value to compare the two cohorts. Chi^2 test was used for categorical variables; *t-test* for normally distributed variables; Wilcoxon-Mann-Whitney for non-normally distributed variables.

Table 5.1 Formal comparison of characteristics (maternal and infant at birth) of the MRI cardiac cohort with that of the SWS birth cohort (still births, neonatal deaths, and intrauterine deaths excluded).

	MRI Cohort		SWS Birth Cohort		
Characteristic	n	Median (IQR) Mean (SD)*	n	Median (IQR) Mean (SD)*	p-value
Maternal					
BMI (kg/m^2)	336	24.4(22.4,27.7)	2784	24.1(21.8,27.3)	0.09
Early pregnancy: oily fish (portions/week)	276	0.5(0.2, 1.5)	1938	0.5(0.1, 1.5)	0.58
Late pregnancy: oily fish (portions/week)	328	0.5(0.3, 1.5)	2310	0.5(0.0, 1.5)	0.06
Ethnic origin, n (%)					
White	327 (96.6)		2680 (95.4)		0.26
Social class, n (%)					
Professional/Management/Technical	155 (46.7)		1056 (38.8)		
Skilled non-manual/manual	136 (41.0)		1286 (47.3)		
Partly skilled/unskilled	41 (12.4)		378 (13.9)		0.02
Qualification level, n (%)					
None/CSE	33 (9.8)		359 (12.8)		
Secondary education	189 (55.9)		1668 (59.6)		
HND or degree	116 (34.3)		774 (27.6)		0.02
Pre-pregnancy smoking status, n (%)					
Smoker	84 (24.9)		792 (28.2)		0.19
Parity, n (%)					
1+	168 (49.7)		1369 (48.8)		0.75

The relationships between developmental influences (maternal, and childhood) and left ventricular measures of cardiac structure and function; left ventricular systolic mass, left ventricular end-diastolic volume (LVEDV), left ventricular stroke volume (LVSV), left ventricular cardiac output (LVCO) and left ventricular ejection fraction (LVEF) at 9 years of age are presented in this chapter.

Measures of LV systolic mass, LVEDV, LVSV, and LVCO normalised to body surface area (BSA) were included in the analyses. Given the early gestation critical period for cardiac development and publications suggesting maternal stress effects on fetal cardiac development, outlined in section 1.3, maternal influences such as preconception state of health, stress levels, measures of size and nutritional status were considered along with cardiovascular measures at time of MRI (BP and aortic stiffness), and measures of child's size at birth, and at 4 and 9 years of age. Maternal characteristics of the study population are presented in Table 5.2. Infant and childhood characteristics are presented in Table 5.3.

Table 5.2 Maternal characteristics of the LV study population.

Characteristic	n	Median (IQR) / Mean (SD)*
Maternal: pre-pregnancy		
Townsend index	333	-0.22 (-2.52,2.69)
Pre-pregnancy smoking status, n (%)		
Smoker	84 (24.9)	
Ethnic origin, n (%)		
White	327 (96.8)	
Other	11 (3.2)	
Social class, n (%)		
Professional/management & technical	155 (46.7)	
Skilled non-manual/manual	136 (41)	
Partly skilled/unskilled	41 (12.3)	
Qualification level, n (%)		
None/CSE	33 (9.8)	
Secondary education	189 (55.9)	
HND/Degree	116 (34.3)	
Stress in daily living in last 4 weeks, n (%)		
None	30 (8.9)	
Just a bit	175 (51.8)	

Characteristic	n	Median (IQR) / Mean (SD)*
A good bit	60 (17.8)	
Quite a lot	56 (16.6)	
A great deal	17 (5)	
Assessment of general health, n (%)		
Very good	101 (29.9)	
Good	168 (49.7)	
Fair	60 (17.8)	
Bad	9 (2.6)	
Stress in life affecting health, n (%)		
None	86 (25.4)	
Slightly	138 (40.8)	
Moderately	73 (21.6)	
Quite a lot	35 (10.4)	
Extremely	6 (1.8)	
Longstanding illness, n (%)		
Yes	84 (24.9)	
No	253 (75.1)	
Frequency of strenuous exercise per week, n (%)		
< 1/week	187 (55.7)	
1-4 times per week	102 (30.4)	
>4 times per week	47 (14)	
Height (cm)	337	163.6 (6.5)*
BMI (kg/m^2)	336	24.4 (22.4,27.7)
Mid upper arm circumference (cm)	335	29.1 (3.9)*
Triceps skinfold (mm)	337	20.4 (7.3)*
Biceps skinfold (mm)	337	9.8 (7.0,14.0)
Subscapular skinfold (mm)	337	16.7 (11.9,24.8)
Sum of skinfolds (mm)	337	67.3 (51.0,91.5)
Arm muscle area (cm^2)	335	33.9 (29.0,39.2)
Waist hip ratio (cm)	335	0.77 (0.74,0.81)
Maternal: pregnancy		
Vitamin D (nmol/l)	316	61.0 (44.7,89.7)
Vitamin A (umol/l)	272	1.4 (0.3)*
Early pregnancy: oily fish portions/week	276	0.5 (0.2,1.5)
Late pregnancy: oily fish portions/week	328	0.5 (0.3,1.5)

Characteristic	n	Median (IQR) / Mean (SD)*
EDPS >11, n (%)	124 (27.4)	
EDPS (continuous)	332	10.2 (5.7)*
Age at childbirth	338	30.4 (3.7)*
Maternal: post natal		
EPDS > 13 n (%)	32 (21.1)	
EPDS (continuous)	152	10 (7,13)

Table 5.3 Infant and childhood characteristics of the LV study population.

Characteristic	n	Median (IQR) / Mean (SD)*
Infant		
Birth weight (g)	335	3483 (545)*
Placental weight (g)	312	485 (114)*
Ponderal index (kg/m^3)	328	27.9 (2.4)*
Breast feeding duration (months)	329	3 (0,7)
Total lean mass at birth (kg)	154	3.0 (0.3)*
Total fat mass at birth (kg)	154	0.6 (0.2)*
Total lean mass at 4 years (kg)	233	12.0 (1.5)*
Total fat mass at 4 years (kg)	233	1.9 (1.2)*
Child at 9 years		
Age (y)	338	9.5 (0.23)*
Height (cm)	337	137.0 (6.5)*
Weight (kg)	337	33.7 (6.6)*
Body mass index (kg/m2)	337	17.9 (2.7)*
Total lean mass (kg)	298	22.9 (3.3)*
Total fat mass (kg)	298	8.0 (5.9,10.8)
Systolic blood pressure (mmHg)	301	98.9 (8.8)*
Diastolic blood pressure (mmHg)	301	58.2 (7.1)*
Pulse pressure (mmHg)	301	40.7 (9.4)*
Mean arterial pressure (estimated) (mmHg)	301	71.8 (6.3)*
Mean arterial pressure (measured) (mmHg)	179	74.2 (7.7)*
Heart rate (beats per minute)	338	81.3 (10.4)*
Distensibility ($10^{-3}mmHg^{-1}$)	301	11.5 (4.3)*
Pulse wave velocity (descending aorta) (m/s)	338	3.5 (0.5)*

5.1.1 Sex

Left ventricular functional and structural measures are presented by sex in Table 5.4. All measures were checked for a normal distribution, and means and standard deviations calculated. *t*-tests were performed to compare left ventricular structure and function in male and female subgroups. These revealed significant sex differences for all measures (with the exception of ejection fraction), with boys measuring larger than girls; LV mass and LV mass normalised by body surface area ($p<0.001$), LV end-diastolic volume and end-diastolic volume normalised for body surface area ($p<0.001$), LV end-systolic volume ($p=0.03$), LV end-systolic volume normalised to body surface area ($p=0.01$), LV stroke volume ($p=0.002$), LV stroke volume normalised to body surface area ($P<0.001$), LV cardiac output ($p=0.01$), and LV cardiac output normalised to body surface area ($p=0.002$). Further analyses to assess relationships between maternal and childhood characteristics with LV structural and functional measures were therefore adjusted for sex.

Table 5.4 Left ventricular structural and functional measures at 9 years of age - formal comparison by sex.

	Male (n=162)	**Female (n=176)**	
	Mean (SD)	Mean (SD)	p-value
LVsysmass (g)	53.1 (10.2)	46.8 (7.9)	<0.001
LVsysmass (g/m²)†	46.9 (7.1)	41.5 (5.9)	<0.001
LVEDV (ml)	73.4 (12.2)	68.9 (11.0)	<0.001
LVEDV (ml/m²)†	65.1 (9.7)	61.0 (7.7)	<0.001
LVESV (ml)	22.9 (6.0)	21.5 (5.4)	0.03
LVESV (ml/m²)†	20.3 (5.1)	19.0 (4.4)	0.01
LVSV (ml)	50.5 (9.30)	47.5 (8.2)	0.002
LVSV (ml/m²)†	44.7 (7.5)	42.0 (5.9)	<0.001
LVCO (l/min)	3.9 (0.7)	3.7 (0.7)	0.01
LVCO (l/min/m²)†	3.5 (0.6)	3.3 (0.5)	0.002
LVEF (%)	68.8 (6.1)	68.9 (5.6)	0.85

† Adjusted for body surface area

Univariate regression analyses, adjusted for child's sex, were performed to assess infant, childhood, and maternal factors in relation to measures of LV structure and function; myocardial mass (systolic), end-diastolic volume, stroke volume, cardiac output, and ejection fraction. β coefficients reflect the change in outcome when compared with the reference.

5.1.2 Ethnicity

Regression analyses of mother's ethnicity in relation to MRI measures of child's cardiac volumes and mass are presented in Table 5.5. Children of non-white mothers had significantly lower measures of LVsysmass, LVEDV, LVEDV adjusted for BSA, and LVSV (β = -6.44 g, [95% CI -11.89, -0.99], p=0.02, n=309), (β = -10.83 ml, [95% CI -17.74, -3.92], p<0.01, n=309), (β = -6.44ml/m^2, [95% CI -11.66, -1.22], p=0.02, n=309), and (β = -6.17 ml, [95% CI -11.41, -0.93], p=0.02, n=309), respectively. Whilst the significance of the association with LVsysmass and LVSV was attenuated following adjustment for body surface area there was a weak trend for smaller volumes in non-white children.

Table 5.5 Regression analyses of mother's ethnicity (reference white) in relation to childhood measures of cardiac structure and function.

	n	Coefficient	95% CI	p-value
LVsysmass (g)	309	-6.44	-11.89, -0.99	0.02
LVsysmass (g/m^2)†	309	-3.27	-7.19, 0.64	0.1
LVEDV (ml)	309	-10.83	-17.74, -3.92	<0.01
LVEDV (ml/m^2)†	309	-6.44	-11.66, -1.22	0.02
LVSV (ml)	309	-6.17	-11.41, -0.93	0.02
LVSV (ml/m^2)†	309	-2.95	-6.79, 0.88	0.13
LVCO (ml/min)	309	-0.31	-0.73, 0.11	0.15
LVCO (ml/min/m^2)†	309	0.01	-0.40, 0.25	0.64
LVEF (%)	309	2.24	-1.29, 5.77	0.21

*Adjusted for sex of child

† Adjusted for body surface area

5.1.3 Child's haemodynamic measures

Univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to heart rate, and BP at time of MRI, taking account of child's sex, are presented in Appendix 1 and Appendix 2 respectively.

Children of mothers who reported poorer general health had higher heart rates. Longer duration of breastfeeding was associated with lower heart rates and lower SBP in the child at the age of 9 years. Faster heart rate was linked to higher BP. Increased arterial stiffness (lower distensibility and higher PWV) was associated with faster heart rates and higher SBP. Children of mothers who had no or minimal qualifications had higher systolic and diastolic BP compared to those of mothers who had standard school qualifications or higher educational qualifications. Taller children and those who were heavier or had greater BMI had higher SBP.

5.2 LV Systolic Mass

Univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to LVsysMass (adjusted for sex) are presented in Table 5.6. Univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to LVsysMass (adjusted for sex and heart rate) are presented in Appendix 3.

Table 5.6 Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular systolic mass (g) at age 9 years (adjusted for sex).

		LV systolic mass (g)			LV systolic mass (normalised to BSA) (g/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	-0.1	-0.44, 0.23	0.54	-0.12	-0.36, 0.12	0.34
Currently smoking	309	1.93	-0.42, 4.28	0.11	0.33	-1.37, 2.02	0.7
Woman's ethnic group, 2 groups	309	-6.44	-11.89, -0.99	0.02	-3.27	-7.19, 0.64	0.1

		LV systolic mass (g)			LV systolic mass (normalised to BSA) (g/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		0.18	-2.03, 2.39	0.87	-0.29	-1.89, 1.30	0.72
Partly skilled / unskilled		-2.85	-6.10, 0.409	0.09	-1.49	-3.84, 0.85	0.21
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		0.73	-2.91, 4.37	0.69	-0.11	-2.72, 2.49	0.93
Secondary education		-0.44	-2.64, 1.76	0.69	-0.75	-2.33, 0.82	0.35
How much stress in daily living in last 4 weeks	309	-0.87	-1.84, 0.11	0.08	-0.93	-1.62, -0.23	<0.01
Assessment of general health	309	-1.1	-2.42, 0.22	0.1	-1.07	-2.01, -0.13	0.03
Stress in life affected health	309	-0.61	-1.63, 0.41	0.24	-0.4	-1.13, 0.33	0.28
GHQ > 13	141	-1.49	-5.21, 2.22	0.43	-1.19	-3.76, 1.39	0.36
GHQ score Likert	141	-0.12	-0.49, 0.26	0.54	-0.14	-0.40, 0.12	0.28
Long-standing illness	309	-1.01	-3.37, 1.36	0.4	-0.85	-2.54, 0.84	0.32
Frequency of strenuous exercise per week, two groups	307	-1.12	-3.19, 0.95	0.29	-0.41	-1.89, 1.07	0.59
Height (cm)	308	0.27	0.12, 0.43	<0.001	0.02	-0.09, 0.13	0.74
Body mass index (kg/m²)	307	-0.02	-0.24, 0.21	0.89	-0.13	-0.29, 0.04	0.13
Mid-upper arm circumference (cm)	306	0.04	-0.23, 0.31	0.78	-0.17	-0.36, 0.02	0.08
Triceps skinfold (mm) (average of three closest)	308	-0.04	-0.18,0.10	0.61	-0.08	-0.18, 0.02	0.11
Biceps skinfold (mm) (average of three closest)	308	-0.04	-0.21,0.13	0.63	-0.14	-0.26, -0.01	0.03
Subscapular skinfold (mm) (average of three closest)	308	-0.01	-0.12, 0.09	0.81	-0.04	-0.11, 0.03	0.29
Sum of skinfolds (mm)	308	-0.01	-0.04, 0.03	0.68	-0.02	-0.05, 0.00	0.09
Arm muscle area (cm²)	306	0.04	-0.07, 0.14	0.5	-0.05	-0.13, 0.03	0.19
Waist-Hip ratio	306	-9.63	-27.46, 8.20	0.29	-9.22	-21.92, 3.49	0.15
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.01	-0.02, 0.05	0.44	0.01	-0.01, 0.04	0.27
Late pregnancy Retinol (vitamin A) (umol/l)	249	0.58	-2.93, 4.10	0.74	1.26	-1.28, 3.80	0.33
Early pregnancy: oily fish potions/week	252	-0.04	-1.06,0.97	0.94	0.23	-0.50, 0.95	0.54
Late pregnancy: oily fish portions/week	299	-0.06	-0.92,0.80	0.89	0.3	-0.32, 0.92	0.34

		LV systolic mass (g)			LV systolic mass (normalised to BSA) (g/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-2.72	-4.82, -0.63	0.01	-1.60	-3.11, -0.09	0.04
EPDS: EPDS score	303	-0.13	-0.31, 0.05	0.16	-0.09	-0.22, 0.0	0.2
Paternal							
Height (cm)	295	0.18	0.03, 0.32	0.02	-0.03	-0.13, 0.08	0.6
Birth weight (g)	263	0	-0.00, 0.00	0.27	0	-0.00, 0.00	0.72
Infant							
Birth weight (kg)	307	2.72	0.84, 4.59	<0.01	0.43	-0.93,1.78	0.53
Placental weight (g)	286	0.01	0.00, 0.02	0.02	0	-0.00, 0.01	0.26
Ponderal index (kg/m³)	300	0.23	-0.20, 0.66	0.29	0.23	-0.08, 0.54	0.14
Age last breast fed, (months)	300	-0.05	-0.24, 0.15	0.64	0.08	-0.06, 0.22	0.27
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	6.60	1.86, 11.34	<0.01	1.02	-2.58, 4.62	0.58
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	3.27	-3.55, 10.08	0.34	-0.01	-5.07, 5.05	1
4 year DXA: Total lean (kg), adjusted for sex	212	3.43	2.76, 4.09	<0.001	0.75	0.17, 1.32	0.01
4 year DXA: Total fat (kg), adjusted for sex	212	2.59	1.57, 3.60	<0.001	-0.14	-0.91, 0.63	0.72
Child at 9 years							
Height (cm)	309	0.77	0.64, 0.90	<0.001	0.07	-0.04, 0.18	0.21
Weight (kg)	309	0.77	0.64, 0.90	<0.001	-0.01	-0.13, 0.10	0.81
BMI (kg/m²)	309	1.26	0.89, 1.63	<0.001	-0.17	-0.45, 0.11	0.23
Total lean mass (SD)	271	5.79	4.96, 6.62	<0.001	0.95	0.16, 1.73	0.02
Total fat mass (SD)	271	2.99	1.95, 4.04	<0.001	-0.91	-1.71, -0.11	0.03
Child cardiovascular measures							
Systolic BP (mmHg)	286	0.20	0.08, 0.31	<0.01	0.05	-0.04, 0.13	0.29
Diastolic BP (mmHg)	286	-0.04	-0.19, 0.10	0.57	-0.04	-0.15, 0.06	0.44
Pulse pressure (mmHg)	286	0.21	0.10, 0.31	<0.001	0.08	-0.00, 0.16	0.06
Mean arterial pressure (estimated) (mmHg)	286	0.09	-0.07, 0.25	0.29	-0.01	-0.13, 0.11	0.92
Mean arterial pressure (measured) (mmHg)	169	0.05	-0.13, 0.24	0.56	-0.01	-0.14, 0.12	0.88
Heart rate (beats per minute)	309	-0.19	-0.29, -0.09	<0.001	-0.18	-0.25, -0.10	<0.001
Aortic distensibility (10^{-3}mmHg^{-1})	286	-0.28	-0.53, -0.03	0.03	-0.1	-0.28, 0.09	0.31
Pulse wave velocity (m/s)	308	1.07	-1.08, 3.23	0.33	-0.72	-2.27, 0.82	0.36

5.2.1 Paternal influences

Children with taller fathers had greater LVsysmass (β = 0.18 g/cm, [95% CI 0.03, 0.32], p=0.02, n=295). This effect was attenuated when LVsysmass was adjusted for child's BSA and the association was no longer significant. Father's birth weight was not associated with child's LVsysmass.

5.2.2 Maternal influences

Children of mother's reporting higher levels of stress in daily living and those with a poorer assessment of general health tended to have smaller LV mass when normalised to body surface area (β = -0.93 (g/m^2)/level, [95% CI -1.62, -0.23], p<0.01, n=309), and (β = -1.07 (g/m^2)/level, [95% CI -2.01, -0.13], p=0.03, n=309) respectively (Figure 5.1).

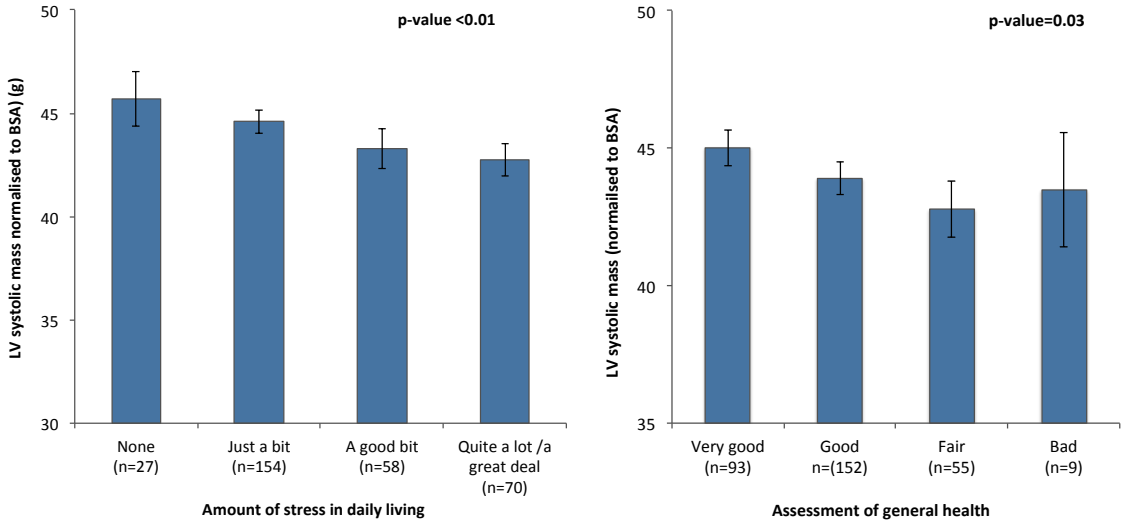

Figure 5.1 Maternal assessment of amount of stress in daily living (left) and general health (right) in relation to child's LV systolic mass normalised to body surface area. Values are means and SEM.

LV mass was smaller in children of mothers who had an Edinburgh Post Natal Depression Score (EPDS) of greater than 11 (β = 2.72 g, [95% CI -4.82, -0.63], p=0.01, n=303). When normalised to BSA LV mass showed a similar association (β = -1.60 (g/m^2), [95% CI -3.11, -0.09], p=0.04, n=303).

Children of taller mothers had greater LV mass (β = 0.27 g/cm, [95% CI 0.12, 0.43], p<0.001, n=308). When LV mass was normalised to BSA there was no association with maternal height.

5.2.3 Childhood - cardiovascular measures

Faster childhood heart rate was associated with smaller LV mass ((β = -0.19 g/bpm, [95% CI -0.029, -0.09], p<0.01, n=309). When normalised for BSA the association strengthened ((β = (-0.18 g/m^2)/bpm, [95% CI 0.25, 0.10], p<0.001, n=309).

Higher PP was associated with greater LV mass (β=0.21 g/mmHg, [95% CI 0.10, 0.31], p<0.001, n=286). The significance persisted when adjusted for heart rate. The trend remained but was not significant when LV mass normalised to child's body surface area (BSA). When adjusted for heart rate there was a significant positive association between PP and LV mass adjusted for BSA (β = 0.08 (g/m^2)/mmHg, [95% CI 0.001, 0.16], p=0.04, n=286).

Greater arterial aortic distensibility (less stiff arteries) was associated with smaller LV mass (β = -0.28 g/10^{-3}mmHg^{-1}, [95% CI -0.53, -0.03], p=0.03, n=286). This relationship persisted when adjusted for heart rate. There was no significant association between heart rate and LV mass adjusted for BSA, although there was a weak trend for lower LV mass (normalised to BSA) with greater distensibility.

Higher SBP was associated with greater LV mass ((β = 0.29 g/mmHg, [95% CI 0.08, 0.31], p<0.01, n=286). This relationship persisted when adjusted for heart rate. There was no association between SBP and LV mass normalised to BSA. There was no association with DBP or PWV.

5.2.4 Childhood – size and body composition

Greater birth and placental weight were associated with greater LV mass (β = 2.72 g/kg, [95% CI 0.84, 4.59], p<0.01, n=307) and (β = 0.01 g/g, [95% CI 0.001, 0.02], p=0.02, n=286). These associations were no longer apparent when LV mass was adjusted for BSA.

Greater total lean mass measured by DXA at birth, 4 years of age and 9 years of age were all associated with greater LV mass (β = -6.6 g/kg, [95% CI 1.86, 11.34], p<0.001, n=135), ((β = 3.43 g/kg, [95% CI 2.76, 4.09], p<0.001, n=212), and ((β = 5.79 g/SD, [95% CI 4.96, 6.62], p<0.001, n=271) respectively (Figure 5.2).

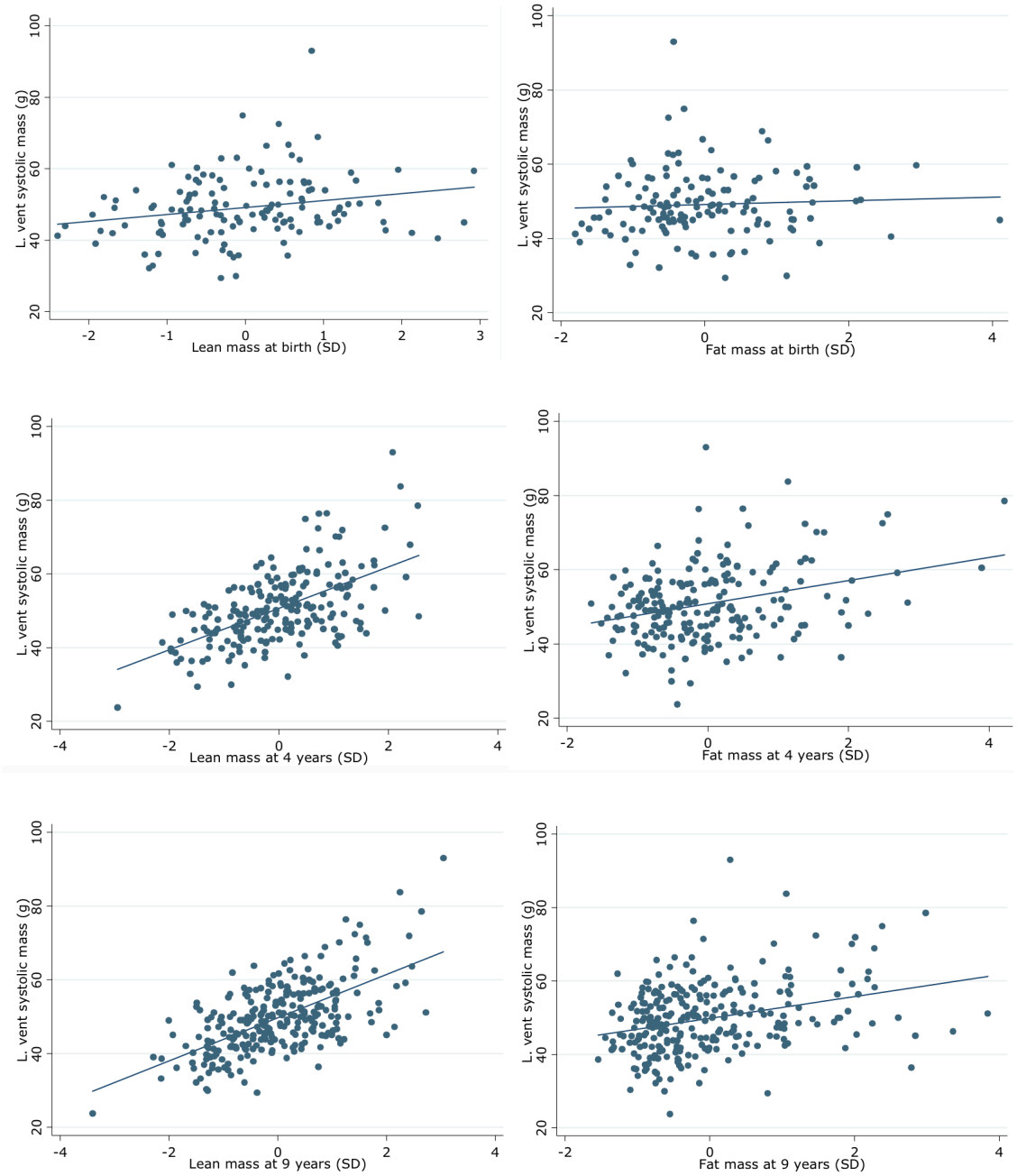

Figure 5.2 Scatter plots showing lean and fat mass (measured at birth, 4 years and 9 years), in relation to LV systolic mass at 9 years of age.

There was a similar, but weaker association with lean mass measured at both 4 and 9 years of age and LV mass normalised to BSA (β = 0.75 (g/m^2)/kg, [95% CI 0.17, 1.32], p=0.01, n=286) and (β = 0.95 (g/m^2)/SD, [95% CI 0.16, 0.1.73], p=0.02, n=286) respectively. However there was no association between lean mass at birth and LV mass normalised to BSA.

Similar associations were seen with total fat mass measured by DXA at 4 and 9 years of age (Figure 5.2). Greater fat mass was associated with greater LV mass ((β = 2.59 g/kg, [95% CI 1.57, 3.60], p<0.001, n=212) and (β = 2.99 g/SD, [95% CI 1.95, 4.04], p<0.001, n=271) respectively. There was no association with fat mass measured at birth. There was no association between total fat mass at 4 years of age and LV mass normalised for BSA. However greater fat mass at 9 years of age was associated with lower LV mass normalised for BSA (β = -0.91 (g/m^2)/SD, [95% CI -1.71, -0.11], p=0.03, n=271).

Taller height, heavier weight and greater BMI at time of scan were associated with greater LV mass (β = 0.77 g/cm, [95% CI 0.64, 0.9], p<0.001, n=309), (β = 0.77 g/kg, [95% CI 0.64, 0.90], p<0.001, n=309), and (β = 1.26 g/(kg/m^2), [95% CI 0.89 1.63], p<0.001, n=309) respectively. There was no association between height, weight, and BMI with LV mass normalised to BSA.

5.3 LV End-diastolic Volume

Univariate analyses of maternal, infant and childhood characteristics, and childhood cardiovascular measures in relation to LVEDV (adjusted for sex) are presented in Table 5.7. Univariate analyses of maternal, infant and childhood characteristics, and childhood cardiovascular measures in relation to LVEDV (adjusted for sex and heart rate) are presented in Appendix 4.

Table 5.7 Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular end-diastolic volume (ml) at age 9 years (adjusted for sex).

		LV end-diastolic volume (ml)			LV end-diastolic volume (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	0.18	-0.25, 0.61	0.4	0.09	-0.23, 0.41	0.59
Currently smoking	309	1.7	-1.31, 4.71	0.27	-0.54	-2.81, 1.72	0.64
Woman's ethnic group (2 groups) ref white	309	-10.83	-17.7, -3.92	<0.01	-6.44	-11.7, -1.22	0.02
Social class – 3 groups (ref Professional)	309						
Skilled non-manual / manual		-1.06	-3.90, 1.78	0.47	-1.42	-3.55, 0.71	0.19
Partly skilled / unskilled		-0.62	-4.80, 3.57	0.77	1.33	-1.81, 4.47	0.41
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		-3.12	-7.76, 1.51	0.19	-3.68	-7.15, -0.21	0.04
Secondary education		-1.27	-4.07, 1.53	0.37	-1.47	-3.57, 0.62	0.17
How much stress in daily living in last 4 weeks	309	-0.9	-2.15, 0.35	0.16	-1.15	-2.09, -0.22	0.02
Assessment of general health	309	-1.44	-3.12, 0.24	0.09	-1.44	-2.70, -0.18	0.03
Stress in life affected health	309	-1.59	-2.88, -0.30	0.02	-1.26	-2.23, -0.29	0.01
GHQ > 13	141	2.16	-1.91, 6.24	0.3	2.06	-1.19, 5.32	0.21
GHQ score Likert	141	0.16	-0.25, 0.57	0.44	0.07	-0.25, 0.40	0.66
Long-standing illness	309	-0.87	-3.89, 2.15	0.57	-0.86	-3.13, 1.41	0.46
Frequency of strenuous exercise per week, two groups	307	1.37	-1.27, 4.01	0.31	1.73	-0.25, 3.71	0.09
Height (cm)	308	0.44	0.24, 0.63	<0.001	0.08	-0.07, 0.23	0.29
Body mass index (kg/m²)	307	-0.16	-0.45, 0.13	0.29	-0.32	-0.53, -0.10	<0.01
Mid-upper arm circumference (cm)	306	-0.08	-0.42, 0.26	0.65	-0.38	-0.63, -0.12	<0.01
Triceps skinfold (mm) (average of three closest)	308	-0.06	-0.24, 0.12	0.5	-0.14	-0.28, -0.01	0.04
Biceps skinfold (mm) (average of three closest)	308	-0.08	-0.30, 0.13	0.45	-0.23	-0.39, -0.07	0.01
Subscapular skinfold (mm) (average of three closest)	308	-0.07	-0.20, 0.06	0.31	-0.11	-0.21, -0.01	0.03
Sum of skinfolds (mm)	308	-0.01	-0.06, 0.03	0.56	-0.04	-0.07, -0.00	0.03
Arm muscle area (cm²)	306	-0.02	-0.16, 0.11	0.75	-0.14	-0.24, -0.04	0.01
Waist-Hip ratio	306	-14.72	-37.38, 7.95	0.2	-16.11	-33.11, 0.88	0.06

Exposure	n	LV end-diastolic volume (ml)			LV end-diastolic volume (normalised to BSA) (ml/m²)		
		β	95% CI	p value	β	95% CI	p value
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.03	-0.01, 0.07	0.19	0.03	-0.00, 0.06	0.09
Late pregnancy Retinol (Vitamin A) (umol/l)	249	0.55	-3.71, 4.81	0.8	1.59	-1.74, 4.92	0.35
Early pregnancy: oily fish portions/week	252	-0.17	-1.42, 1.08	0.79	0.14	-0.81, 1.08	0.78
Late pregnancy: oily fish portions/week	299	-0.04	-1.14, 1.05	0.94	0.35	-0.47, 1.18	0.4
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-0.99	-3.68, 1.70	0.47	-0.01	-2.04, 2.02	0.99
EPDS: EPDS score	303	0	-0.24, 0.23	0.97	0.02	-0.15, 0.20	0.8
Paternal							
Height (cm)	295	0.20	0.01, 0.38	0.04	-0.09	-0.23, 0.05	0.22
Birth weight (g)	263	0	-0.00, 0.00	0.15	0	-0.00, 0.00	0.54
Infant							
Birth weight (kg)	307	4.88	2.53, 7.24	<0.001	1.44	-0.37, 3.25	0.12
Placental weight (g)	286	0.01	-0.00, 0.02	0.13	0	-0.01, 0.01	0.88
Ponderal index (kg/m³)	300	0.38	-0.17, 0.93	0.18	0.37	-0.05, 0.78	0.08
Age last breast fed, (months)	300	0.11	-0.14, 0.35	0.39	0.26	0.08, 0.44	<0.01
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	10.67	4.20, 17.13	<0.01	2.69	-2.47, 7.85	0.3
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	7.27	-2.08, 16.61	0.13	2.31	-4.95, 9.57	0.53
4 year DXA: Total lean (kg), adjusted for sex	212	4.41	3.53, 5.30	<0.001	0.72	-0.08, 1.52	0.08
4 year DXA: Total fat (kg), adjusted for sex	212	2.81	1.44, 4.17	<0.001	-0.86	-1.91, 0.19	0.11
Child at 9 years							
Height (cm)	309	0.95	0.78, 1.12	<0.001	0	-0.16, 0.15	0.95
Weight (kg)	309	0.91	0.74, 1.08	<0.001	-0.16	-0.31, -0.01	0.04
BMI (kg/m²)	309	1.46	0.98, 1.94	<0.001	-0.50	-0.88, -0.13	<0.01
Total lean mass (SD)	271	6.73	5.59, 7.86	<0.001	0.16	-0.91, 1.23	0.77
Total fat mass (SD)	271	3.27	1.91, 4.63	<0.001	-2.05	-3.11, -1.00	<0.001
Child cardiovascular measures							
Systolic BP (mmHg)	286	0.16	0.01, 0.32	0.03	-0.03	-0.15, 0.08	0.58
Diastolic BP (mmHg)	286	-0.08	-0.27, 0.11	0.41	-0.08	-0.23, 0.06	0.25

Exposure	n	LV end-diastolic volume (ml)			LV end-diastolic volume (normalised to BSA) (ml/m²)		
		β	95% CI	p value	β	95% CI	p value
Pulse pressure (mmHg)	286	0.19	0.05, 0.33	<0.01	0.03	-0.08, 0.14	0.59
Mean arterial pressure (estimated) (mmHg)	286	0.04	-0.17, 0.25	0.73	-0.09	-0.25, 0.07	0.26
Mean arterial pressure (measured) (mmHg)	169	-0.03	-0.24, 0.17	0.76	-0.1	-0.26, 0.06	0.23
Heart rate (beats per minute)	309	-0.34	-0.46, -0.21	<0.001	-0.30	-0.39, -0.21	<0.001
Aortic distensibility (10^{-3}mmHg^{-1})	286	0.01	-0.31, 0.34	0.93	0.22	-0.02, 0.47	0.07
Pulse wave velocity (m/s)	308	-0.97	-3.72, 1.79	0.49	-3.18	-5.22, -1.13	<0.01

5.3.1 Paternal influences

Taller paternal height was associated with greater LVEDV (β = 0.20 ml/cm, [95% CI 0.01, 0.38], p=0.04, n=295). There was no association after LVEDV was adjusted for child's BSA. There was no association with father's birth weight.

5.3.2 Maternal influences

With regard to mother's educational attainment, children of mothers who had none or minimal qualifications had lower LVEDV normalised to BSA when compared to those whose mothers had HND or degrees (β = -3.68 ml, [95% CI -7.15, -0.21], p=0.04, n=309). Children of mothers who had secondary education had similar LVEDV to those born to mothers with HND or degrees. There was no association with LVEDV non-normalised.

Greater stress in life affecting maternal health was associated with lower LVEDV (β =-1.59 ml/level, [95% CI -2.88, -0.30], p=0.02, n=309). Children of mothers who reported increased stress in life affecting health and higher levels of stress in daily living, had lower LVEDV normalised to BSA (β = -1.26 ml/level, [95% CI -2.23, -0.29], p=0.01, n=309) and (β = -1.15 ml/level, [95% CI -2.09, -0.22], p=0.02, n=309) respectively (Figure 5.3).

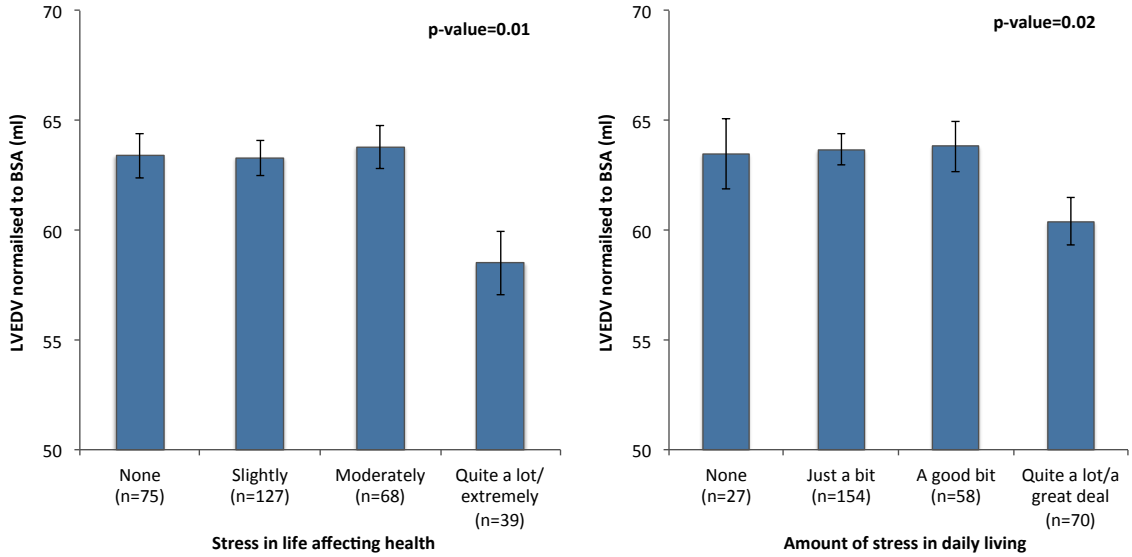

Figure 5.3 Maternal assessment of amount of stress in life affecting health (left) and amount of stress in daily living (right) in relation to child's left ventricular end-diastolic volume (normalised to body surface area).

Children of mothers who had poorer general health had lower LVEDV (β = -1.44 ml/level, [95% CI -2.70, -0.18], p=0.03, n=309) (Figure 5.4)

Taller maternal height was associated with greater LVEDV (β = 0.44 ml/cm, [95% CI 0.24, 0.63], p<0.001, n=308), but not after LVEDV was normalised to BSA.

Greater maternal BMI was not associated with LVEDV but was associated with lower LVEDV when normalised to BSA (β = -1.15 (ml/m^2)/(kg/m^2), [95% CI -0.53, -0.10], p<0.01, n=307) (Figure 5.5). Similarly greater measures of mid-upper arm circumference, triceps skinfold, biceps skinfold, subscapular skinfold, sum of skinfolds, and arm muscle area were not associated with LVEDV but were associated with lower LVEDV when normalised to BSA (β = -0.38 (ml/m^2)/cm, [95% CI -0.63, -0.12], p<0.01, n=306), (β = -0.14 (ml/m^2)/mm, [95% CI -0.28, -0.01 p=0.04, n=308), (β = -0.23 (ml/m^2)/mm, [95% CI -0.39, -0.07], p<0.01, n=308), (β = -0.11 (ml/m^2)/mm, [95% CI -0.21, -0.01], p=0.03, n=308), (β = -0.04 (ml/m^2)/mm, [95% CI -0.07, -0.001], p=0.03, n=308) and (β = -0.14 (ml/m^2)/cm^2 , [95% CI -0.24, -0.04], p<0.01, n=306) respectively.

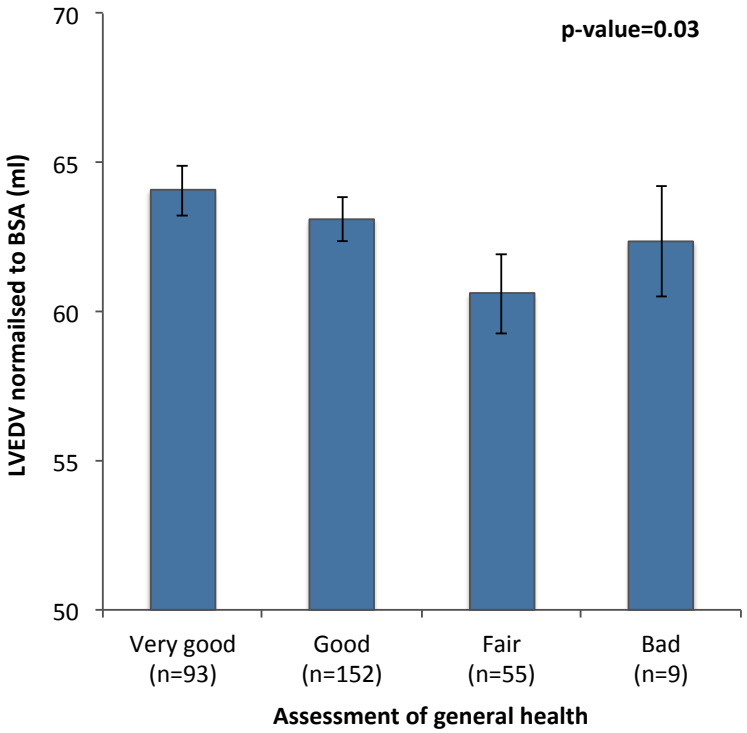

Figure 5.4 Maternal assessment of general health in relation to left ventricular end-diastolic volume in the offspring at age 9 years (normalised to body surface area).

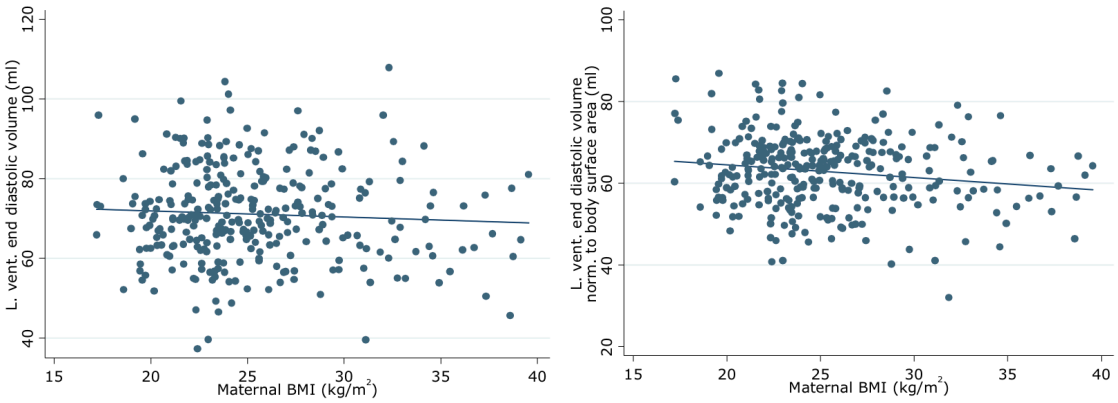

Figure 5.5 Maternal BMI in relation to left ventricular end-diastolic volume (left) and left ventricular end-diastolic volume normalised to child's body surface area (right).

5.3.3 Childhood - cardiovascular measures

Higher SBP and higher PP were associated with greater LVEDV (β = 0.16 ml/mmHg, [95% CI 0.01, 0.32], p=0.03, n=286) and (β = 0.19 ml/mmHg, [95% CI 0.05, 0.33], p<0.01, n=286) respectively. This association was independent of heart rate but did not persist when LVEDV was normalised to BSA. There was no association with DBP.

Faster heart rate was associated with lower LVEDV, and lower LVEDV normalised to BSA (β = -0.34 ml/bpm, [95% CI -0.46, -0.21], p<0.001, n=309) and (β = -0.30 (ml/m^2)/bpm [95% CI -0.39, -0.21], p<0.001, n=309).

There was no LVEDV association with arterial distensibility or PWV, however greater PWV (stiffer arteries) was associated with lower LVEDV normalised to BSA (β = -0.97 (ml/m^2)/(m/s), [95% CI -5.22, -1.13], p<0.01, n=308).

5.3.4 Childhood – size and body composition

Heavier birth weight was associated with greater LVEDV (β = 4.88 ml/kg, [95% CI 2.53, 7.20], p<0.001, n=307) (Figure 5.6) but there was no association with LVEDV normalised to BSA. There was no association with placental weight.

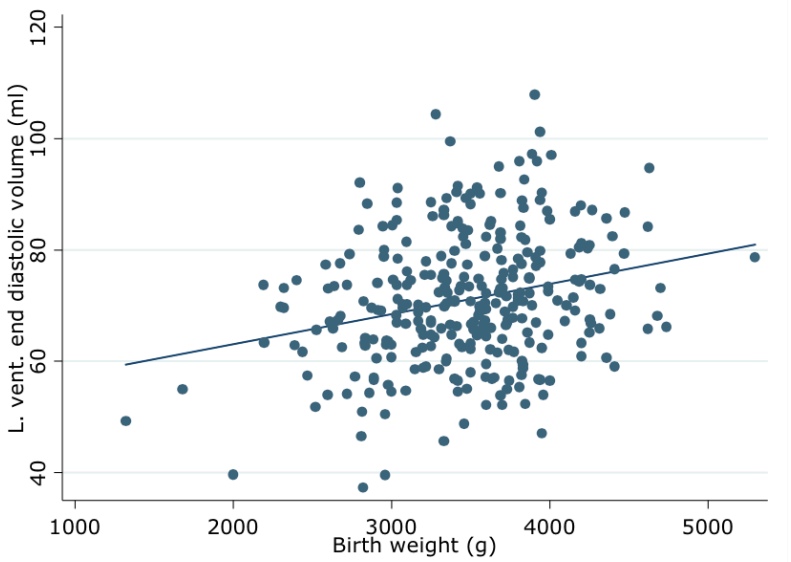

Figure 5.6 Scatter plot showing birth weight in relation to left ventricular end-diastolic volume at 9 years of age.

Greater total lean mass at birth, 4 years and 9 years was associated with greater LVEDV (β = 10.67 ml/kg, [95% CI 4.20, 17.13], p<0.01, n=135), (β = 4.41ml/kg, [95% CI 3.53, 5.30], p<0.001, n=212) and (β = 6.73 ml/SD, [95% CI 5.59, 7.86], p<0.001, n=271) respectively (Figure 5.7). There was no association with LVEDV when normalised to BSA.

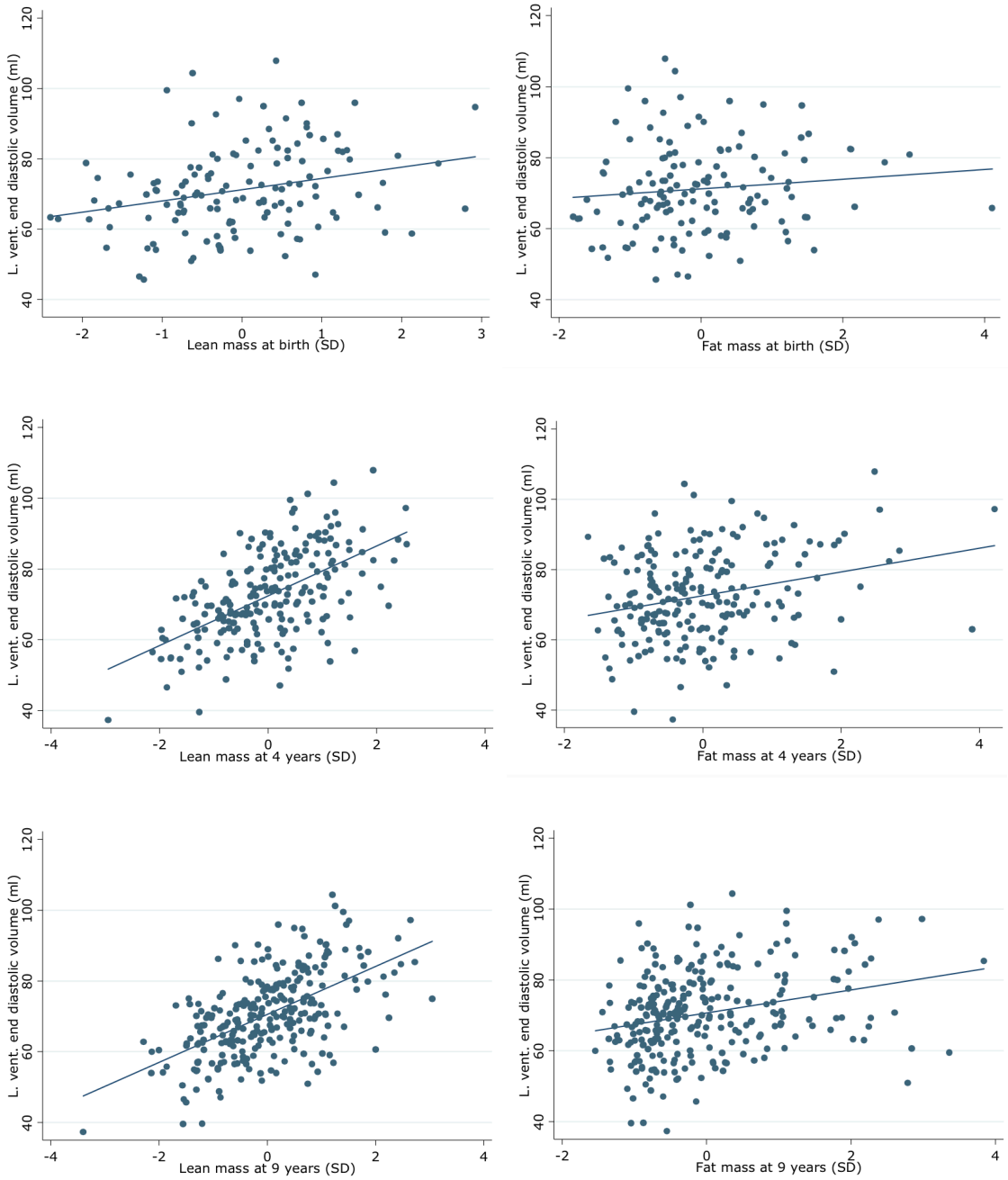

Figure 5.7 Scatter plots showing lean and fat mass, measured at birth, 4 and 9 years of age, in relation to left ventricular end-diastolic volume.

There was no association with fat mass measured at birth, however greater fat mass measured at both 4 and 9 years was associated with greater LVEDV (β = 2.81ml/kg, [95% CI 1.44, 4.17], p<0.001, n=212) and (β = 3.27 ml/SD, [95% CI 1.91, 4.63], p<0.001, n=271) respectively (Figure 5.7). Greater fat mass at 9 years was associated with smaller LVEDV normalised to BSA (β = -2.05 (ml/m^2)/SD, [95% CI -3.11, -1.0], p<0.001, n=271).

Taller height, heavier weight and greater BMI were associated with greater LVEDV (β = 0.95 ml/cm, [95% CI 0.78, 1.12], p<0.001, n=309), (β = 0.91 ml/kg, [95% CI 0.74, 1.08], p<0.001, n=309), and (β = 1.46 ml/ (kg/m^2), [95% CI 0.98, 1.94], p<0.001, n=309) respectively. Heavier weight and Greater BMI were associated with lower LVEDV when normalised to BSA (β = -0.16 (ml/m^2)/kg, [95% CI 0.77, 1.09], p<0.001, n=309) and (β = -0.50 (ml/m^2)/ (kg/m^2), [95% CI -0.88, -0.13], p<0.01, n=309) respectively. There was no association with height.

5.4 LV Stroke Volume

Table 5.8 presents results of linear regression models examining the associations of maternal, infant and childhood characteristics, and childhood cardiovascular characteristics, with LVSV (adjusted for sex). Associations of maternal, infant and childhood characteristics and childhood characteristics with LVSV (adjusted for sex and heart rate) are presented in Appendix 5.

Table 5.8 Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular stroke volume (ml) at age 9 years (adjusted for sex).

		LV stroke volume (ml)			LV stroke volume (normalised to BSA) (ml/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	-0.1	-0.42, 0.23	0.55	-0.14	-0.39, 0.11	0.27
Currently smoking	309	0.56	-1.71, 2.84	0.63	-0.84	-2.58, 0.90	0.34
Woman's ethnic group, 2 groups	309	-6.17	-11.41, -0.93	0.02	-3.29	-7.32, 0.74	0.11

		LV stroke volume (ml)			LV stroke volume (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		-1.48	-3.62, 0.65	0.172	-1.58	-3.21, 0.047	0.057
Partly skilled / unskilled		-0.60	-3.75, 2.54	0.705	0.91	-1.49, 3.31	0.457
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		-4.04	-7.51, -0.56	0.023	-4.18	-6.82, -1.53	0.002
Secondary education		-0.99	-3.08, 1.11	0.356	-1.15	-2.75, 0.44	0.156
How much stress in daily living in last 4 weeks	309	-0.48	-1.42, 0.46	0.32	-0.7	-1.42, 0.02	0.06
Assessment of general health	309	-0.85	-2.12, 0.42	0.19	-0.87	-1.84, 0.10	0.08
Stress in life affected health	309	-1.15	-2.13, -0.18	0.02	-0.91	-1.65, -0.16	0.02
GHQ > 13	141	1.37	-1.85, 4.6	0.4	1.26	-1.33, 3.84	0.34
GHQ score Likert	141	0.16	-0.16, 0.48	0.33	0.09	-0.17, 0.35	0.49
Long-standing illness	309	-0.46	-2.74, 1.81	0.69	-0.38	-2.12, 1.37	0.67
Frequency of strenuous exercise per week, two groups	307	0.76	-1.23, 2.75	0.45	0.96	-0.56, 2.48	0.22
Height (cm)	308	0.27	0.12, 0.42	<0.001	0.03	-0.08, 0.15	0.59
Body mass index (kg/m²)	307	-0.11	-0.33, 0.11	0.33	-0.22	-0.38, -0.05	<0.01
Mid-upper arm circumference (cm)	306	-0.1	-0.36, 0.16	0.44	-0.30	-0.49, -0.10	<0.01
Triceps skinfold (mm) (average of three closest)	308	-0.05	-0.18, 0.09	0.49	-0.10	-0.21, -0.00	0.05
Biceps skinfold (mm) (average of three closest)	308	-0.06	-0.22, 0.11	0.49	-0.16	-0.28, -0.03	0.01
Subscapular skinfold (mm) (average of three closest)	308	-0.05	-0.15, 0.05	0.35	-0.08	-0.15, -0.00	0.05
Sum of skinfolds (mm)	308	-0.01	-0.04, 0.03	0.66	-0.02	-0.05, 0.00	0.06
Arm muscle area (cm²)	306	-0.04	-0.14, 0.06	0.45	-0.11	-0.19, -0.04	<0.01
Waist-Hip ratio	306	-5.26	-22.34,11.81	0.54	-7.05	-20.08, 5.99	0.29
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.02	-0.02, 0.05	0.31	0.02	-0.01, 0.04	0.19
Late pregnancy Retinol (Vitamin A) (umol/l)	249	-0.58	-3.82, 2.67	0.73	0.15	-2.43, 2.73	0.91
Early pregnancy: oily fish portions/week	252	0.04	-0.90, 0.99	0.93	0.23	-0.51, 0.96	0.54
Late pregnancy: oily fish portions /week	299	-0.07	-0.89, 0.76	0.87	0.2	-0.43, 0.84	0.53
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-1.06	-3.09, 0.97	0.31	-0.26	-1.83,1.30	0.74

		LV stroke volume (ml)			LV stroke volume (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
EPDS: EPDS score	303	-0.05	-0.23, 0.12	0.55	-0.02	-0.16, 0.11	0.75
Paternal							
Height (cm)	295	0.14	0.00, 0.28	0.05	-0.05	-0.16, 0.05	0.32
Birth weight (g)	263	0	-0.00, 0.00	0.31	0	-0.00, 0.00	0.81
Infant							
Birth weight (kg)	307	3.57	1.80,5.34	<0.001	1.23	-0.15, 2.61	0.08
Placental weight (g)	286	0	-0.00, 0.01	0.31	0	-0.01, 0.01	0.63
Ponderal index (kg/m³)	300	0.37	-0.04, 0.78	0.08	0.35	0.04, 0.67	0.03
Age last breast fed, (months)	300	0.11	-0.08, 0.29	0.25	0.21	0.07, 0.35	<0.01
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	7.56	2.25, 12.87	0.01	1.96	-2.26, 6.17	0.36
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	5.99	-1.61, 13.60	0.12	2.43	-3.48, 8.35	0.42
4 year DXA: Total lean (kg), adjusted for sex	212	2.75	2.04, 3.45	<0.001	0.23	-0.38, 0.84	0.45
4 year DXA: Total fat (kg), adjusted for sex	212	1.92	0.90, 2.94	<0.001	-0.58	-1.38, 0.22	0.16
Child at 9 years							
Height (cm)	309	0.69	0.57,0.82	<0.001	0.03	-0.08, 0.15	0.6
Weight (kg)	309	0.66	0.53,0.79	<0.001	-0.08	-0.20, 0.03	0.15
Body mass index (kg/m²)	309	1.05	0.68,1.41	<0.001	-0.31	-0.60, -0.02	0.03
Total lean mass (SD)	271	4.48	3.57,5.39	<0.001	-0.04	-0.86, 0.78	0.93
Total fat mass (SD)	271	2.42	1.39,3.45	<0.001	-1.29	-2.11, -0.47	<0.01
Child cardiovascular measures							
Systolic BP (mmHg)	286	0.20	0.09,0.32	<0.001	0.06	-0.03, 0.15	0.21
Diastolic BP (mmHg)	286	-0.05	-0.19,0.09	0.49	-0.05	-0.16, 0.06	0.37
Pulse pressure (mmHg)	286	0.20	0.09,0.30	<0.001	0.08	-0.01, 0.16	0.07
Mean arterial pressure (estimated) (mmHg)	286	0.08	-0.08,0.24	0.3	-0.01	-0.13, 0.12	0.92
Mean arterial pressure (measured) (mmHg)	169	0.04	-0.12,0.19	0.65	-0.01	-0.13, 0.11	0.83
Heart rate (beats per minute)	309	-0.24	-0.33, -0.14	<0.001	-0.21	-0.28, -0.14	<0.001
Aortic distensibility (10^{-3}mmHg^{-1})	286	-0.09	-0.34,0.15	0.46	0.06	-0.13, 0.26	0.51
Pulse wave velocity (m/s)	308	0.46	-1.62,2.53	0.66	-1.23	-2.82, 0.36	0.13

5.4.1 Paternal influences

Children of taller fathers tended to have higher LVSV (β = 0.14 ml/cm, [95% CI 0.00, 0.28], p=0.05, n=295). This association was attenuated when LVSV was adjusted for child's BSA.

5.4.2 Maternal influences

Children of mothers who had no, or minimal, qualifications had lower LVSV and LVSV normalised to BSA when compared to those whose mothers had HND or degrees (β = -4.04 ml/level, [95% CI -7.51, -0.56], p=0.023, n=309) and (β = -4.18 (ml/m^2)/level, [95% CI -6.82, -1.53], p=0.002, n=309) respectively. Children of mothers who had secondary education had similar LVSV to those born to mothers with HND or degrees.

Increased stress in life affecting health was associated with lower LVSV and LVSV normalised to BSA (β = -1.15 ml/level, [95% CI -2.13, -0.18], p=0.02, n=309) and (β = -0.91 (ml/m^2)/level, [95% CI -1.65, -0.16], p=0.02, n=309) respectively. This relationship persisted when adjusted for heart rate.

Children of taller mothers had greater LVSV (β = 0.27 ml/cm, [95% CI 0.12, 0.42], p<0.001, n=308). There was no association when LVSV normalised to BSA.

Children of mothers with greater BMI had lower LVSV normalised to BSA (β = -0.22 (ml/m^2)/(kg/m^2), [95% CI -0.38, -0.05], p<0.01, n=307).

Greater maternal measures of mid upper arm circumference, triceps skinfold, biceps skinfold, subscapular skinfold, and arm muscle area were associated with lower LVSV normalised to BSA (β = -0.3 (ml/m^2)/cm, [95% CI -0.38, -0.05], p<0.01, n=306), (β = -0.10 (ml/m^2)/mm, [95% CI -0.49, -0.10], p=0.05, n=308), (β = -0.16 (ml/m^2)/mm, [95% CI -0.28, -0.03], p=0.01, n=308), (β = -0.08 (ml/m^2)/mm, [95% CI -0.15, -0.001], p=0.05, n=308) and (β = -0.11 (ml/m^2)/cm^2 [95% CI -0.19, -0.04], p<0.001, n=306) respectively.

5.4.3 Childhood - cardiovascular measures

Higher SBP and PP were associated with larger LVSV (β = 2.42ml/mmHg, [95% CI 0.09, 0.32], p<0.001, n=286) and (β = 0.20 ml/mmHg, [95% CI 0.09, 0.30],

p<0.001, n=286) respectively. There was no association with LVSV normalised to BSA. However higher SBP and PP were associated with larger LVSV normalised to BSA when adjusted for heart rate (β = 0.09 (ml/m^2)/mmHg, [95% CI 0.001, 0.17], p=0.05, n=286) and (β=0.08 (ml/m^2)/mmHg, [95% CI 0.001, 0.16], p<0.01, n=286) respectively. There were no associations with DBP.

Faster heart rate was associated with lower LVSV and lower LVSV normalised to BSA (β = -0.24 ml/bpm, [95% CI -0.33, -0.14], p<0.001, n=309) and (β=-0.21 (ml/m^2)/bpm, [95% CI -0.28, -0.14], p<0.001, n=309) respectively.

There was no association with measures of arterial stiffness.

5.4.4 Childhood – size and body composition

Heavier birth weight was associated with larger LVSV (β = 3.57 ml/kg [95% CI 1.8, 5.34], p<0.001, n=307); this was independent of heart rate but there was no association with LVSV normalised to BSA.

Greater lean mass at birth, 4 and 9 years were associated with greater LVSV (β = 7.56 ml/kg, [95% CI 2.25, 12.87], p<0.01, n=135), (β = 2.75 ml/kg, [95% CI 2.04, 3.45], p<0.001, n=212), and (β = 4.48 ml/SD, [95% CI 3.57, 5.39], p<0.001, n=271) respectively. When LVSV was normalised to BSA there was no association.

There was no association with fat mass at birth but greater fat mass at 4 and 9 years was associated with larger LVSV (β = 1.92 ml/kg, [95% CI 0.90, 2.94], p<0.001, n=212) and (β = 2.42 ml/SD, [95% CI 1.39, 3.45], p<0.001, n=271) respectively. Greater fat mass at 9 years was associated with lower LVSV normalised for BSA (β = -1.29 (ml/m^2)/SD [95% CI -2.11, -0.47], p<0.01, n=271).

Taller child's height, heavier weight and greater BMI were associated with greater LVSV (β = 0.69 ml/cm, [95% CI 0.57, 0.82], p<0.001, n=309), (β = 0.66 ml/kg [95% CI 0.53, 0.79], p<0.001, n=309) and (β = 1.05ml/(kg/m^2), [95% CI 0.68, 1.41], p<0.001, n=309) respectively. Greater BMI was associated with lower LVSV normalised to BSA (β = -0.31 (ml/m^2)/(kg/m^2), [95% CI -0.6, -0.02], p<0.001, n=309). There was no association with height and weight.

5.5 LV Cardiac Output

Univariate analyses of maternal, infant and childhood characteristics, and childhood cardiovascular measures in relation to LVCO (adjusted for sex) are presented in Table 5.9

Table 5.9 Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular cardiac output (ml) at age 9 years (adjusted for sex).

		LV cardiac output (l/min)			LV cardiac output (normalised to BSA) (l/min/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	-0.01	-0.03, 0.02	0.7	-0.01	-0.03, 0.01	0.41
Currently smoking	309	0.03	-0.15, 0.21	0.76	-0.07	-0.21, 0.07	0.31
Woman's ethnic group, 2 groups	309	-0.31	-0.73, 0.11	0.15	-0.08	-0.40, 0.25	0.64
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		-0.12	-0.29, 0.05	0.17	-0.13	-0.26, -0.002	0.047
Partly skilled / unskilled		-0.11	-0.37, 0.14	0.37	0.01	-0.18, 0.20	0.931
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		-0.27	-0.55, 0.013	0.06	-0.29	-0.5, -0.07	0.008
Secondary education		-0.03	-0.195, 0.14	0.76	-0.06	-0.19, 0.07	0.378
How much stress in daily living in last 4 weeks	309	0.03	-0.05, 0.10	0.5	0.01	-0.05, 0.06	0.86
Assessment of general health	309	0.04	-0.06, 0.14	0.45	0.02	-0.05, 0.10	0.54
Stress in life affected health	309	-0.03	-0.11, 0.05	0.48	-0.01	-0.07, 0.05	0.66
GHQ > 13	141	0.14	-0.11, 0.40	0.26	0.14	-0.06, 0.33	0.17
GHQ score Likert	141	0.01	-0.01, 0.04	0.25	0.01	-0.01, 0.03	0.32
Long-standing illness	309	0.04	-0.14, 0.23	0.64	0.03	-0.11, 0.17	0.63
Frequency of strenuous exercise per week, two groups	307	0.03	-0.13, 0.19	0.73	0.05	-0.07, 0.17	0.44
Height (cm)	308	0.02	0.00, 0.03	0.01	0	-0.01, 0.01	0.59
Body mass index	307	-0.01	-0.02, 0.01	0.53	-0.01	-0.03, -0.00	0.04
Mid-upper arm circumference (cm)	306	-0.01	-0.03, 0.01	0.43	-0.02	-0.04, -0.01	<0.01
Triceps skinfold (mm) (average of three closest)	308	0	-0.01, 0.01	0.51	-0.01	-0.02, 0.00	0.06
Biceps skinfold (mm) (average of	308	0	-0.02, 0.01	0.73	-0.01	-0.02, 0.00	0.05

Exposure	n	LV cardiac output (l/min) β	95% CI	p value	LV cardiac output (normalised to BSA) (l/min/m²) β	95% CI	p value
three closest)							
Subscapular skinfold (mm) (average of three closest)	308	0	-0.01, 0.00	0.42	-0.01	-0.01, 0.00	0.08
Sum of skinfolds (mm)	308	0	-0.00, 0.00	0.65	0	-0.00, 0.00	0.08
Arm muscle area (cm^2)	306	0	-0.01, 0.01	0.5	-0.0	-0.02, -0.00	<0.01
Waist-Hip ratio	306	-0.37	-1.74, 1.01	0.6	-0.45	-1.50, 0.61	0.41
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0	-0.00, 0.00	0.45	0	-0.00, 0.00	0.34
Late pregnancy Retinol (Vitamin A) (umol/l)	249	-0.08	-0.34, 0.18	0.56	-0.02	-0.22, 0.19	0.87
Early pregnancy: oily fish portions/week	252	-0.03	-0.11, 0.05	0.42	-0.01	-0.07, 0.05	0.72
Late pregnancy: oily fish portions/week	299	-0.03	-0.10, 0.03	0.33	-0.01	-0.06, 0.04	0.79
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-0.07	-0.23, 0.10	0.42	0	-0.13, 0.12	0.96
EPDS: EPDS score	303	-0.01	-0.02, 0.01	0.25	-0.01	-0.02, 0.01	0.35
Paternal							
Height (cm)	295	0	-0.01, 0.02	0.48	-0.01	-0.02, -0.00	0.02
Birth weight (g)	263	0	-0.00, 0.00	0.85	0	-0.00,0.00	0.55
Infant							
Birth weight (kg)	307	0.30	0.16, 0.44	<0.001	0.11	-0.00, 0.22	0.05
Placental weight (g)	286	0	-0.00, 0.00	0.16	0	-0.00, 0.00	0.94
Ponderal index (kg/m^3)	300	0.03	-0.00, 0.06	0.06	0.03	0.00, 0.05	0.02
Age last breast fed, (months)	300	0	-0.01, 0.02	0.9	0.01	-0.00, 0.02	0.07
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	0.62	0.21, 1.03	<0.01	0.17	-0.16, 0.50	0.31
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	0.43	-0.16, 1.02	0.15	0.14	-0.32, 0.60	0.55
4 year DXA: Total lean (kg), adjusted for sex	212	0.20	0.14, 0.26	<0.001	0	-0.05, 0.05	0.87
4 year DXA: Total fat (kg), adjusted for sex	212	0.15	0.07, 0.24	<0.001	-0.03	-0.10, 0.03	0.31
Child at 9 years							
Height (cm)	309	0.05	0.04, 0.06	<0.001	0	-0.01, 0.01	0.57
Weight (kg)	309	0.05	0.04, 0.06	<0.001	0	-0.01, 0.01	0.39

Exposure	n	LV cardiac output (l/min)			LV cardiac output (normalised to BSA) (l/min/m²)		
		β	95% CI	p value	β	95% CI	p value
Body mass index (kg/m²)	309	0.10	0.07, 0.13	<0.001	-0.01	-0.03, 0.02	0.48
Total lean mass (SD)	271	0.32	0.25, 0.40	<0.001	-0.03	-0.10, 0.04	0.36
Total fat mass (SD)	271	0.25	0.17, 0.34	<0.001	-0.04	-0.11, 0.02	0.2
Child cardiovascular measures							
Systolic BP (mmHg)	286	0.02	0.01, 0.03	<0.001	0.01	0.00, 0.02	0.02
Diastolic BP (mmHg)	286	0	-0.01, 0.02	0.54	0	-0.01, 0.01	0.58
Pulse pressure (mmHg)	286	0.01	0.01, 0.02	<0.01	0.01	-0.00, 0.01	0.12
Mean arterial pressure (estimated) (mmHg)	286	0.02	0.00, 0.03	0.02	0.01	-0.00, 0.02	0.14
Mean arterial pressure (measured) (mmHg)	169	0.01	-0.00, 0.02	0.16	0	-0.00, 0.01	0.33
Heart rate (beats per minute)	309	0.03	0.02, 0.03	<0.001	0.02	0.02, 0.03	<0.001
Aortic distensibility (10^{-3}mmHg^{-1})	286	-0.03	-0.05, -0.01	<0.01	-0.01	-0.03, 0.00	0.12
Pulse wave velocity (m/s)	308	0.26	0.10, 0.43	<0.01	0.1	-0.03, 0.23	0.13

5.5.1 Paternal influences

Children of taller fathers had lower cardiac output adjusted to BSA (β = -0.01 (l/min/m²)/cm, [95% CI -0.02, -0.00], p=0.02). There was no association with child's birth weight.

5.5.2 Maternal influences

Children of mothers with lower educational attainment had lower CO. When compared to children of mothers who had a HND or degree, children of mothers who had minimal or no qualifications had lower CO normalised for BSA (β = -0.29 l/min, [95% CI -0.5, -0.07], p=0.008) children of mothers who had secondary education had similar CO to those of mothers who had HND or degrees (β = -0.06 l/min, [95% CI -0.19, 0.07], p=0.378).

Children of taller mothers had greater CO (β = 0.02 (l/min)/cm [95% CI 0.001, 0.03], p=0.01, n=308). There was no association with CO normalised to BSA.

Greater maternal BMI, mid-upper arm circumference, biceps skinfold and arm muscle area were associated with lower CO normalised to BSA (β=0.01 (l/min/m^2)/(kg/m^2), [95% CI -0.03, -0.00], p=0.04, n=30307), (β = -0.02 (l/min/m^2)/cm, [95% CI -0.04, -0.01], p<0.01, n=306), (β = -0.01 (l/min/m^2)/mm, [95% CI -0.02, 0.00], p=0.05, n=308), and (β = -0.01 (l/min/m^2) /cm^2, [95% CI -0.02, -0.00], p<0.01, n=306) respectively. Greater measures of triceps skinfold, subscapular skinfold and sum of skinfolds were not significant but tended to have lower CO normalised to BSA.

5.5.3 Childhood - cardiovascular measures

Unsurprisingly, faster heart rate was associated with greater CO (β = 0.03 (l/min)/bpm, [95% CI 0.02, 0.03, p<0.001, n=309). Higher SBP, PP and estimated MAP were associated with greater CO (β = 0.02 (l/min)/mmHg, [95% CI 0.01, 0.0.03, p<0.001, n=286), (β = 0.01 (l/min)/mmHg, [95% CI 0.01, 0.02], p<0.01, n=286) and (β = 0.02 (l/min)/mmHg, [95% CI 0.00, 0.03], p=0.02, n=286) respectively. When CO was normalised to BSA there was no association with PP or MAP but the associations with heart rate and SBP persisted.

Greater arterial stiffness (lower distensibility, and higher PWV) was associated with higher CO (β = -0.103 (l/min)/$10^{-3}$$mmHg^{-1}$, [95% CI -0.05, -0.01], p<0.01, n=286) and (β = 0.26 (l/min)/(m/s), [95% CI 0.10, 0.43], p<0.01, n=308), respectively. These associations did not persist when CO was adjusted for heart rate. Greater arterial stiffness (lower distensibility and higher PWV) was associated with faster heart rate (β = -0.30 bpm/($10^{-3}$$mmHg^{-1}$), [95% CI -0.58, -0.01], p=0.04, n=301), and (β = 5.5 bpm/(m/s), [95% CI 3.39, 7.68], p<0.001, n=337) respectively.

5.5.4 Childhood – size and body composition

Heavier birth weight was associated with greater CO and greater CO normalised to BSA (β = 0.30 (l/min)/kg [95% CI 0.16, 0.44], p<0.001, n=307) and (β = 0.11 (l/min/m^2)/kg, [95% CI -0.00, 0.22], p=0.05, n=307) respectively.

Greater lean mass at birth, 4 and 9 years were associated with greater CO (β = 0.62 (l/min)/kg, [95% CI 0.21, 1.03], p<0.01, n=135), (β =0.20 (l/min)/kg [95% CI0.14, 0.26], p<0.001, n=212), and (β = 0.32 (l/min)/SD, [95% CI 0.25, 0.40],

p<0.001, n=271) respectively. These associations did not persist when CO normalised to BSA.

There was no association with fat mass at birth but greater fat mass at 4 and 9 years was associated with greater CO (β = 0.15 (l/min)/kg, [95% CI 0.07, 0.24], p<0.001, n=212) and (β = 0.25 (l/min)/SD, [95% CI 0.17, 0.34], p<0.001, n=271) respectively. These associations did not persist when CO was normalised to BSA.

Taller height, heavier weight and greater BMI at 9 years were associated with greater CO (β = 0.05 (l/min)/cm, [95% CI0.04, 0.06], p<0.001, n=309), (β = 0.05 (l/min)/kg, [95% CI 0.04, 0.06], p<0.001, n=309) and (β = 0.10 (l/min)/(kg/m^2), [95% CI 0.07, 0.13 p<0.001, n=309) respectively. No associations were apparent when CO normalised to BSA.

5.6 LV Ejection Fraction

Univariate analyses of maternal, infant and childhood characteristics, and childhood cardiovascular measures in relation to LVEF (adjusted for sex), and LVEF adjusted for heart rate, are presented in Table 5.10.

Table 5.10 Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular ejection fraction (ml) at age 9 years (adjusted for sex, and adjusted for sex and heart rate).

Exposure	n	LV ejection fraction (%)			LV ejection fraction (%) (adjusted for heart rate)		
		β	95% CI	p value	β	95% CI	p value
Maternal: pre-pregnancy							
Townsend index	304	-0.32	-0.53, -0.10	<0.01	-0.32	-0.53, -0.10	<0.01
Currently smoking	309	-0.82	-2.35, 0.70	0.29	-0.82	-2.35, 0.70	0.29
Woman's ethnic group, 2 groups (ref white)	309	2.24	-1.29, 5.77	0.21	2.25	-1.29, 5.78	0.21
Social class - 3 groups (ref Professional)	303						
Skilled non-manual / manual		-0.99	-2.42, 0.43	0.17	-0.10	-2.42, 0.43	0.17
Partly skilled / unskilled		-0.17	-2.27, 1.93	0.87	-0.18	-2.28, 1.92	0.87
Qualification level - 3 groups (ref	309						

Exposure	n	LV ejection fraction (%)			LV ejection fraction (%) (adjusted for heart rate)		
		β	95% CI	p value	β	95% CI	p value
HND or degree)							
None / CSE		-2.80	-5.13, -0.48	0.02	-2.80	-5.13, -0.48	0.02
Secondary education		-0.34	-1.74, 1.06	0.63	-0.34	-1.74, 1.06	0.64
How much stress in daily living in last 4 weeks	309	0.18	-0.46, 0.81	0.58	0.18	-0.45, 0.81	0.57
Assessment of general health	309	0.2	-0.65, 1.05	0.65	0.21	-0.64, 1.06	0.63
Stress in life affected health	309	-0.11	-0.77, 0.55	0.74	-0.11	-0.77, 0.55	0.74
GHQ > 13	141	-0.33	-2.56, 1.91	0.77	-0.33	-2.56, 1.91	0.77
GHQ score Likert	141	0.05	-0.17, 0.27	0.66	0.05	-0.17, 0.27	0.66
Long-standing illness	309	0.26	-1.27, 1.78	0.74	0.26	-1.26, 1.79	0.73
Frequency of strenuous exercise per week, two groups	307	-0.35	-1.68, 0.99	0.61	-0.35	-1.68, 0.99	0.61
Height (cm)	308	-0.03	-0.13, 0.07	0.51	-0.03	-0.13, 0.07	0.51
Body mass index (kg/m^2)	307	-0.01	-0.16, 0.14	0.92	-0.01	-0.15, 0.14	0.93
Mid-upper arm circumference (cm)	306	-0.07	-0.24, 0.10	0.42	-0.07	-0.24, 0.10	0.42
Triceps skinfold (mm)	308	-0.01	-0.10, 0.08	0.9	-0.01	-0.10, 0.09	0.91
Biceps skinfold (mm)	308	0	-0.11, 0.11	1	0	-0.11, 0.11	1
Subscapular skinfold (mm)	308	0	-0.07, 0.07	0.99	0	-0.07, 0.07	1
Sum of skinfolds (mm)	308	0	-0.02, 0.02	0.86	0	-0.02, 0.02	0.86
Arm muscle area (cm^2)	306	-0.04	-0.11, 0.03	0.28	-0.04	-0.11, 0.03	0.28
Waist-Hip ratio	306	5.74	-5.69,17.17	0.32	5.78	-5.65, 17.21	0.32
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0	-0.03, 0.02	0.68	0	-0.03, 0.02	0.68
Late pregnancy Retinol (Vitamin A) (umol/l)	249	-1.55	-3.80, 0.70	0.17	-1.55	-3.80, 0.70	0.18
Early pregnancy: oily fish portions/week	252	0.26	-0.38, 0.90	0.42	0.26	-0.38, 0.90	0.43
Late pregnancy: oily fish portions/week	299	-0.05	-0.61, 0.51	0.86	-0.05	-0.61, 0.51	0.86
EPDS: Depression EPDS > 11	303	-0.59	-1.96, 0.78	0.4	-0.59	-1.96, 0.78	0.4
EPDS: EPDS score	303	-0.07	-0.19, 0.05	0.24	-0.07	-0.19, 0.05	0.24
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-0.59	-1.96, 0.78	0.4	-0.59	-1.96, 0.78	0.4
EPDS: EPDS score	303	-0.07	-0.19, 0.05	0.24	-0.07	-0.19, 0.05	0.24
Paternal							
Height (cm)	295	0.01	-0.08, 0.11	0.76	0.01	-0.08, 0.11	0.77
Birth weight (g)	263	0	-0.00, 0.00	0.61	0	-0.00, 0.00	0.61
Infant							

Exposure	n	LV ejection fraction (%)			LV ejection fraction (%) (adjusted for heart rate)		
		β	95% CI	p value	β	95% CI	p value
Birth weight (kg)	307	0.35	-0.87, 1.57	0.57	0.35	-0.87,1.57	0.57
Placental weight (g)	286	0	-0.01, 0.00	0.49	0	-0.01, 0.00	0.49
Ponderal index (kg/m^3)	300	0.15	-0.13, 0.42	0.3	0.15	-0.13, 0.42	0.3
Age last breast fed, (months)	300	0.05	-0.07, 0.18	0.42	0.05	-0.07, 0.17	0.42
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	0.08	-3.29, 3.46	0.96	0.09	-3.29, 3.46	0.96
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	1.35	-3.38, 6.08	0.57	1.35	-3.38, 6.08	0.57
4 year DXA: Total lean (kg), adjusted for sex	212	-0.37	-0.87, 0.13	0.15	-0.37	-0.87, 0.13	0.15
4 year DXA: Total fat (kg), adjusted for sex	212	0.02	-0.64, 0.69	0.94	0.02	-0.64, 0.69	0.94
Child at 9 years							
Height (cm)	309	0.07	-0.03, 0.17	0.16	0.07	-0.03, 0.17	0.16
Weight (kg)	309	0.06	-0.04, 0.16	0.27	0.06	-0.04, 0.16	0.27
Body mass index (kg/m^2)	309	0.07	-0.18, 0.33	0.59	0.07	-0.18, 0.33	0.58
Total lean mass (SD)	271	-0.16	-0.87, 0.54	0.65	-0.17	-0.87, 0.54	0.64
Total fat mass (SD)	271	0.29	-0.43, 1.00	0.43	0.29	-0.42, 1.01	0.42
Child's cardiovascular measures							
Systolic BP (mmHg)	286	0.12	0.05, 0.20	<0.01	0.13	0.05, 0.20	<0.01
Diastolic BP (mmHg)	286	0.01	-0.09, 0.11	0.82	0.01	-0.09, 0.11	0.81
Pulse pressure (mmHg)	286	0.09	0.02, 0.16	0.02	0.09	0.02, 0.16	0.02
Mean arterial pressure (estimated) (mmHg)	286	0.09	-0.02, 0.20	0.11	0.09	-0.02, 0.20	0.11
Mean arterial pressure (measured) (mmHg)	169	0.09	-0.01, 0.19	0.09	0.09	-0.01, 0.19	0.09
Heart rate (beats per minute)	309	-0.01	-0.07, 0.06	0.87	0	-0.07, 0.07	0.98
Aortic distensibility (10^{-3}mmHg^{-1})	286	-0.13	-0.30, 0.04	0.13	-0.13	-0.30, 0.04	0.12
Pulse wave velocity (m/s)	308	1.51	0.14, 2.88	0.03	1.53	0.16, 2.90	0.03

5.6.1 Paternal influences

There were no associations with paternal height or birth weight.

5.6.2 Maternal influences

Higher maternal Townsend Index (greater deprivation) was associated with lower EF in the child (β = -0.32%, [95% CI -0.53, -0.10], p<0.01, n=304).

Higher educational attainment was associated with higher EF; children of mothers who had minimal or no qualifications had lower EF when compared to those of mothers with HND or degree (β = -2.8%, [95% CI -5.13, -0.48], p=0.02). There was no association with measures of maternal size. Adjusting for heart rate had little effect on the findings.

5.6.3 Childhood - cardiovascular measures

Higher SBP and PP were associated with higher EF (β = 0.12 % /mmHg, [95% CI 0.05, 0.54], p<0.01, n=286) and (β = 0.09 %/mmHg, [95% CI 0.02, 0.16], p=0.02, n=286) respectively.

Greater PWV (stiffer arteries) was associated with higher EF (β = 1.51 %/(m/s), [95% CI 0.14, 2.88], p=0.03, n=308). These associations persisted following adjustment for heart rate.

5.6.4 Childhood – size and body composition

There were no associations with child's size at birth, 4 years of age, or at 9 years of age at time of MRI.

5.7 Discussion

This study investigated the associations between developmental influences (maternal, infant and childhood characteristics) and measures of cardiac structure and function, assessed using CMR.

5.7.1 Summary of findings

An overview of maternal, infant and childhood characteristics in relation to childhood measures of LV structure and function measured at 9 years of age is presented in Table 5.11, with bold arrows indicating the direction of findings significant to p<0.05, and smaller arrows indicating the trend of findings significant at p=0.05 to p<0.2 showing that following adjustment for child's

body surface area the significance of many findings was attenuated, however the trend remained.

Table 5.11 Overview of maternal, infant and childhood characteristics in relation to LV measures of structure and function. (⬇⬆ significant at p<0.05, ↓↑ p = 0.05 to 0.2). (n) = values normalised to child's body surface area. LVM = LV mass, LVEDV = LV end-diastolic volume, LVSV = LV stroke volume, LVCO = LV cardiac output, LVEF = LV ejection fraction.

Exposure	**LVM**	**LVM (n)**	**LVEDV**	**LVEDV (n)**	**LVSV**	**LVSV (n)**	**LVCO**	**LVCO (n)**	**LVEF**
Maternal pre-pregnancy									
Smoking	↓								
Lower educational attainment			↓	⬇	⬇	⬇	↓	⬇	⬇
Lower social class	↓			↓	↓	↓	↓	⬇	↓
Higher Townsend Index									⬇
Greater stress affecting health			⬇	⬇	⬇	⬇			
Greater Stress in daily living	↓	⬇	↓	⬇		↓			
Poorer general health	↓	⬇	↓	⬇	↓	↓			
Increased exercise frequency				↑					
Taller height	⬆		⬆		⬆		⬆		
Greater BMI				⬇		⬇		⬇	
Greater waist: hip ratio		↓		↓					
Greater arm circumference		↓		⬇		⬇		⬇	
Greater arm muscle area		↓		⬇		⬇		⬇	
Greater Biceps skinfold		⬇		⬇		⬇		⬇	
Greater Triceps skinfold		↓		⬇		⬇		↓	
Greater Subscapular skinfold									
Greater sum of skin folds		↓		⬇		↓		↓	

Exposure	LVM	LVM (n)	LVEDV	LVEDV (n)	LVSV	LVSV (n)	LVCO	LVCO (n)	LVEF
Paternal									
Taller height	↑↑		↑↑		↑↑			↓↓	
Greater birth weight			↑						
Maternal: pregnancy									
Higher vitamin D status			↑	↑		↑			
Higher vitamin A status								↓	
Maternal: postnatal									
EPDS >11	↓↓	↓↓							
Higher EPDS	↓								
Infant									
Greater birth weight	↑↑		↑↑	↑	↑↑	↑	↑↑	↑↑	
Greater Ponderal index		↑	↓	↑	↑	↑↑	↑	↑↑	
Greater placental weight			↓				↑		
Shorter duration of breastfeeding				↓↓		↓↓		↓	
Greater total lean mass at birth	↑↑		↑↑		↑↑		↑↑		
Greater total lean mass at 4 years	↑↑	↑↑	↑↑	↑	↑↑		↑↑		↓
Total fat mass at birth			↓		↑		↑		
Greater total fat mass at 4 years	↑↑		↑↑	↓	↑↑	↓	↑↑		
Child at 9 years									
Greater total lean mass	↑↑	↓↓	↑↑		↑↑		↑↑		
Greater total fat mass	↑↑	↓↓	↑↑	↓↓	↑↑	↓↓	↑↑		
Taller height	↑↑		↑↑		↑↑		↑↑		↑
Heavier weight	↑↑		↑↑	↓↓	↑↑	↓	↑↑		
Greater BMI	↑↑		↑↑	↓↓	↑↑	↓↓	↑↑		
Childhood cardiovascular measures									
Higher MAP (E)							↑↑	↑	↑
Higher MAP (M)							↑		↑
Higher systolic BP	↑↑		↑↑		↑↑		↑↑	↑↑	↑↑

Exposure	LVM	LVM (n)	LVEDV	LVEDV (n)	LVSV	LVSV (n)	LVCO	LVCO (n)	LVEF
Higher pulse pressure	↑	↓	↑		↑	↑	↑	↑	↑
Faster heart rate	↓	↓	↓	↓	↓	↓	↑	↑	
Lower distensibility	↑			↓			↑	↑	↑
Higher PWV				↓		↓	↑	↑	↑

Main findings of the study are summarised below:

- Left ventricular mass and volumes were smaller in non-white children.
- Left ventricular volumetric measures were strongly associated with sex, measures being greater in males than in females, and were greater after adjustment for body size.
- Adjusting for sex, increased maternal stress perceived to be affecting health was associated with smaller LVEDV (and after adjusting for BSA), and greater stress in general life was associated with smaller LV mass when normalised to BSA, smaller LVEDV, smaller LVSV, and smaller LVSV adjusted for BSA.
- Adjusting for sex, poor self-reported maternal health was associated with smaller LV mass when normalised to BSA and smaller LVEDV. Children of mothers scoring greater than 11 on the EPDS had smaller LV mass when normalised to BSA.
- Adjusting for sex, lower maternal educational attainment was associated with smaller LVEDV, LVSV, LVSV normalised to BSA, and lower LVCO.
- Adjusting for sex, maternal size was related to LV volumetric measures. Taller maternal height was associated with greater LV mass, LVEDV, LVSV and LVCO but not when normalised to BSA. Greater BMI and larger arm measures (skin fold thickness and arm muscle area) were associated with smaller LVEDV, LVSV and LVCO when all were normalised to BSA.
- Adjusting for sex, child's size at birth and 9 years was positively associated with LV mass and volumes, however when normalised to BSA, heavier weight and greater BMI was associated with lower LVEDV and LVSV. Total lean mass measured at birth, 4 and 9 years was associated with greater LV mass and volumes. Total fat mass measured at 4 and 9

years was associated with greater LV mass and volumes. However when LV measures were normalised to BSA total fat mass at 9 years was associated with smaller LV mass, LVEDV and LVSV.

- Adjusting for sex, higher SBP was associated with greater LV mass, LVEDV, LVSV and CO. Faster heart rate was associated with lower LV mass, LVEDV and LVSV when all were normalised to BSA.
- Lower aortic root distensibility (greater arterial stiffness) was associated with greater LV mass and increased LVCO. Higher PWV (greater arterial stiffness) was associated with lower LVEDV.

5.7.2 Ethnicity

Children of non-white mothers tended to have smaller measures of LV mass and small LV volumes. Published reference values in adults suggest that LV volumes are lower in Indian-Asian populations when compared to European populations (Chahal et al., 2012). However, as the children in this study were predominantly white Caucasian, only 11 (3.2%) were of non-white descent, the study was not adequately powered to address this issue further.

5.7.3 Sex

All left ventricular volumetric and functional measures with the exception of ejection fraction were smaller in females. These sex differences are similar to those reported in adults where volumetric measures EDV and LV mass were greater in men than in women with no differences in global EF regardless of adjustment for BSA (Salton et al., 2002).

Sex differences in fetal growth are well recognised (Lubchenco et al., 1963). Ultrasound measurements in both the second and third trimesters have shown that female fetuses have a slower growth trajectory than males throughout pregnancy (Melamed et al., 2013). Thus the difference in heart size in males would appear to track from early development.

5.7.4 Maternal stress and health

Children of mothers reporting higher chronic stress levels, and higher levels of stress affecting their health, had smaller hearts. Higher self-perceived levels of on-going maternal stress in daily living over a period of 4 weeks pre-

conception was associated with smaller measures of LV mass and LVEDV in the offspring in relation to current body size. Although not significant, there was a weak trend for smaller heart size (LV mass and LVEDV) in the child with higher levels of maternal stress as demonstrated in Table 5.11. Greater stress experienced over time is associated with poor health, and smaller heart mass and volumes were measured in children of mothers reporting poorer health. Stress is linked with depression and children of mothers scoring higher than 11 on the EPDS scale had smaller LV mass when normalised to BSA.

Maternal stress was self-reported pre-pregnancy; the women were asked to rate the amount of stress they had experienced in daily living over the last 4 weeks from 1 to 5 with 1 being none and 5 a great deal. This provided a self-perceived measure of chronic stress. Salivary cortisol measures would have provided a more objective measure of maternal stress, however 'stress' is a matter of perception and self-reporting is considered an accurate indication of level of stress. In fact, findings in the ALSPAC longitudinal cohort study in the UK showed that self-reported maternal anxiety at 32 weeks gestation predicted cortisol concentrations in children at the age of 10 years (O'Connor et al., 2005).

Exposure to stress provokes a classic endocrine response in the secretion of glucocorticoids (GCs) (Sapolsky et al., 2000) causing a rise in plasma GC levels. In humans the main GC is cortisol, which functions as the primary hormonal mediator of stress. Cortisol enhances an organism's resistance to stress by suppressing non-essential functions in the fight or flight response. Glucocorticoids are known to regulate growth and development, and fetal cortisol strongly influences cellular and organ differentiation (Challis et al., 2001). GCs therefore play a vital role in normal fetal development and facilitate maturational changes in fetal organ systems, which can be induced prematurely by exogenous GC administration (Ballard, 1979, Fowden, 1995). Dexamethasone is often administered to accelerate lung maturation and the production of surfactant necessary for extra-uterine lung function in the infant in threatened preterm delivery. Studies suggest that excess exposure to adrenal glucocorticoids (glucocorticoids released by the adrenal gland in response to stress) during development is associated with an increased risk of cardiovascular disease in later life (Harris and Seckl, 2011, Cottrell and Seckl, 2009, Meaney et al., 2007) due to developmental cardiovascular adaptations

leading to cardiovascular dysfunction in later life. Findings in this research suggest that stress-induced increases in maternal and fetal cortisol levels may accelerate cardiac maturation resulting in smaller heart mass and volumes in the offspring.

Although there is a strong correlation between maternal and fetal cortisol levels in humans (de Vries et al., 2006), GC levels in the maternal circulation are 5-10 times greater than those in the fetal circulation. Under normal conditions most of the cortisol crossing the placenta is converted to biologically inactive cortisone and the fetus is protected from the growth inhibiting effects of maternal GCs (Edwards et al., 1993). Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-OHSD2), which converts physiological GCs to inactive products, acts to protect the fetus from exposure to the growth inhibiting effects of maternal GC levels. Placental 11β-OHSD2 does not however provide a complete barrier to maternal GCs. Exposure to higher levels of maternal GCs is associated with attenuated 11β-OHSD2 activity and increased permeability of the placenta to cortisol results in higher fetal GC levels (Benediktsson et al., 1993). Thus higher maternal stress may result in fetal exposure to higher cortisol levels and the associated growth inhibiting effects.

Growth of fetal myocardium is achieved by replication of cardiomyocytes throughout gestation. Terminal differentiation occurs in late pregnancy when cardiomyocytes become binucleated and stop dividing – the number of cells at birth is therefore the number of cells for the lifespan (Thornburg et al., 2011). As there is little evidence for cardiomyocyte renewal in humans (Bergmann et al., 2009) the number of cardiomyocytes in the adult heart is determined by factors that influence the rate of terminal differentiation. A heart with fewer cardiomyocytes at birth is more vulnerable when subjected to increased workloads due to normal physiological demands of growth and physical activity or in response to pathologic processes. A reduction in the number of cardiomyocytes limits the capacity for cardiac growth in adulthood and renders the heart more susceptible to any pathological processes associated with an increase in cardiac demand. In keeping with this theory neonatal rats exposed to dexamethasone showed a marked suppression of cardiomyocyte proliferation on days 2 and 4 (de Vries et al., 2006) and cardiomyocyte hypertrophy by week 50 (de Vries et al., 2002). Similarly, in maternal protein

restricted models a reduced heart size is often found in younger rats (Nutter et al., 1979, Corstius et al., 2005) and cardiac hypertrophy is seen at 3 and 11 months of age (Manning and Vehaskari, 2001, Cheema et al., 2005).

Porello et al. (2009) demonstrated that the hypertrophic rat heart in adulthood is associated with cardiac growth restriction at birth due to excess cardiomyocyte attrition during critical growth windows, and postulate that the identification of determinants of physiological growth of the heart in early life may permit early therapeutic intervention to prevent cardiac hypertrophy in later life. Crispi et al. (2010, 2014) reported structural changes in the cardiac ventricles, and a significant reduction in LVSV at the age of 5 years in children who had experienced intrauterine growth restriction. They also noted increased heart rates, compensating for the less efficient hearts. In the SWS higher levels of stress affecting maternal health were associated with significantly lower birth weights (β = -14.69 [95% CI -216.87, -419.04], p=0.036, n=309), however whilst lower birth weight was associated with smaller LV mass at 9 years, there was no association in relation to the child's body size at 9 years of age.

It should be considered that lower physical activity in the child might in part explain smaller measures of LV mass. Lower levels of physical activity in children is associated with higher levels of parental stress (Stenhammar et al., 2010) and with lower LV mass (DeMaria et al., 1978).

Exposure to multiple psychosocial stressors during pregnancy has been linked to higher systolic and diastolic BP and higher MAP in children aged 5-7 years (van Dijk et al., 2012). More objective measures of stress during pregnancy have shown that higher maternal salivary cortisol in early pregnancy is associated with increased vascular resistance and lower arterial elasticity in children of the same age (Rondo et al., 2010).

Tomanek et al. (1999) reported that the rate of coronary vascularisation during development is regulated by the magnitude of cardiac growth, inferring that there are vascular repercussions of smaller heart size. MRI measures of increased arterial stiffness (lower distensibility and higher PWV) in young adults whose mothers had received antenatal steroids suggest that there may be on going vascular effects in response to GC exposure (Kelly et al., 2012). Interestingly, the same study found no association with LV measures. Cardiac

images in that study were acquired using prospectively gated sequences and therefore not acquiring data across the entire cardiac cycle, which may explain these findings.

Rat studies have shown that offspring of mothers exposed to the stress of protein deficiency had smaller hearts, reduced numbers of cardiomyocytes and an increase in cardiac interstitial fibrosis (Corstius et al., 2005, Lim et al., 2006). Maternal psychosocial factors responsible for increased stress levels are often associated with higher levels of inflammatory markers (van Dijk et al., 2012) which may explain the associated increase in fibrosis.

Few studies relate pre-pregnancy stress to cardiovascular measures in the offspring. A study by Taal et al. (2013) found that both higher maternal and higher paternal psychological distress during pregnancy were associated with lower childhood left ventricular mass at the age of 6 years but found no association with BP or PWV. They did not measure LV volumes, however concluded that the association could be due to unmeasured social and environmental factors although they did not investigate this further.

There is limited knowledge, and conflicting results in the literature on the implications of exposure to increased cortisol levels in response to maternal stress for fetal cardiovascular development. The timing of stressors in development is crucial and may explain the differences in findings. In this research stress levels were deemed chronic and on going and may have influenced cardiac development throughout gestation rather than during shorter specific exposures to endogenous or exogenous GCs reported in studies to date.

In summary, although this is a complicated area, I conclude that my data suggest that increased fetal cortisol exposure as a result of maternal stress could lead to premature cardiac maturation in the offspring and have important implications for cardiac structure and function and associated cardiovascular risk in later life.

5.7.5 Maternal education

Lower maternal educational attainment is associated with smaller heart size. Educational attainment in women is strongly linked with diet (Robinson et al.,

2004), which may explain the link to smaller heart size in the offspring and potentially poorer cardiac health. In this study there was a lack of association of LV structure and function with maternal diet in terms of oily fish consumption and late pregnancy vitamin D status. Oily fish consumption and maternal vitamin D status in late pregnancy are associated with large artery structure (see sections 4.2.1 and 4.3.2 respectively) but have no influence yet on heart structure and function at the age of 9 years.

5.7.6 Maternal size

When corrected for size by indexing to body surface area heart size was smaller in children of 'fatter' mothers.

Obesity rates are rising in both developed and developing countries. Trend data from the Health Survey for England (HSE) shows an increase in the prevalence of obesity (defined as BMI>30kg/m^2) in women of childbearing age (16-44 years) from 1993 to 2013 (HSE, 2014). Maternal obesity pre and during pregnancy is associated with impaired cardiometabolic health in the offspring in both childhood (Gaillard et al., 2014) and in young adults (Hochner et al., 2012, Drake and Reynolds, 2010), increasing the risk of CVD in later life.

Evidence from both human and animal studies suggests that maternal obesity has an adverse effect on the cardiovascular health of offspring. Maternal obesity has been linked with hypertension, endothelial dysfunction, dyslipidaemia, insulin resistance and greater adiposity in the offspring (Hochner et al., 2012, Fraser et al., 2010, Samuelsson et al., 2008), however the physiological mechanisms explaining this are not clear.

Animal studies have demonstrated structural changes in both the heart and vasculature of offspring following maternal overnutrition (Samuelsson et al., 2008). Huang et al. (2010b) found that maternal obesity in sheep was associated with increased collagen accumulation in the myocardium and suggest that this accumulation of connective tissue and myocardial fibrosis and associated impaired function could lead to cardiac dysfunction in later life. Although there was no association with LV mass and maternal size, this may explain the smaller volumes measured in children of mothers with higher BMIs.

There was no association between maternal BMI and childhood BP, however BP changes may manifest later in the life course as a consequence of vascular dysfunction and increased arterial stiffness.

5.7.7 Childhood - cardiovascular measures

Higher BP was associated with larger cardiac measures. Faster heart rate was associated with smaller cardiac measures in relation to body size. This may indicate a compensatory mechanism to maintain sufficient cardiac output. This compensatory mechanism has allowed the child to achieve the cardiac output required by the physiological demands of growth. At the age of 9 years the child has not yet been exposed to increased demand required by pathological changes, for example aortic stenosis. It is reasonable to speculate that a smaller, less efficient, heart, whilst compensating at this age with an increase in heart rate in order to maintain acceptable cardiac output, will be more susceptible to hypertrophic changes when exposed to increased demand with aging, and the acquisition of CV risk burden with age.

5.7.8 Childhood – size and body composition

Low birth weight is linked with increased risk of CVD. Low birth weight was associated with smaller hearts. Bigger children (taller, heavier, and greater BMI at 9 years) had larger hearts. This suggests tracking of child size and heart size across the life course. LV mass has a strong positive linear association with age, weight, height and BP (Malcolm et al., 1993) and up to 50% of the variation in LV dimensions in adults may be accounted for by body size (Pelliccia et al., 1999). Measures of LV mass, LVEDV, LVSV, LVCO were therefore indexed for BSA as previously described in order to assess LV structural and functional measures independently of the effect of body size. Unsurprisingly the effect of birth weight, and of height and weight at 9 years, on LV volumes was attenuated when adjusted for BSA.

Greater total lean mass measured at birth, 4 years and 9 years was associated with larger hearts, but similar to measures of child's size, there was no association when normalised to BSA. Children who had greater total fat mass at 4 and 9 years, and greater BMI at 9 years had larger hearts. When cardiac measures were normalised to BSA children who had greater total fat mass at 4

years of age tended to have smaller hearts but the relationship was no longer significant. However children who had greater BMI at 9 years and those who had greater fat mass at 9 years had smaller hearts in relation to body size (following normalisation to BSA). Gaillard et al. (2014) suggest that BMI may not be the most appropriate measure of fat mass in childhood. Maternal BMI is known to be an important determinant of total fat mass in the offspring (Davey Smith et al., 2007) independent of child's BMI.

This conflicts with other studies that found that excess weight (Urbina et al., 1995), and increased lean and fat mass in children were associated with greater LV mass (Daniels et al., 1995, Dai et al., 2009) in relation to body size.

5.7.9 Strengths and weaknesses

The imaging technique employed for the assessment of LV structure and function is a major strength of the study. CMR permits accurate assessment of LV structure and function and has been shown in this study to have high repeatability and reliability in children as described in section 3.1. CMR has high spatial and temporal resolution imaging capabilities, does not use ionizing radiation and is the gold standard method of assessing cardiovascular structure and function. Detailed records of prenatal and perinatal maternal and offspring characteristics were available and included follow-up data in childhood.

Multiple testing is a weakness of this study, which increases the possibility of chance findings. Replication of the research is necessary to confirm the findings in this research.

A potential weakness of the study is that measures of maternal health, stress in life affecting health, and amount of stress in daily living were self-reported and were recorded pre-conception. However these measures give an indication of chronic on-going levels of stress in life. Self-reported measures of anxiety during pregnancy in a large cohort study have been associated with higher salivary cortisol levels in the offspring at the age of 10 years (Talge et al., 2007). Measures of stress (self-reported), or biomarkers such as maternal cortisol levels were not available during pregnancy. However no previous studies have assessed the impact of chronic stress levels from pre-conception on the cardiovascular outcomes of the offspring. I examined postnatal data for

the extensively validated EPDS to examine effects of maternal mental state on child's later cardiac structure and function.

6. Discussion

This thesis reports on research to investigate developmental influences on cardiovascular structure and function in children at the age of 9 years measured using magnetic resonance imaging. Cardiac and aortic MRI protocols were optimised for paediatric imaging, and appropriate image segmentation techniques and tools identified. The influence of maternal (and some paternal) and childhood characteristics on measures of arterial stiffness, and left ventricular structure and function in the child were assessed.

Higher levels of maternal stress, and poorer maternal general health pre-conception were associated with smaller measures of LV mass and volumes in the child at 9 years. Late pregnancy nutrition measures were associated with MRI measures of arterial stiffness in the child; lower maternal oily fish consumption in late pregnancy was associated with higher PWV (increased arterial stiffness). Children of mothers who had lower vitamin D and vitamin A concentrations measured in late pregnancy had stiffer arteries, indicated by lower aortic root distensibility.

6.1 LV function

A standard CMR imaging protocol was optimised for paediatric imaging. Cardiovascular MRI is the gold standard imaging modality for the quantification of cardiovascular structure and function. However, the pursuit of high quality images in MRI requires a constant trade-off between signal-to-noise-ratio, spatial resolution and image acquisition time. Any improvement in one area is achieved at the expense of one or both of the other factors.

Optimisation of the CMRI protocol for use in paediatric research demanded a reduction in image acquisition time in order to achieve realistic and achievable breath hold durations for the SSFP cine acquisitions. Parameter adaptations to minimise breath hold demands were achieved at the expense of both signal-to-noise and spatial (and temporal) resolution. Analysis of repeat measures of LV structure and function in 10 children permitted the assessment of inter and intra-operator coefficients of variation in order to determine protocol accuracy and repeatability, and hence the suitability of the protocol for research measurements in children. Variability between subjects was greater than the

inter-study and inter-observer variability combined for all measures of LV structure and function and the protocol was therefore considered suitable for this research study. Image segmentation tools and techniques were assessed and it was determined that a manual segmentation tool with exclusion of papillary muscles and trabeculae from the blood pool was the most accurate and reliable technique.

The children were imaged using a 5-channel phased array anterior body coil in conjunction with a posterior phased array spine coil. Clinically, at this site, children are imaged using a 32-channel coil which affords greater signal, permitting greater reductions in acquisition time and the use of smaller fields of view, and therefore improved spatial resolution, whilst maintaining acceptable levels of signal. The anterior 16-channel coil is considerably heavier than the body phased array and was not used for this reason. In future it would be advantageous to assess the tolerance for this coil.

6.2 Vascular structure and function

Regional and global arterial stiffness was measured by distensibility and PWV respectively.

Aortic distensibility was measured at a single site, across the aortic root. A high resolution SSFP cine sequence was employed. The increase in spatial resolution unfortunately demanded a longer breath hold duration for the scan, which not all of the children were able to achieve. The aortic root is the most distensible portion of the aorta; distensibility was measured in this portion in an attempt to identify more subtle changes. In future studies it would be valuable to acquire additional measures of distensibility in the descending thoracic, and abdominal aorta in order to map changes in arterial stiffness across the aortic pathway. A cross-section of the descending thoracic aorta was included in the scan however the slice was not angled perpendicular to the long axis of the descending aorta, deeming cross-sectional area measurements here inaccurate and the data was not used.

A velocity encoded phase contrast flow mapping sequence was acquired at three sites in the aorta and PWV, a measure known to be relevant to later CV outcomes (Mitchell et al., 2010), measured across three sections of the aorta,

namely, the arch, descending aorta and entire aorta. Due to difficulties in accurately measuring distance across the aortic arch, measures of PWV across the descending thoracic and abdominal aorta were used in this study. The abdominal portion of the aorta is the site of most lipid deposition (Napoli et al., 1997) and is more likely to be affected by fatty streaks in early life. Further, studies have shown more significant changes in aortic stiffness in the abdominal aorta (Lewandowski et al., 2011), therefore measures of PWV across the descending thoracic and abdominal aorta were more likely to demonstrate changes in aortic stiffness at this age.

Coronary artery imaging proved unfeasible in this study. A 3D navigator sequence, dependent on regular respiration and lack of motion in the child, was performed to acquire coronary data in the first 39 children who were scanned. Objective assessment of these images found that the sequence was able to visually demonstrate the coronary arteries well in 27 of the image datasets. However, the images were not of sufficiently high resolution to permit accurate measurements of coronary artery dimensions. Image quality of the remaining 12 datasets was degraded either by motion or poor positioning of the imaging slab. Coronary artery imaging was thus abandoned following assessment of the initial scans.

6.3 Study Findings

Comprehensive MRI measures of arterial stiffness and cardiovascular structure and function were acquired in 9-year old children, participants in the SWS. These measures were then related to maternal and childhood characteristics in an attempt to determine developmental influences on cardiovascular structure and function, and cardiovascular risk in childhood. Normal variations in maternal nutrition have been linked in this research to measures of arterial stiffness at the age of 9 years, and maternal stress associated with smaller heart size in the child.

6.3.1 The pathway to cardiovascular disease

Cardiovascular disease in later life is not always attributable to current lifestyle or established behavioural and metabolic risk factors including unhealthy diet, physical inactivity and smoking. The extent to which CVD can be attributed to

a genetic predisposition or current lifestyle is not known. It has become clear that the early developmental environment influences the susceptibility of an individual to acquiring cardiovascular disease in later life, although the mechanism by which this occurs is not clear.

Animal studies have highlighted several potential mechanisms by which the developmental environment may influence cardiovascular development and cardiovascular risk in the offspring, namely in response to maternal nutritional challenges and the effect of maternal stress on the intrauterine environment. Increased fetal glucocorticoids in response to maternal stress have been shown to influence cardiac growth in utero. Rodent studies suggest that growth inhibiting effects of glucocorticoids in response to maternal stress induced premature maturation of the heart in the offspring, and therefore a reduction in cardiomyocyte proliferation (de Vries et al., 2006), which was associated with reduced heart size (Corstius et al., 2005). Maternal over and under nutrition has been associated with changes in cardiovascular structure and function in the offspring; altered endothelial function, hypertension and ventricular hypertrophy.

The early developmental environment may induce phenotypic changes through epigenetic processes such as DNA methylation (Low et al., 2011), thereby ‘tuning’ gene expression to produce a phenotype best suited to meet challenges of the predicted later environment (Gluckman and Hanson, 2004b).These epigenetic changes are highly gene-specific (Hanson et al., 2011) and may permit the identification of individuals at increased risk of CVD in later life. The changes in DNA methylation levels, or ‘memory’ of early developmental changes persist throughout life and can manifest as altered disease risk by influencing gene expression in response to challenges later in the life course (Godfrey et al., 2015). A proposed ‘pathway to cardiovascular disease’ is outlined in the flowchart in Figure 6.1, which considers potential influences across the life course from early development to adulthood, which may contribute to the development of cardiovascular disease.

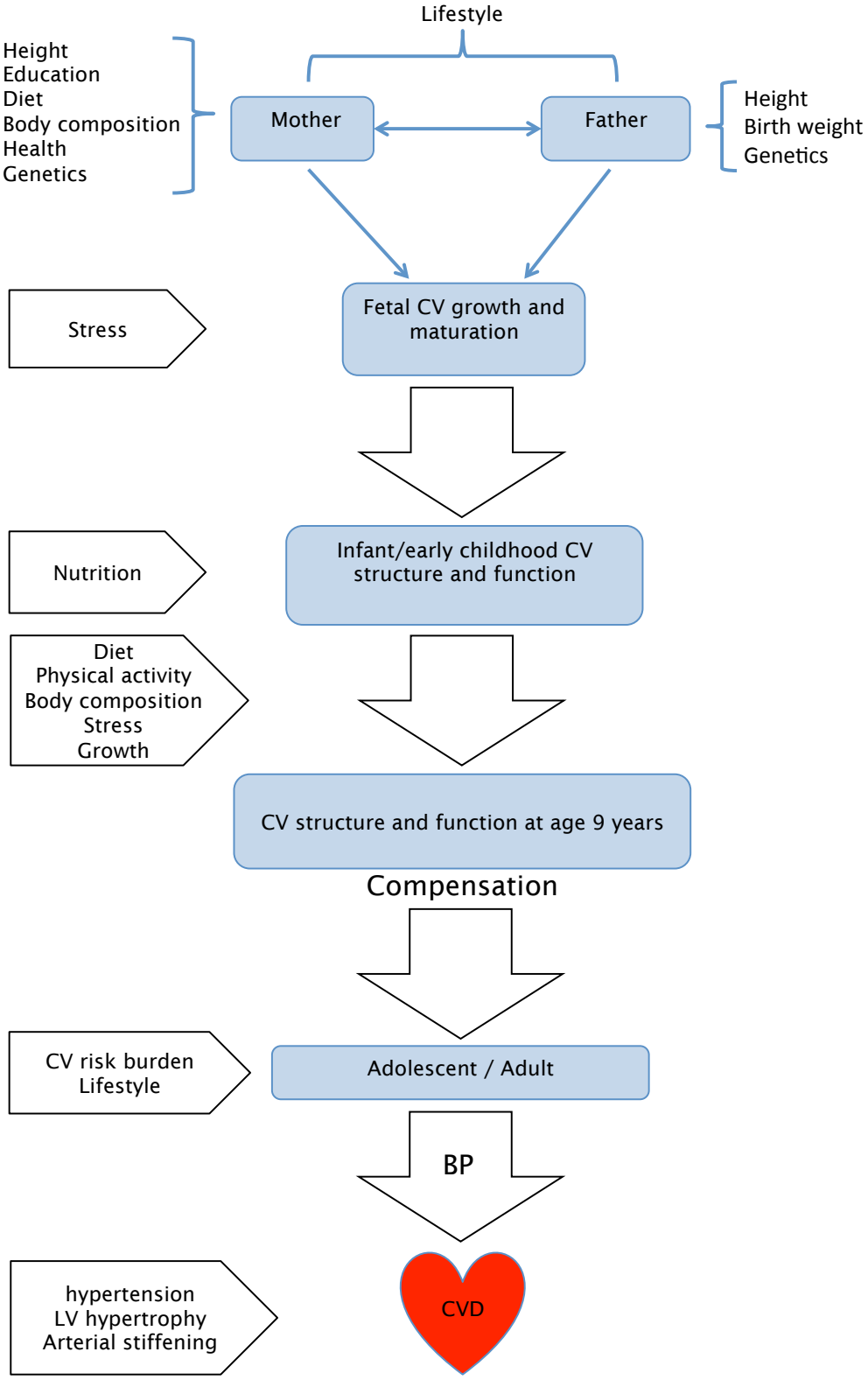

Figure 6.1 The cardiovascular disease pathway depicting potential influences at various time points throughout the life course, which may contribute to cardiovascular disease (CVD).

The incidence of cardiovascular disease cannot be fully explained by influences at the periphery of 'the pathway', namely parental genetic influences in the early phase, and lifestyle and acquired cardiovascular risk burden later in life. Elder et al. (2009) suggest that genetic influences play an important role in development of components of the cardiometabolic syndrome. However, findings in this research suggest that developmental influences may play a significant role in the development of cardiovascular structure and function and therefore on the likelihood of developing cardiovascular disease later in life.

As depicted in Figure 6.1 maternal and paternal factors influence cardiovascular development in utero resulting in changes in cardiovascular structure and function measured by MRI at the age of 9 years. Although measures were within normal reported ranges, increased arterial stiffness, lower distensibility and higher PWV, and smaller LV mass and volumes present as measures of increased cardiovascular risk. Already at this age smaller hearts are associated with higher heart rates suggesting a compensatory mechanism in order to maintain sufficient cardiac output.

The main findings of this research are displayed in Figure 6.2. The modified flowchart of the cardiovascular disease pathway focuses on maternal influences and shows that children of mothers who reported higher levels of perceived stress in daily living from pre-conception had smaller hearts measured by MRI at the age of 9 years. Measures of LV mass and LVEDV in relation to current body size were smaller; suggesting that chronic stress in the mother may influence the rate of fetal cardiac development and maturation. A possible explanation for this would be an increase in maternal, and fetal, cortisol levels in response to higher levels of stress which may accelerate cardiac maturation resulting in smaller heart mass and volumes in the offspring. No previous study has reported on the influence of chronic stress on CV development.

As highlighted in (Figure 6.2), smaller LV mass and LV volumes were associated with faster child heart rates at the age of 9 years suggesting a compensatory mechanism in play an increase in heart rate serves to maintain adequate cardiac output in smaller less efficient hearts. Faster child heart rates were associated with increased arterial stiffness but there was no association

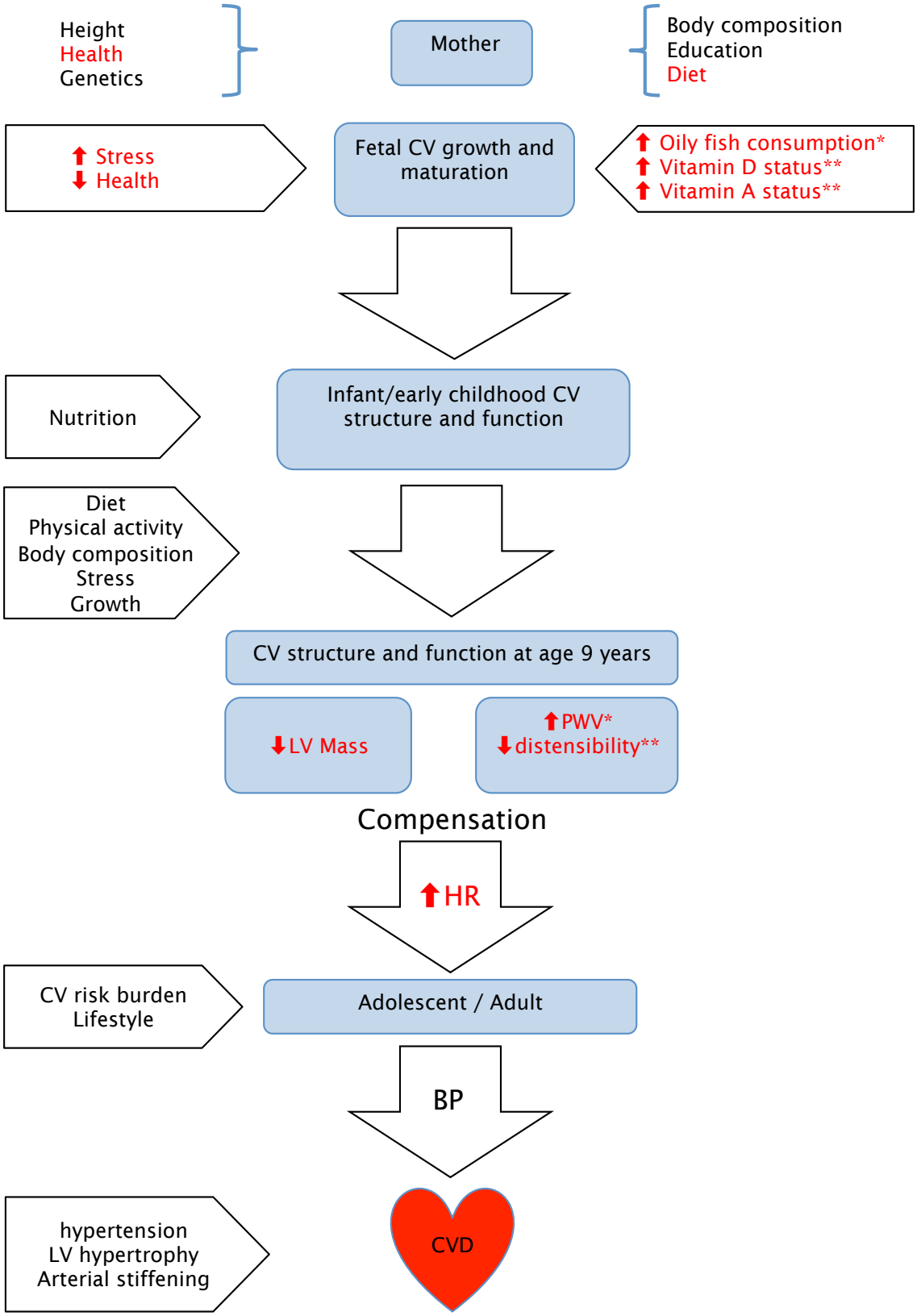

Figure 6.2 A modified cardiovascular disease pathway depicting associations of maternal health and nutrition with MRI measures of LV mass and aortic stiffness.

seen yet between LV mass and volumes, with measures of arterial stiffness. The perceived levels of stress in daily living over a 4-week period were self-reported by the women preconception. 'Stress' may be interpreted differently and thus was a very subjective measure. The time between these measures and conception was not considered and it was assumed that the levels of stress indicated were chronic and on going, not limited to specific life events. Previous studies have shown that exposure to stress at specific time points in utero is associated with changes in cardiac structure in the offspring but no previous study has linked chronic maternal stress levels to cardiac growth in the offspring. In order to investigate this further future studies need to be conducted using objective measures of stress such as measures of salivary cortisol levels at several time points prior to and throughout gestation.

Figure 6.2 further demonstrates that measures of maternal micronutrient status were associated with childhood arterial stiffness. Lower maternal serum vitamin D and lower serum vitamin A concentrations in late pregnancy were associated with lower measures of aortic root distensibility and greater aortic stiffness in the child at 9 years. Similarly lower maternal oily fish consumption in late pregnancy was associated with greater aortic stiffness in the descending aorta (higher PWV). Maternal socioeconomic status and educational attainment were independently associated with child's PWV, with higher educational attainment and social class associated with lower PWV.

It was interesting that the association with vitamin D status and arterial stiffness was limited to aortic root distensibility and there was no association with PWV measures. Conversely the association of oily fish consumption was limited to descending aorta PWV and there was no association with distensibility. Studies have shown that lipid deposits are first seen in the descending aorta and that the abdominal aorta is the first site to demonstrate increased stiffness, which may explain the link between aortic PWV and LC n-3 PUFAs. Changes in ascending aortic distensibility could be explained by altered elastin deposition in the perinatal period. Elastin is an essential component of the arterial wall and determines visco-elastic properties of the vessel wall. Animal studies have suggested a role for vitamin A in the regulation of elastin development in utero (Antipatis et al., 1998).

Whilst maternal oily fish consumption and late pregnancy vitamin D status were associated with changes in vascular structure there were no associations with cardiac parameters at this age. There was also no association of arterial stiffness with LV structure and functional measures. It would be of value to re-scan these children later in life to assess implications of increased measures of arterial stiffness at this early age.

Behaviour and lifestyle in childhood contribute to the pathway of acquired disease risk. Diet and level of physical activity are linked to obesity and body composition. Child's size at 9 years was associated with heart size; children with greater BMI had larger hearts, but after LV mass was normalised to body surface area the heart was smaller in relation to body size. Similarly total fat mass was associated with smaller LV mass in relation to body size, indicating that there is more stress on the heart to maintain cardiac output for increased demands imposed by larger body size.

The changes in LV mass and volumes and arterial stiffness seen in this study were within normal ranges, modest compared to those seen in disease processes. Nonetheless, within these normal ranges there were significant associations with maternal and childhood factors which may predispose the children to CVD in later life. The possibility that the smaller heart is compensating at this stage must be considered. The long-term impact of smaller cardiac volumes and measures of LV mass is uncertain, however these hearts may be susceptible to compensatory hypertrophy. It is speculated that with additional acquisition of risk burden throughout adolescence and into adulthood there will come a point where the heart is no longer able to compensate with tachycardia alone. Increased demand on smaller hearts due to increased risk burden with age or pathological processes will result in increasing BP and increased arterial stiffness ultimately leading to hypertension and cardiovascular disease. Increased ventricular afterload as a result of increasing arterial stiffness and associated hypertension may also lead to LV hypertrophy. Thus the adverse CV structural and functional changes, although within normal ranges at the age of 9 years are potentially a precursor to hypertension.

In summary, the effect sizes seen in this research are subclinical at this age; it may be too early to demonstrate the impact of increased arterial stiffness on

cardiac structure and function whilst the cardiovascular system is able to compensate for variations in heart size with increased heart rates in order to maintain required levels of cardiac output in childhood. The effect sizes seen in this study may be further amplified by acquired risk burden in later life, contributing to the development of cardiovascular disease. Thus it would be useful to obtain repeat measures of cardiovascular structure and function in these children later in life in order to assess the impact of increased arterial stiffness on LV mass.

6.3.2 Mismatch

Full investigation of the mismatch phenomenon was beyond the scope of this thesis. There are two main scenarios by which a mismatch in environments may manifest; through a poor developmental environment and a healthy later environment, or a 'normal' developmental environment followed by either an obesogenic unhealthy, or a poor unhealthy, later environment. In this study measures of cardiovascular structure and function were within normal ranges previously published, and changes in arterial structure and function were evident as a result of small changes in normal developmental conditions.

The changes evident from normal variations in maternal nutrition are perhaps indicative of a modest level of mismatch. It remains uncertain which component of the mismatch to address. There is a wealth of data available from the SWS to date and further research is needed to establish the dietary or other components of lifestyle which it may be important to modify, and the opportune time points for such intervention. It would also be valuable to determine the influence of changes in growth trajectories throughout gestation and childhood. By assessing the impact of growth trajectories at various stages of development, fetal growth during early and late pregnancy, and growth throughout infancy and early childhood, on measures of cardiovascular structure and function at the age of 9 years, it may be possible to assess the effect of mismatch on cardiovascular risk in children. This could identify opportunities for intervention and even provide the opportunity to track potential mismatch interventions.

6.3.3 Potential interventions

There are several time points in the life course at which intervention may be feasible; in the developmental phase and before with regard to mothers lifestyle pre-conception, during childhood, and in adolescence with regard to modifying adverse lifestyle burden. A natural point to intervene might be in secondary school. With a third of young women overweight, an opportunity to improve nutritional education and push a healthy lifestyle agenda before reproduction may have far reaching beneficial effects.

Level of educational attainment has been shown to be strongly predictive of quality of diet in women (Robinson et al., 2004). In conjunction with findings in this research that maternal vitamin D levels and oily fish consumption are linked with offspring cardiovascular risk, measures of aortic distensibility and PWV respectively, this suggests that intervention in the form of improved nutritional education in adolescence could reduce risk not only for the woman later in life, but also for the next generation.

Alternatively, nutritional interventions during pregnancy may improve offspring cardiovascular health. Early gestation in particular has been identified as a vulnerable period and exposure to famine during this developmental period has been associated with an increased risk of CHD (Roseboom et al., 2006).

Interventions should not be limited to early gestation; the association observed in this research whereby higher late pregnancy maternal oily fish consumption was associated with reduced arterial stiffness in the offspring suggests that nutritional intervention throughout pregnancy may improve childhood cardiovascular structure and function. The Salmon in Pregnancy Study (SiPS) (Miles et al., 2011), an oily fish intervention during the later half of pregnancy whereby women with low consumption of oily fish were randomly assigned to consume 2 portions of salmon per week, or to continue with their normal diet, found that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) percentages were higher not only in maternal plasma phosphatidylcholine in late pregnancy but also in umbilical cord plasma phosphatidylcholine in the salmon group. Cardiovascular follow-up of offspring in such studies would be worthwhile to assess the long-term influence of maternal nutritional

interventions during pregnancy on cardiovascular structure and function in the offspring.

The postnatal period and early childhood present further important opportunities for nutritional intervention. Exposure to famine during early childhood has been associated with hypertension in adult life (Huang et al., 2010a, Hult et al., 2010). The association is often stronger in those who have an obesogenic lifestyle later in life (Li et al., 2011) as a result of a 'mismatch' in environments. People who were small at birth and in the first 2 years of life have an increase risk of CHD, particularly if the impaired early growth is followed by rapid childhood weight gain (Barker et al., 2005) suggestive of an early life 'mismatch'. Similarly, Eriksson et al. (2001) found that men in the Helsinki cohort who were small at birth had a greater risk of CHD later in life and that this risk was increased in those who had experienced rapid weight gain or 'catch-up' growth after the age of 1 year. These findings suggest that by controlling the growth trajectory throughout the first few years of life in children who were small at birth it may be possible to reduce the risk of CHD in later life.

6.3.4 Comparison with other studies

The SWS is unique in that data were collected prior to conception. Other mother-child birth cohorts investigating developmental influences report on measures during gestation and offspring CV outcomes. In this research measures of aortic dimensions, arterial stiffness and cardiac volumes and mass by MRI were considered.

Aortic root dimensions were associated with child's size at 9 years, and were smaller in children who weighed less at birth. This is consistent with previous studies reporting that constrained early growth was associated with long term structural changes in major arteries and coronary vessels (Jiang et al., 2006).

Few studies have investigated the influence of maternal 25(OH)D concentration on childhood CV outcomes. Williams et al. (2013) report that whilst maternal vitamin D concentrations are inversely associated with SBP in the offspring at the age of 9 years, there is no effect seen by the age of 15 years. Gale et al. (2008) found no association between maternal vitamin D concentrations in pregnancy and offspring arterial stiffness measured by cfPWV. Aortic root

distensibility may be more sensitive in identifying small changes in structure and function. Measurements by MRI are more accurate than by ultrasound.

Interesting associations seen in this study with lower maternal vitamin A concentrations related to greater arterial stiffness measured by aortic root distensibility have not been reported previously. A randomised controlled study in Nepal found that maternal vitamin A supplementation had no effect on childhood CV risk factors (BP and cholesterol) measured at 9-13 years of age (Stewart et al., 2010). Arterial stiffness is increasingly being recognised as an early marker of CV risk and changes in arterial stiffness may be evident before changes in BP. For example in this research higher maternal oily fish consumption was associated with lower PWV measured by MRI and although PWV was linked to BP, maternal oily fish consumption was not associated with BP.

Measures of stress in daily living were reported pre-conception in the SWS and greater perceived stress was associated with smaller hearts in the offspring. No previous study has reported on the influence of chronic maternal stress on cardiac structure and function. However, higher measures of maternal psychological distress during pregnancy has been associated with smaller LV mass in 6-year old offspring (Taal et al., 2013). Further, self-reported maternal anxiety during pregnancy has ben associated with salivary cortisol measures in the offspring at the age of 9 years suggesting a lasting influence. Objective measures of maternal stress were not available in the SWS cohort, however a study by Rondo et al. (2010) found that maternal cortisol levels were associated with offspring systemic vascular resistance.

6.4 Limitations of Study

This research has several potential limitations. The SWS is a longitudinal cohort and may suffer from recruitment and retention bias, making the MRI cohort not representative of a true cross section of the general population. Women in the MRI cohort were likely to be more highly educated and of a higher social class than the original SWS birth cohort, which was representative of the Southampton population. Attrition rates in similar studies have found that more highly educated women and those in a higher social class are more likely to attend follow-up clinics (Golding et al., 2001). However it is felt that this

would not bias findings and conversely may result in associations being under reported. Socioeconomic status is strongly linked to quality of health with individuals of a lower social class more likely to have poor health (Adler et al., 1994). The higher incidence of unhealthy behaviours reported in people of lower socioeconomic status may in part explain this association (Pampel et al., 2010). Smoking, lower physical activity, poor diet (Lantz et al., 1998), and higher levels of self-reported perceived stress (Adler et al., 1994) contribute to poor health outcomes. Although the MRI cohort may not be completely representative of the original SWS birth cohort the effects of educational attainment, quality of health and levels of stress, and maternal diet were still clear.

Although multiple testing is a limitation, with the associated increased possibility of chance findings, the key findings in this study arose from a priori hypotheses. Nonetheless, further studies in similar cohorts with greater numbers would be advantageous to confirm the findings in this research. It would be beneficial to investigate the pertinent developmental influences in this study on other phenotypes associated with CVD.

The accuracy of MRI aortic distensibility measures is limited by the use of brachial BP in calculations, which can differ significantly from central aortic BP measures. However, invasive BP measures were not appropriate for this study, brachial BP has been considered an acceptable substitute with many studies reporting accurate and reproducible results using MRI measures of aortic distensibility in the study of CVD (Groenink et al., 1998, Groenink et al., 2001, Toikka et al., 2002).

The extent of progression of childhood measures of aortic stiffness throughout the life course is unknown. However BP has been shown to track throughout the life course (Bao et al., 1995, Berenson, 2002, Berenson et al., 2005). BP is linked to arterial stiffness so it is reasonable to assume that childhood measures of arterial stiffness will track in a similar fashion to BP, and are a reliable measure of future cardiovascular risk.

CMR measurements are not as reliable or accurate in children as compared to those in adults due to reduced ability to comply with breath hold requirements. However, development of a scan protocol optimised for pediatric imaging permitted accurate quantification of cardiac structure and

function in children at the age of 9 years. Between-subject variability was greater than inter-study and inter-observer variability demonstrating that meaningful measurements could be obtained in this study. PWV values obtained in this study are similar to those previously reported using cfPWV applanation tonometry measurements in children (Reusz et al., 2010). There are limited data on normal childhood measures of distensibility however our measures were within the range of those reported by Voges et al. (2012) measured by MRI.

Although detailed data on maternal intake of fish oil supplements were available, these were only taken by a small minority of SWS subjects and therefore not considered in the analysis. Detailed measurements of maternal pregnancy serum fatty acid concentrations, particularly in early pregnancy are now available on the SWS database and future work could examine the associations with maternal oily fish consumption and with offspring cardiovascular parameters.

Measures of the child's vitamin D status were not available however season at the time of MRI was considered as a proxy of child's vitamin D status and there was no association found with measures of arterial stiffness. The main focus of the research was on the influence of maternal nutrition on cardiovascular outcomes in the child at age 9 years. Severe malnutrition in utero has been associated with endothelial dysfunction in adulthood (Stanner et al., 1997); however, it is unclear which components of maternal diet influence offspring vascular structure and function. Long-chain n-3 (omega-3) polyunsaturated fatty acids (LC PUFAS) found in fatty fish and fish oils have been shown to reduce aortic stiffness in studies of patient and healthy populations (Pase et al., 2011). Further, variation in maternal fatty acid intake in rat studies has been shown to influence aortic structure and function in the offspring (Armitage et al., 2005b). Similarly low vitamin D levels have been associated with arterial stiffness (Giallauria et al., 2012, Dong et al., 2010). Animal studies indicate that maternal consumption of a diet low in vitamin D is associated with endothelial dysfunction in the offspring (Tare et al., 2011). The influence of maternal serum vitamin D and serum vitamin A concentrations, and oily fish consumption, in late pregnancy on arterial structure and function were thus assessed in this study.

6.5 Strengths of Study

The participants are part of a large prospective longitudinal cohort study covering a wide socioeconomic background, ensuring minimal recall bias. The women were characterised before conception providing a wealth of information on diet and lifestyle factors. The SWS provides detailed information on both pre- and post-natal growth trajectories of the children. The SWS has data on assessment of growth trajectories in utero, at birth, throughout childhood to date, and data recorded of maternal exposures, whereas adult studies are reliant on recall and subject to associated bias.

The MRI cohort was representative of the initial SWS birth cohort. The children were all the same age at MRI enabling direct comparison of outcomes. The children were assessed at the age of 9 years; old enough to tolerate the MRI environment and comply with breath hold requirements, before the onset of puberty, and before the acquisition of significant risk burden, ensuring minimal influence of other acquired lifestyle risk factors such as smoking. Results are therefore less likely to be confounded by the influence of other CV risk factors than in adult studies. It is well established that CV risk factors are definable in childhood and are predictive of future CV risk (Berenson, 2002).

MRI is considered the gold standard imaging modality for assessing cardiac structure and function - it is non-invasive and involves no ionising radiation. PWV assessment by MRI is a well-validated, accurate and reproducible means of determining arterial stiffness. Aortic PWV was measured across the descending thoracic and abdominal aorta – the area most likely to be affected by early changes in vascular stiffness due to atherogenic processes.

MRI permits the accurate automated measurement of change in aortic area throughout the cardiac cycle, providing accurate measures of minimum and maximum area. Previous studies have measured aortic diameter with ultrasound – the aorta is not necessarily a perfect circle, which questions the accuracy of the measurement of diameter and manual measurement is subject to operator error. It is also often not reported in which cardiac phase the measurements are acquired.

6.6 Future Work

Extensive imaging data acquired in this study provides the opportunity for further research. Similarly, further interrogation of extensive data and samples collected throughout the SWS offer the opportunity to investigate associations with cardiovascular parameters measured with MRI.

6.6.1 Normal values for ventricular morphology and function in children aged 9 years

There is limited information published on normal ranges of cardiovascular structure and function in children measured by MRI. Three groups have published suggested normative values for LV and RV volumes from SSFP sequences in children however these studies report on small numbers of children across large age ranges; 50 children aged 7 months to 18 years (Beuchel et al., 2009), 60 children aged 8 to 17 years (Robbers-Visser et al., 2009), and 114 children aged 4 to 20 years (Sarikouch et al., 2010). Due to continuous changes in weight and height with age it is difficult to establish accurate normal childhood values in small studies (Kawel-Boehm et al., 2015). A single paper reports on normal values for aortic distensibility and PWV in a small group of 71 children and young adults (Voges et al., 2012) ranging in age from 2.3 years to 28.3 years.

The data acquired in this research will allow the generation of the first population-based ranges for normal ventricular morphology and function, and vascular structure for children at the age of 9 years. Although data are restricted to a single age group it would be a valuable contribution towards the development of comprehensive data on normal CV measures across childhood.

6.6.2 Quantification of pericardial fat as a predictor of CV risk

Epicardial adipose tissue surrounds the heart and coronary arteries and may influence the development of atherosclerosis (Mookadam et al., 2010). Studies in adults have found that epicardial fat is associated with cardiovascular risk factors such as higher BP, fasting insulin (Iacobellis et al., 2003), and triglycerides. A study of over 4,000 adults found that increased epicardial fat was associated with an increased risk of coronary events (Mahabadi et al., 2013). Epicardial fat thickness of greater than 5mm measured on the right

ventricular free wall by echocardiography was associated with left atrial enlargement, lower ejection fraction and higher left ventricular mass in a study by Mookadam et al. (2010).

These studies suggest a role for epicardial adipose quantification as a measure of cardiovascular risk, and demonstrate an influence on measures of cardiac structure and function. No studies have quantified epicardial adipose tissue in children.

The SSFP cine images acquired in this research demonstrate adipose tissue very clearly due to the high signal from fat characteristic of this sequence permitting delineation of fat from surrounding tissue. The imaging data acquired therefore offer an opportunity to interrogate the relationship between epicardial adipose tissue and cardiac morphology, function and cardiovascular risk markers in children. Dey et al. (2012) suggest that epicardial adipose tissue can be quantified by a simple measure of thickness, or a volumetric measure from SAX images acquired from base to apex of the heart as for LV and RV analysis; the quantification of epicardial adipose tissue volume performed in a similar manner as that used to measure myocardial mass.

From extensive imaging data acquired on these children, it is possible to quantify pericardial fat, which is an interesting predictor of cardiovascular risk and would add further weight to the research.

6.6.3 Follow-up MRI

Measures of vascular structure and function were acquired and related to developmental influences. However, while BP measures are known to track through childhood into adulthood, it is not known whether measures of arterial stiffness track in a similar fashion. The SWS offers the opportunity to assess vascular structure and function by MRI again in the future to determine whether arterial stiffness tracks, and to confirm whether the findings from this study persist throughout adolescence.

There was no association seen between measures of arterial stiffness and LV measures of function and structure - it may be too early in the CVD pathway, at the age of 9 years, to demonstrate the impact of increased arterial stiffness on cardiac structure and function. Repeated measures of arterial stiffness and LV

structure and function later in life would be valuable in order to assess the impact of increased arterial stiffness on LV mass and confirm the findings in the research at this stage. The effect size found in this study may be further amplified by acquired risk burden in later life and further assessment may offer an opportunity to assess the contribution of developmental influences on cardiovascular risk in comparison to those conferred by acquired risk burden.

6.6.4 Influence of growth trajectories on CV structure and function in childhood

It would be of great value to further explore the mismatch theory by assessing pre- and postnatal growth trajectories against measures of cardiovascular structure and function. MRI measurements of arterial stiffness in this research offer early biomarkers of CV risk. Impaired fetal growth in the first trimester has been associated with an adverse cardiovascular risk profile in school aged children (Jaddoe et al., 2014).

6.6.5 Perinatal epigenetic biomarkers and CV structure and function at 9 years of age

The early developmental environment may induce phenotypic changes through epigenetic processes such as DNA methylation (Low et al., 2011), thereby 'tuning' gene expression to produce a phenotype best suited to meet challenges of the predicted later environment (Gluckman and Hanson, 2004b). Perinatal DNA methylation has been related to maternal diet and childhood adiposity in two independent cohorts (Godfrey et al., 2011) suggesting that it may be possible to identify a predisposition to later obesity and metabolic disease. Umbilical cord tissue collected at birth in the SWS offers the opportunity to extract genomic DNA giving scope for relating perinatal epigenetic biomarkers to childhood cardiovascular structure and function measurements acquired by MRI.

6.6.6 Maternal pregnancy serum fatty acid concentrations and offspring arterial stiffness

Detailed measurements of maternal pregnancy serum fatty acid concentrations, particularly in early pregnancy are now available on the SWS

database presenting an opportunity to interrogate further the association between pregnancy oily fish consumption and offspring arterial stiffness. Future work could examine the associations of maternal pregnancy serum fatty acid concentrations with maternal oily fish consumption, and with offspring cardiovascular parameters.

6.7 Conclusions

This research confirms that maternal health and nutrition have a strong influence on the development of the child. Maternal oily fish consumption, maternal vitamin D and vitamin A concentrations during late pregnancy can influence development in utero changing arterial function with a lasting effect on vascular compliance in childhood, and having potentially important implications for cardiovascular disease risk in later life. Maternal nutrition is strongly linked to educational attainment and social class; therefore maternal nutritional education may be one way to achieve early intervention. Further, nutritional interventions during critical periods of early development may induce changes in vascular structure and function with major implications for future cardiovascular disease risk and possible reduction of cardiovascular disease burden.

Although the effect sizes seen were modest, the prenatal environment may alter cardiovascular development in utero, by changing arterial structure and/or influencing cardiac growth in the offspring. Small changes in both childhood aortic structure and cardiac growth may have important implications for cardiovascular risk in later life.

Although further work is necessary to confirm the findings in this research, the data suggest that what are regarded as normal biological variations in early growth and development may have long-term effects on cardiovascular health, and even far reaching effects on the next generation. The findings indicate that individuals with an increased risk of later disease can be identified early in the life course, enabling targeting of childhood intervention to individuals at risk. Further, interventions earlier in the life course before and/or during pregnancy may improve CV health in the offspring at an earlier age.

Appendices

Appendix 1

	Heart Rate (beats per minute)			
Exposure	**n**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy				
Townsend index	333	0	-0.36, 0.36	1
Currently smoking	338	0.8	-1.78, 3.37	0.54
Woman's ethnic group, 2 groups	338	1.24	-5.03, 7.52	0.7
Social class - 3 groups (Ref Professional)	332			
Skilled non-manual / manual		0.61	-1.79,3.0	0.62
Partly skilled / unskilled		0.24	-3.32, 3.81	0.89
Qualification level - 3 groups (Ref HND or degree)	338			
None / CSE		2.13	-1.90, 6.17	0.3
Secondary education		1.44	-.98, 3.85	0.24
How much stress in daily living in last 4 weeks	338	0.85	-0.23, 1.93	0.12
Assessment of general health	338	2.08	0.63, 3.53	<0.01
Stress in life affected health	338	0.96	-0.16, 2.08	0.09
GHQ > 13	152	-0.33	-4.37, 3.70	0.87
GHQ score Likert	152	-0.05	-0.46, 0.35	0.79
Long-standing illness	338	0.51	-2.07, 3.09	0.7
Frequency of strenuous exercise per week, two groups	336	-1.19	-3.44, 1.06	0.3
Height (cm)	337	-0.05	-0.22, 0.12	0.58
Body mass index (kg/m^2)	336	0.11	-0.13, 0.35	0.37
Mid-upper arm circumference (cm)	335	0.06	-0.23, 0.35	0.67
Triceps skinfold (mm) (average of three closest)	337	0.1	-0.06, 0.25	0.21
Biceps skinfold (mm) (average of three closest)	337	0.09	-0.09, 0.27	0.33
Subscapular skinfold (mm) (average of three closest)	337	0.09	-0.03, 0.20	0.13
Sum of skinfolds (mm)	337	0.03	-0.01, 0.06	0.18
Arm muscle area (cm^2)	335	-0.01	-0.13, 0.10	0.82
Waist-Hip ratio	335	6.69	-13.27, 26.66	0.51
Maternal: pregnancy				
Late pregnancy Vitamin D (nmol/l)	316	0	-0.04,0.03	0.82

	Heart Rate (beats per minute)			
Exposure	**n**	**β**	**95% CI**	**p value**
Late pregnancy Retinol (Vitamin A) (umol/l)	272	-0.52	-4.22,3.18	0.78
Early pregnancy: oily fish portions/week	276	-0.76	-1.85,0.34	0.17
Late pregnancy: oily fish portions/week	328	-0.78	-1.73,0.18	0.11
Maternal: postnatal				
EPDS: Depression EPDS > 11	332	0.84	-1.48, 3.17	0.48
EPDS: EPDS score	332	0	-0.20, 0.20	0.99
Paternal				
Height (cm)	323	-0.14	-0.31, 0.02	0.09
Birth weight (g)	290	0	-0.00, 0.00	0.87
Infant				
Birth weight (kg)	335	1.03	-1.05, 3.10	0.33
Placental weight (g)	312	0	-0.01, 0.01	0.45
Ponderal index (kg/m^3)	328	-0.05	-0.53, 0.44	0.85
Age last breast fed, (completed months)	329	-0.25	-0.46, -0.04	0.02
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	154	2.55	-3.17, 8.27	0.38
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	154	2.12	-5.79,10.03	0.6
4 year DXA: Total lean (kg), adjusted for sex	233	-0.69	-1.58, 0.21	0.13
4 year DXA: Total fat (kg), adjusted for sex	233	-0.04	-1.20, 1.12	0.95
Child at 9 years				
Height (cm)	337	-0.11	-0.28, 0.06	0.22
Weight (kg)	337	0.03	-0.14, 0.20	0.77
Body mass index (kg/m^2)	337	0.23	-0.19, 0.65	0.28
Total lean mass (SD)	298	-0.79	-2.00, 0.41	0.2
Total fat mass (SD)	298	0.95	-0.25, 2.16	0.12
Systolic BP (mmHg)	301	0.14	0.01, 0.27	0.04
Diastolic BP (mmHg)	301	0.16	-0.00, 0.33	0.05
Pulse pressure (mmHg)	301	0.02	-0.10, 0.15	0.7
Mean arterial pressure (estimated) (mmHg)	301	0.22	0.04, 0.41	0.02

	Heart Rate (beats per minute)			
Exposure	**n**	**β**	**95% CI**	**p value**
Mean arterial pressure (measured) (mmHg)	179	0.06	-0.13, 0.25	0.54
Heart rate (beats per minute)				
Arterial distensibility (10^{-3}mmHg^{-1})	301	-0.30	-0.58, -0.01	0.04
Pulse wave velocity (m/s)	337	5.53	3.39, 7.68	<0.001

Appendix 2

Taking account of child's sex, univariate analyses of maternal, infant and childhood characteristics, and cardiovascular measures in relation to systolic and diastolic BP.

		Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	296	0.18	-0.15, 0.51	0.29	-0.02	-0.28, 0.25	0.89
Currently smoking	301	2	-0.35, 4.35	0.1	0.8	-1.09, 2.70	0.41
Woman's ethnic group, 2 groups	301	-2.22	-7.53, 3.08	0.41	1.15	-3.12, 5.42	0.6
Social class - 3 groups (Ref Professional)	295						
Skilled non-manual / manual		2.62	0.49, 4.76	0.02	1	-0.75, 2.75	0.26
Partly skilled / unskilled		2.46	-0.65, 5.57	0.12	2.63	0.084, 5.17	0.04
Qualification level - 3 groups (Ref HND or degree)	301						
None / CSE		5.0	1.47, 8.53	0.006	3.19	0.34, 6.03	0.03
Secondary education		1.37	-0.75, 3.5	0.204	1.66	-0.06, 3.37	0.06
How much stress in daily living in last 4 weeks	301	-0.29	-1.27,0.69	0.56	-0.12	-0.91,0.67	0.77
Assessment of general health	301	0.93	-0.37,2.23	0.16	0.66	-0.39,1.71	0.22
Stress in life affected health	301	-0.08	-1.10, 0.94	0.87	0.15	-0.67, 0.97	0.71
GHQ > 13	139	0.63	-2.66, 3.93	0.71	1.05	-1.69, 3.78	0.45
GHQ score Likert	139	0.04	-0.29, 0.37	0.8	0.06	-0.22, 0.33	0.68
Long-standing illness	301	0.75	-1.56, 3.06	0.52	0.26	-1.60, 2.12	0.78
Frequency of strenuous exercise per week, two groups	299	-1.4	-3.40, 0.61	0.17	0.07	-1.56, 1.70	0.93
Height (cm)	300	0	-0.15, 0.15	0.99	-0.02	-0.14, 0.10	0.75
Body mass index (kg/m^2)	299	0.12	-0.11, 0.34	0.31	0	-0.18, 0.18	0.98
Mid-upper arm circumference (cm)	299	0.13	-0.12, 0.39	0.31	0.06	-0.14, 0.27	0.55
Triceps skinfold (mm) (average of three closest)	301	0.05	-0.09, 0.18	0.49	-0.01	-0.12, 0.10	0.9
Biceps skinfold (mm) (average of three closest)	301	0.09	-0.07, 0.25	0.28	0.02	-0.11, 0.15	0.77
Subscapular skinfold	301	0.01	-0.09, 0.11	0.8	0	-0.08, 0.08	0.96

		Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
(mm) (average of three closest)							
Sum of skinfolds (mm)	301	0.01	-0.02, 0.04	0.53	0	-0.03, 0.02	0.9
Arm muscle area (cm^2)	299	0.04	-0.06, 0.14	0.41	0.04	-0.04, 0.12	0.34
Waist-Hip ratio	299	0.64	-17.43, 8.71	0.94	-5.3	-19.72, 9.12	0.47
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	282	0	-0.03, 0.03	0.89	0.02	-0.01, 0.05	0.13
Late pregnancy Retinol (Vitamin A) (umol/l)	243	1.26	-1.98, 4.49	0.44	1.84	-0.74, 4.41	0.16
Early pregnancy: oily fish portions/week	243	1.38	0.47, 2.29	<0.01	0.21	-0.54, 0.95	0.59
Late pregnancy: oily fish portions/week	292	0.23	-0.61, 1.07	0.6	-0.18	-0.86, 0.50	0.61
Maternal: postnatal							
EPDS: Depression EPDS > 11	296	-0.16	-2.26, 1.93	0.88	0.44	-1.23,2.11	0.6
EPDS: EPDS score	296	-0.06	-0.24, 0.13	0.54	0.03	-0.11, 0.18	0.66
Paternal							
Height (cm)	288	0	-0.14, 0.15	0.95	-0.03	-0.15, 0.08	0.56
Birth weight (g)	255	0	-0.00, 0.00	0.89	0	-0.00, 0.00	0.92
Infant							
Birth weight (kg)	299	0.84	-1.04, 2.72	0.38	0.3	-1.22, 1.82	0.69
Placental weight (g)	278	0.01	0.00, 0.02	0.03	<0.01	-0.00, 0.01	0.36
Ponderal index (kg/m^3)	292	0.17	-0.25, 0.59	0.43	0.03	-0.31, 0.36	0.88
Age last breast fed, (months)	293	-0.19	-0.38, -0.00	0.05	-0.09	-0.24, 0.06	0.25
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	5.1	-0.00,10.21	0.05	-1.15	-5.00, 2.70	0.56
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	0.96	-6.10, 8.03	0.79	0.11	-5.15, 5.37	0.97
4 year DXA: Total lean (kg), adjusted for sex	209	0.97	0.15, 1.79	0.02	-0.17	-0.77, 0.43	0.57
4 year DXA: Total fat (kg), adjusted for sex	209	1.22	0.12, 2.33	0.03	0.3	-0.51, 1.10	0.47
Child at 9 years							
Height (cm)	300	0.17	0.02, 0.33	0.02	-0.02	-0.15, 0.10	0.72
Weight (kg)	300	0.29	0.14, 0.44	<0.001	0.03	-0.09, 0.16	0.59
Body mass index (kg/m^2)	300	0.68	0.31, 1.06	<0.001	0.17	-0.14, 0.48	0.29

		Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Total lean mass (SD)	266	1.44	0.44, 2.43	<0.01	-0.32	-1.15, 0.51	0.45
Total fat mass (SD)	266	1.71	0.72, 2.70	<0.001	0.44	-0.39,1.27	0.3
Systolic BP (mmHg)	301	1	1.00, 1.00	.	0.27	0.19, 0.36	<0.001
Diastolic BP (mmHg)	301	0.42	0.29, 0.56	<0.001	1	1.00, 1.00	.
Pulse pressure (mmHg)	301	0.64	0.57, 0.72	<0.001	-0.33	-0.41, -0.25	<0.001
MAP (E) (mmHg)	301	0.99	0.88, 1.10	<0.001	1.00	0.95, 1.06	<0.001
MAP (M) (mmHg)	179	0.58	0.44, 0.73	<0.001	0.60	0.49, 0.71	<0.001
Heart rate (beats per minute)	301	0.10	0.00, 0.20	0.04	0.08	-0.00, 0.15	0.05
Arterial distensibility (10^{-3}mmHg^{-1})	301	-0.80	-1.03, -0.58	<0.001	0.51	0.32, 0.70	<0.001
PWV (descending aorta) (m/s)	300	4.01	1.97, 6.04	<0.001	3.74	2.11, 5.36	<0.001

Appendix 3

Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular systolic mass adjusted for sex and heart rate.

		LV systolic mass Adjusted for heart rate (g)			LV systolic mass Adjusted for heart rate (normalised to BSA) (g/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	-0.1	-0.43, 0.23	0.55	-0.11	-0.35, 0.12	0.34
Currently smoking	309	1.93	-0.37, 4.24	0.1	0.33	-1.30, 1.96	0.69
Woman's ethnic group, 2 groups	309	-6.11	-11.45, -0.77	0.03	-2.97	-6.75, 0.82	0.12
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		-0.02	-2.18, 2.15	0.99	-0.48	-2.01, 1.06	0.541
Partly skilled / unskilled		-2.89	-6.07, 0.29	0.08	-1.53	-3.80, 0.73	0.183
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		0.87	-2.70, 4.43	0.63	0.01	-2.50, 2.53	0.991
Secondary education		-0.36	-2.52, 1.79	0.74	-0.68	-2.20, 0.83	0.377
Stress in daily living in last 4 weeks	309	-0.63	-1.59, 0.33	0.2	-0.71	-1.38, -0.03	0.04
Assessment of general health	309	-0.62	-1.92, 0.67	0.34	-0.63	-1.54, 0.28	0.17
Stress in life affected health	309	-0.43	-1.43, 0.57	0.4	-0.24	-0.94, 0.47	0.51
GHQ > 13	141	-1.49	-5.15, 2.17	0.42	-1.19	-3.67, 1.30	0.35
GHQ score Likert	141	-0.13	-0.49, 0.24	0.5	-0.15	-0.40, 0.10	0.23
Long-standing illness	309	-0.73	-3.05, 1.59	0.53	-0.59	-2.23, 1.04	0.47
Frequency of strenuous exercise/week, two groups	307	-1.14	-3.17, 0.89	0.27	-0.43	-1.86, 1.00	0.56
Height (cm)	308	0.27	0.12, 0.42	<0.001	0.01	-0.10, 0.12	0.84
Body mass index (kg/m^2)	307	0.01	-0.21, 0.24	0.92	-0.1	-0.26, 0.06	0.2
Mid-upper arm circumference (cm)	306	0.05	-0.21, 0.31	0.71	-0.16	-0.34, 0.03	0.09
Triceps skinfold (mm) (average of three closest)	308	-0.02	-0.16, 0.12	0.78	-0.07	-0.16, 0.03	0.18
Biceps skinfold (mm)	308	-0.02	-0.19, 0.14	0.79	-0.12*	-0.23, -0.00	0.05

		LV systolic mass Adjusted for heart rate (g)			LV systolic mass Adjusted for heart rate (normalised to BSA) (g/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
(average of three closest)							
Subscapular skinfold (mm) (average of three closest)	308	0	-0.10, 0.11	0.95	-0.03	-0.10, 0.05	0.49
Sum of skinfolds (mm)	308	0	-0.04, 0.03	0.88	-0.02	-0.04, 0.01	0.16
Arm muscle area (cm²)	306	0.03	-0.07, 0.14	0.52	-0.05	-0.13, 0.02	0.15
Waist-Hip ratio	306	-7.75	-25.24, 9.73	0.38	-7.48	-19.76, 4.80	0.23
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.01	-0.02, 0.05	0.39	0.01	-0.01, 0.04	0.22
Late pregnancy Retinol (Vitamin A) (umol/l)	249	0.67	-2.79, 4.13	0.7	1.34	-1.12, 3.80	0.28
Early pregnancy oily fish portions/week	252	-0.12	-1.11, 0.87	0.81	0.15	-0.54, 0.84	0.66
Late pregnancy oily fish portions/week	299	-0.19	-1.03, 0.65	0.65	0.18	-0.42, 0.77	0.56
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-2.60	-4.65, -0.54	0.01	-1.49	-2.95, -0.03	0.05
EPDS: EPDS score	303	-0.15	-0.32, 0.03	0.11	-0.1	-0.22, 0.03	0.13
Paternal							
Height (cm)	295	0.14	-0.00, 0.29	0.05	-0.06	-0.16, 0.04	0.24
Birth weight (g)	263	0	-0.00, 0.00	0.31	0	-0.00, 0.00	0.82
Infant							
Birth weight (kg)	307	2.85	1.02, 4.68	<0.01	0.55	-0.76, 1.86	0.41
Placental weight (g)	286	0.01	0.00, 0.02	0.01	0	-0.00, 0.01	0.22
Ponderal index (kg/m³)	300	0.22	-0.20, 0.64	0.3	0.22	-0.08, 0.51	0.14
Age last breast fed, (months)	300	-0.09	-0.28, 0.10	0.36	0.04	-0.09, 0.17	0.56
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	6.82	2.24, 11.39	<0.01	1.22	-2.21, 4.65	0.48
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	3.34	-3.27, 9.94	0.32	0.05	-4.77, 4.87	0.98
4 year DXA: Total lean (kg), adjusted for sex	212	3.36	2.70, 4.02	<0.001	0.68	0.12, 1.25	0.02
4 year DXA: Total fat (kg), adjusted for sex	212	2.58	1.57, 3.58	<0.001	-0.15	-0.91, 0.60	0.69

		LV systolic mass Adjusted for heart rate (g)			LV systolic mass Adjusted for heart rate (normalised to BSA) (g/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Child at 9 years							
Height (cm)	309	0.74	0.61, 0.87	<0.001	0.05	-0.06, 0.16	0.37
Weight (kg)	309	0.78	0.66, 0.91	<0.001	0	-0.11, 0.11	1
Body mass index (kg/m^2)	309	1.35	0.99, 1.71	<0.001	-0.09	-0.36, 0.18	0.51
Total lean mass (SD)	271	5.68	4.87, 6.49	<0.001	0.84	0.09, 1.59	0.03
Total fat mass (SD)	271	3.26	2.26, 4.27	<0.001	-0.66	-1.43, 0.11	0.09
Systolic BP (mmHg)	286	0.23	0.11, 0.34	<0.001	0.07	-0.01, 0.16	0.09
Diastolic BP (mmHg)	286	-0.01	-0.15, 0.14	0.92	-0.01	-0.11, 0.09	0.85
Pulse pressure (mmHg)	286	0.21	0.10, 0.32	<0.001	0.08*	0.00, 0.16	0.04
Mean arterial pressure (estimated) (mmHg)	286	0.14	-0.02, 0.30	0.1	0.04	-0.08, 0.15	0.52
Mean arterial pressure (measured) (mmHg)	169	0.07	-0.11, 0.25	0.42	0.01	-0.11, 0.13	0.91
Heart rate (beats per minute)	309	0.04	-0.06, 0.14	0.47	0.03	-0.04, 0.10	0.37
Arterial distensibility (10^{-3}mmHg^{-1}	286	-0.35	-0.59, -0.10	<0.01	-0.16	-0.34, 0.02	0.08
PWV (descending aorta) (m/s)	308	2.11	0.01, 4.22	0.05	0.24	-1.26, 1.73	0.75

Appendix 4

Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular end-diastolic volume, adjusted for sex and heart rate.

		LV end-diastolic volume Adjusted for heart rate (ml)			LV end-diastolic volume Adjusted for heart rate (normalised to BSA) (ml/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	0.19	-0.22, 0.60	0.37	0.09	-0.21, 0.40	0.55
Currently smoking	309	1.71	-1.18, 4.59	0.25	-0.54	-2.67, 1.60	0.62
Woman's ethnic group, 2 groups	309	-10.29	-16.9, -3.67	<0.01	-5.96	-10.88, -1.04	0.02
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		-1.38	-4.09, 1.33	0.32	-1.70	-3.70, 0 .29	0.09
Partly skilled / unskilled		-0.69	-4.68, 3.30	0.73	1.27	-1.67, 4.21	0.4
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		-2.90	-7.35, 1.54	0.2	-3.48	-6.74, -0.22	0.04
Secondary education		-1.15	-3.83, 1.54	0.4	-1.36	-3.33, 0.61	0.18
Stress in daily living in last 4 weeks	309	-0.52	-1.72, 0.68	0.4	-0.81	-1.69, 0.07	0.07
Assessment of general health	309	-0.67	-2.29, 0.95	0.42	-0.76	-1.95, 0 .44	0.21
Stress in life affected health	309	-1.30	-2.54, -0.06	0.04	-1.00	-1.92, -0.09	0.03
GHQ > 13	141	2.16	-1.85, 6.17	0.29	2.06	-1.10, 5.22	0.2
GHQ score Likert	141	0.14	-0.26, 0.55	0.48	0.06	-0.26, 0.38	0.71
Long-standing illness	309	-0.43	-3.32, 2.47	0.77	-0.46	-2.60, 1.68	0.67
Frequency of strenuous exercise per week, two groups	307	1.33	-1.19, 3.86)	0.3	1.69	-0.16, 3.55	0.07
Height (cm)	308	0.42	0.24, 0.61	<0.001	0.07	-0.07, 0.21	0.34
Body mass index (kg/cm^2)	307	-0.12	-0.40, 0.17	0.42	-0.28	-0.48, -0.08	<0.01
Mid-upper arm circumference (cm)	306	-0.06	-0.39, 0.27	0.71	-0.36	-0.60, -0.12	<0.01
Triceps skinfold (mm) (average of three closest)	308	-0.03	-0.21, 0.14	0.7	-0.12	-0.24, 0.01	0.07
Biceps skinfold (mm)	308	-0.05	-0.26, 0.15	0.62	-0.20	-0.35, -0.05	0.01

		LV end-diastolic volume Adjusted for heart rate (ml)			LV end-diastolic volume Adjusted for heart rate (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
(average of three closest)							
Subscapular skinfold (mm) (average of three closest)	308	-0.04	-0.17, 0.09	0.51	-0.09	-0.18, 0.01	0.07
Sum of skinfolds (mm)	308	-0.01	-0.05, 0.04	0.8	-0.03	-0.06, 0.00	0.06
Arm muscle area (cm²)	306	-0.03	-0.16, 0.11	0.69	-0.14	-0.24, -0.05	<0.01
Waist-Hip ratio	306	-11.69	-33.40, 10.01	0.29	-13.42	-29.42, 2.58	0.1
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.03	-0.01, 0.07	0.14	0.03	-0.00, 0.06	0.05
Late pregnancy Retinol (Vitamin A) (umol/l)	249	0.69	-3.40, 4.78	0.74	1.71	-1.41, 4.84	0.28
Early pregnancy oily fish portions/week	252	-0.3	-1.50, 0.90	0.62	0.02	-0.86, 0.90	0.96
Late pregnancy oily fish portions/week	299	-0.26	-1.30, 0.79	0.63	0.16	-0.61, 0.94	0.68
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-0.79	-3.38, 1.79	0.55	0.17	-1.74, 2.08	0.86
EPDS: EPDS score	303	-0.03	-0.25, 0.20	0.82	0	-0.16, 0.17	0.97
Paternal							
Height (cm)	295	0.14	-0.04, 0.32	0.12	-0.14*	-0.27, -0.01	0.04
Birth weight (g)	263	0	-0.00, 0.00	0.17	0	-0.00, 0.00	0.64
Infant							
Birth weight (kg)	307	5.10	2.86, 7.33	<0.001	1.63	-0.06, 3.32	0.06
Placental weight (g)	286	0.01	-0.00, 0.02	0.11	0	(-0.01, 0.01	0.92
Ponderal index (kg/m³)	300	0.36	-0.16, 0.89	0.18	0.35	-0.03, 0.74	0.07
Age last breast fed, (completed months)	300	0.04	-0.20, 0.28	0.74	0.20*	0.03, 0.37	0.02
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	11.01	4.89, 17.14	<0.001	3	-1.84, 7.83	0.22
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	7.37	-1.54, 16.29	0.1	2.4	-4.41, 9.22	0.49
4 year DXA: Total lean (kg), adjusted for sex	212	4.31	3.45, 5.16	<0.001	0.63	-0.14, 1.39	0.11
4 year DXA: Total fat (kg), adjusted for sex	212	2.79	1.47, 4.11	<0.001	-0.87	-1.88, 0.14	0.09

		LV end-diastolic volume Adjusted for heart rate (ml)			LV end-diastolic volume Adjusted for heart rate (normalised to BSA) (ml/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Child at 9 years							
Height (cm)	309	0.91	0.75, 1.07	<0.001	-0.04	-0.18, 0.10	0.58
Weight (kg)	309	0.93	0.77, 1.09	<0.001	-0.14	-0.28, 0.00	0.06
Body mass index (kg/m^2)	309	1.60	1.15, 2.05	<0.001	-0.38	-0.73, -0.02	0.04
Total lean mass (SD)	271	6.55	5.46, 7.63	<0.001	0	-1.01, 1.01	1
Total fat mass (SD)	271	3.70	2.41, 4.99	<0.001	-1.67	-2.67, -0.66	<0.01
Systolic BP (mmHg)	286	0.21	0.06, 0.36	<0.01	0.01	-0.10, 0.12	0.88
Diastolic BP (mmHg)	286	-0.02	-0.20, 0.16	0.8	-0.03	-0.17, 0.10	0.63
Pulse pressure (mmHg)	286	0.20	0.06, 0.33	<0.01	0.03	-0.07, 0.14	0.51
Mean arterial pressure (estimated) (mmHg)	286	0.11	-0.09, 0.32	0.27	-0.02	-0.17, 0.13	0.77
Mean arterial pressure (measured) (mmHg)	169	0	-0.20, 0.20	0.97	-0.07	-0.22, 0.08	0.36
Heart rate (beats per minute)	309	0.02	-0.10, 0.15	0.71	0.02	-0.07, 0.12	0.65
Arterial distensibility (10^{-3}mmHg^{-1})	286	-0.09	-0.41, 0.22	0.55	0.13	-0.11, 0.36	0.28
PWV (descending aorta) (m/s)	308	0.7	-1.94, 3.35	0.6	-1.69	-3.63, 0.25	0.09

Appendix 5

Univariate analyses of maternal, infant, and childhood characteristics, and child's cardiovascular measures in relation to left ventricular stroke volume, adjusted for sex and heart rate.

		LV stroke volume Adjusted for heart rate (ml)			LV stroke volume Adjusted for heart rate (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Maternal: pre-pregnancy							
Townsend index	304	-0.1	-0.41, 0.22	0.55	-0.14	-0.38, 0.10	0.25
Currently smoking	309	0.57	-1.62, 2.76	0.61	-0.84	-2.49, 0.82	0.32
Woman's ethnic group, 2 groups	309	-5.79	-10.85, -0.74	0.02	-2.95	-6.79, 0.88	0.13
Social class - 3 groups (Ref Professional)	303						
Skilled non-manual / manual		-1.71	-3.76, 0.35	0.103	-1.78	-3.33, -0.24	0.02
Partly skilled / unskilled		-0.65	-3.68, 2.37	0.67	0.86	-1.41, 3.14	0.46
Qualification level - 3 groups (Ref HND or degree)	309						
None / CSE		-3.88	-7.23, -0.53	0.023	-4.04	-6.55, -1.53	0.002
Secondary education		-0.90	-2.92, 1.12	0.382	-1.07	-2.59, 0.44	0.164
Stress in daily living in last 4 weeks	309	-0.21	-1.12, 0.70	0.65	-0.46	-1.14, 0.23	0.19
Assessment of general health	309	-0.31	-1.54, 0.91	0.62	-0.39	-1.31, 0.54	0.41
Stress in life affected health	309	-0.95	-1.89, -0.01	0.05	-0.73*	-1.44, -0.01	0.05
GHQ > 13	141	1.37	-1.75, 4.49	0.39	1.26	-1.21, 3.72	0.31
GHQ score Likert	141	0.15	-0.16, 0.46	0.34	0.08	-0.17, 0.33	0.52
Long-standing illness	309	-0.15	-2.35, 2.04	0.89	-0.1	-1.76, 1.56	0.9
Frequency of strenuous exercise per week, two groups	307	0.73	-1.18, 2.64	0.45	0.93	-0.50, 2.37	0.2
Height (cm)	308	0.26	0.12, 0.40	<0.001	0.02	-0.09, 0.13	0.68
Body mass index (kg/m²)	307	-0.08	-0.29, 0.13	0.47	-0.19	-0.35, -0.03	0.02
Mid-upper arm circumference (cm)	306	-0.09	-0.34, 0.16	0.48	-0.29	-0.47, -0.10	<0.01
Triceps skinfold (mm) (average of three closest)	308	-0.03	-0.16, 0.10	0.66	-0.09	-0.18, 0.01	0.08
Biceps skinfold (mm) (average of three closest)	308	-0.04	-0.19, 0.12	0.66	-0.14	-0.26, -0.02	0.02
Subscapular skinfold (mm) (average of three closest)	308	-0.03	-0.13, 0.07	0.55	-0.06	-0.13, 0.01	0.1

		LV stroke volume Adjusted for heart rate (ml)			LV stroke volume Adjusted for heart rate (normalised to BSA) (ml/m^2)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Sum of skinfolds (mm)	308	0	-0.03, 0.03	0.89	-0.02	-0.04, 0.00	0.12
Arm muscle area (cm^2)	306	-0.04	-0.14, 0.06	0.41	-0.12	-0.19, -0.04	<0.01
Waist-Hip ratio	306	-3.16	-19.63, 13.31	0.71	-5.16	-17.56, 7.25	0.41
Maternal: pregnancy							
Late pregnancy Vitamin D (nmol/l)	287	0.02	-0.01, 0.05	0.25	0.02	-0.01, 0.04	0.14
Late pregnancy Retinol (Vitamin A) (umol/l)	249	-0.48	-3.62, 2.66	0.76	0.24	-2.22, 2.70	0.85
Early pregnancy oily fish portions/week	252	-0.05	-0.96, 0.87	0.92	0.14	-0.55, 0.84	0.68
Late pregnancy oily fish portions/week	299	-0.22	-1.01, 0.58	0.59	0.07	-0.53, 0.67	0.82
Maternal: postnatal							
EPDS: Depression EPDS > 11	303	-0.92	-2.88, 1.04	0.36	-0.14	-1.63, 1.35	0.86
EPDS: EPDS score	303	-0.07	-0.24, 0.10	0.42	-0.04	-0.16, 0.09	0.58
Paternal							
Height (cm)	295	0.1	-0.03, 0.24	0.14	-0.09	-0.19, 0.01	0.09
Birth weight (g)	263	0	-0.00, 0.00	0.36	0	-0.00, 0.00	0.93
Infant							
Birth weight (kg)	307	3.72	2.03, 5.42	<0.001	1.36	0.06, 2.67	0.04
Placental weight (g)	286	0	-0.00, 0.01	0.28	0	-0.01, 0.01	0.65
Ponderal index (kg/m^3)	300	0.36	-0.04, 0.76	0.08	0.34	0.05, 0.64	0.02
Age last breast fed, (months)	300	0.06	-0.12, 0.24	0.5	0.17	0.04, 0.30	0.01
Baby DXA: Total lean mass (kg), adjusted for gestational age, sex and age	135	7.80	2.75, 12.85	<0.01	2.17	-1.78, 6.13	0.28
Baby DXA: Total fat (kg), adjusted for gestational age, sex and age	135	6.07	-1.19, 13.33)	0.1	2.5	-3.06, 8.06	0.37
4 year DXA: Total lean (kg), adjusted for sex	212	2.67	1.99, 3.36)	<0.001	0.17	-0.42, 0.75	0.58
4 year DXA: Total fat (kg), adjusted for sex	212	1.91	0.92, 2.90)	<0.001	-0.59	-1.36, 0.18	0.13
Child at 9 years							
Height (cm)	309	0.67	0.54, 0.79	<0.001	0.01	-0.10, 0.12	0.91
Weight (kg)	309	0.67	0.55, 0.80	<0.001	-0.07	-0.18, 0.04	0.22
Body mass index (kg/m^2)	309	1.15	0.80, 1.49	<0.001	-0.22	-0.50, 0.05	0.11
Total lean mass (SD)	271	4.35	3.48, 5.23	<0.001	-0.15	-0.94, 0.64	0.71
Total fat mass (SD)	271	2.72	1.74, 3.71	<0.001	-1.02	-1.81, -0.23	0.01

		LV stroke volume Adjusted for heart rate (ml)			LV stroke volume Adjusted for heart rate (normalised to BSA) (ml/m²)		
Exposure	**n**	**β**	**95% CI**	**p value**	**β**	**95% CI**	**p value**
Systolic BP (mmHg)	286	0.23	0.12, 0.34	<0.001	0.09	0.00, 0.17	0.05
Diastolic BP (mmHg)	286	-0.01	-0.15, 0.13	0.88	-0.02	-0.12, 0.09	0.77
Pulse pressure (mmHg)	286	0.20	0.10, 0.30	<0.001	0.08	0.00, 0.16	0.05
Mean arterial pressure (estimated) (mmHg)	286	0.14	-0.02, 0.29	0.08	0.04	-0.08, 0.16	0.49
Mean arterial pressure (measured) (mmHg)	169	0.06	-0.09, 0.21	0.46	0	-0.11, 0.12	0.93
Heart rate (beats per minute)	309	0.02	-0.08, 0.11	0.74	0.01	-0.06, 0.09	0.69
Arterial distensibility (10^{-3}mmHg^{-1})	286	-0.17	-0.41, 0.07	0.17	0	-0.19, 0.18	0.97
PWV (descending aorta) (m/s)	308	1.62	-0.37, 3.61	0.11	-0.18	-1.70, 1.33	0.81

List of References

ADLER, N. E., BOYCE, T., CHESNEY, M. A., COHEN, S., FOLKMAN, S., KAHN, R. L. & SYME, S. L. 1994. Socioeconomic status and health. The challenge of the gradient. *Am Psychol,* 49**,** 15-24.

ANDERSON, C., ARMSTRONG, M. D., GIDDING, S. S., GJESDAL, O., WU, C., BLUEMKE, D. & LIMA, J. A. C. 2012. LV Mass Assessed by Echocardiography and CMR, Cardiovascular Outcomes, and Medical Practice. *J Am Coll Cardiol,* 5**,** 837-848.

ANTIPATIS, C., ASHWORTH, C. J., GRANT, G., LEA, R. G., HAY, S. M. & REES, W. D. 1998. Effects of maternal vitamin A status on fetal heart and lung: changes in expression of key developmental genes. *American Journal of Physiology* 275**,** L1184-L1191.

ARMITAGE, J. A., LAKASING, L., TAYLOR, P. D., BALACHANDRAN, A. A., JENSEN, R. I., DEKOU, V., ASHTON, N., NYENGAARD, J. R. & POSTON, L. 2005a. Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. *Journal of Physiology-London,* 565**,** 171-184.

ARMITAGE, J. A., LAKASING, L., TAYLOR, P. D., BALACHANDRAN, A. A., JENSEN, R. I., DEKOU, V., ASHTON, N., NYENGAARD, J. R. & POSTON, L. 2005b. Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. *J Physiol,* 565**,** 171-84.

AYER, J. G., HARMER, J. A., XUAN, W., TOELLE, B., WEBB, K., ALMQVIST, C., MARKS, G. B. & CELERMAJER, D. S. 2009. Dietary supplementation with n-3 polyunsaturated fatty acids in early childhood: effects on blood pressure and arterial structure and function at age 8 y. *Am J Clin Nutr,* 90**,** 438-46.

BALLARD, P. L. 1979. Glucocorticoids and differentiation. *Monogr Endocrinol,* 12**,** 493-515.

BAO, W., THREEFOOT, S. H., SRINIVASAN, S. R. & BERENSON, G. S. 1995. Essential Hypertension Predicted by Tracking of Elevated Blood Pressure From Childhood to Adulthood: The Bogaluse Heart Study. *AJH,* 8**,** 657-665.

BARKER, D. 1995. Fetal origins of coronary heart disease. *Br Med J,* 311**,** 171-174.

BARKER, D. J. 1998. In utero programming of chronic disease. *Clin Sci (Lond),* 95**,** 115-28.

BARKER, D. J. & OSMOND, C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet,* 1**,** 1077-81.

BARKER, D. J., OSMOND, C., FORSEN, T. J., KAJANTIE, E. & ERIKSSON, J. G. 2005. Trajectories of growth among children who have coronary events as adults. *N Engl J Med,* 353**,** 1802-9.

BARKER, D. J., OSMOND, C., GOLDING, J., KUH, D. & WADSWORTH, M. E. 1989. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ,* 298**,** 564-7.

BARKER, D. J. P. 2004. Developmental origins of adult health and disease. *J Epidemiol Community Health,* 58**,** 114-115.

BATESON, P., BARKER, D., CLUTTON-BROCK, T., DEB, D., D'UDINE, B., FOLEY, R. A., GLUCKMAN, P., GODFREY, K., KIRKWOOD, T., LAHR, M. M., MCNAMARA, J., METCALFE, N. B., MONAGHAN, P., SPENCER, H. G. &

SULTAN, S. E. 2004. Developmental plasticity and human health. *Nature,* 430**,** 419-21.
BENDECK, M. P. & LANGILLE, B. L. 1991. Rapid Accumulation of Elastin and Collagen in the Aorts of Sheep in the Immediate Perinatal Period. *Circulation Research,* 69**,** 1165-1169.
BENEDIKTSSON, R., LINDSAY, R. S., NOBLE, J., SECKL, J. R. & EDWARDS, C. R. 1993. Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet,* 341**,** 339-41.
BERENSON, G. S. 2002. Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. *Am J Cardiol,* 90**,** 3L-7L.
BERENSON, G. S., SRNIVASAN, S. R. & BOGALUSA HEART STUDY, G. 2005. Cardiovascular risk factors in youth with implications for aging: the Bogalusa Heart Study. *Neurobiol Aging,* 26**,** 303-7.
BERGMANN, O., BHARDWAJ, R. D., BERNARD, S., ZDUNEK, S., BARNABE-HEIDER, F., WALSH, S., ZUPICICH, J., ALKASS, K., BUCHHOLZ, B. A., DRUID, H., JOVINGE, S. & FRISEN, J. 2009. Evidence for cardiomyocyte renewal in humans. *Science,* 324**,** 98-102.
BERNANKE, D. H. & VELKEY, J. M. 2002. Development of the coronary blood supply: changing concepts and current ideas. *Anat Rec,* 269**,** 198-208.
BERRY, C. L. & LOOKER, T. 1972. Nucleic acid and scleroprotein content of the developing human aorta. *J Pathol,* 106**,** Pii.
BEUCHEL, E. V., KAISER, T., JACKSON, C., SCHMITZ, A. & KELLENBERGER, C. J. 2009. Normal Right- and Left Ventricular Volumes and Myocardial Mass in Children Measured by Steady State Free Precession Cardiovascular Magnetic Resonance. *Journal of Cardiovascular Magnetic Resonance,* 11.
BHARGAVA, S. K., SACHDEV, H. S., FALL, C. H., OSMOND, C., LAKSHMY, R., BARKER, D. J., BISWAS, S. K., RAMJI, S., PRABHAKARAN, D. & REDDY, K. S. 2004. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N Engl J Med,* 350**,** 865-75.
BLAND, J. & ALTMAN, D. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* i**,** 307-310.
BLOOMFIELD, F. H., OLIVER, M. H. & HARDING, J. E. 2006. The late effects of fetal growth patterns. *Arch Dis Child Fetal Neonatal Ed,* 91**,** F299-304.
BOUTOUYRIE, P. & VERMEERSCH, S. 2010. Reference values for arterial stiffness collaboration. determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: "establishing normal and reference values". *European Heart Journal,* 31**,** 2338-2350.
BRESLAVSKY, A., FRAND, J., MATAS, Z., BOAZ, M., BARNEA, Z. & SHARGORODSKY, M. 2013. Effect of high doses of vitamin D onarterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. *J Clinical Nutrition*.
CALDER, P. C. 2004. n-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clinical Science,* 107**,** 1-11.
CAVALCANTE, J. L., LIMA, J. A., REDHEUIL, A. & AL-MALLAH, M. H. 2011. Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol,* 57**,** 1511-22.
CECELJA, M. & CHOWIENCZYK, P. 2012. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis,* 1.
CETIN, I., ALVINO, G. & CARDELLICCHIO, M. 2009. Long chain fatty acids and dietary fats in fetal nutrition. *Journal of Physiology-London,* 587**,** 3441-3451.

CHAHAL, N. S., LIM, T. K., JAIN, P., CHAMBERS, J. C., KOONER, J. S. & SENIOR, R. 2012. Population-based reference values for 3D echocardiographic LV volumes and ejection fraction. *JACC Cardiovasc Imaging,* 5**,** 1191-7.

CHALLIS, J. R., SLOBODA, D., MATTHEWS, S. G., HOLLOWAY, A., ALFAIDY, N., PATEL, F. A., WHITTLE, W., FRASER, M., MOSS, T. J. & NEWNHAM, J. 2001. The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. *Mol Cell Endocrinol,* 185**,** 135-44.

CHEEMA, K. K., DENT, M. R., SAINI, H. K., AROUTIOUNOVA, N. & TAPPIA, P. S. 2005. Prenatal exposure to maternal undernutrition induces adult cardiac dysfunction. *Br J Nutr,* 93**,** 471-7.

CLEAL, J. K., POORE, K. R., BOULLIN, J. P., KHAN, O., CHAU, R., HAMBIDGE, O., TORRENS, C., NEWMAN, J. P., POSTON, L., NOAKES, D. E., HANSON, M. A. & GREEN, L. R. 2007. Mismatched pre- and postnatal nutrition leads to cardiovascular dysfunction and altered renal function in adulthood. *Proc Natl Acad Sci U S A,* 104**,** 9529-33.

COLOMBO, J., CARLSON, S. E., CHEATHAM, C. L., FITZGERALD-GUSTAFSON, K. M., KEPLER, A. & DOTY, T. 2011. Long-chain polyunsaturated fatty acid supplementation in infancy reduces heart rate and positively affects distribution of attention. *Pediatr Res,* 70**,** 406-10.

CORSTIUS, H. B., ZIMANYI, M. A., MAKA, N., HERATH, T., THOMAS, W., VAN DER LAARSE, A., WREFORD, N. G. & BLACK, M. J. 2005. Effect of intrauterine growth restriction on the number of cardiomyocytes in rat hearts. *Pediatr Res,* 57**,** 796-800.

COTTRELL, E. C. & SECKL, J. R. 2009. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci,* 3**,** 19.

COX, J., HOLDEN, J. & SAGOVSKY, R. 1987. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal depression Scale. *The British Journal of Psychiatry,* 150**,** 782-786.

CRISPI, F., BIJNENS, B., FIGUERAS, F., BARTRONS, J., EIXARCH, E., LE NOBLE, F., AHMED, A. & GRATACOS, E. 2010. Fetal growth restriction results in remodeled and less efficient hearts in children. *Circulation,* 121**,** 2427-36.

CRISPI, F., BIJNENS, B., SEPULVEDA-SWATSON, E., CRUZ-LEMINI, M., ROJAS-BENAVENTE, J., GONZALEZ-TENDERO, A., GARCIA-POSADA, R., RODRIGUEZ-LOPEZ, M., DEMICHEVA, E., SITGES, M. & GRATACOS, E. 2014. Postsystolic shortening by myocardial deformation imaging as a sign of cardiac adaptation to pressure overload in fetal growth restriction. *Circ Cardiovasc Imaging,* 7**,** 781-7.

CROWTHER, M. A. 2005. Pathogenesis of atherosclerosis. *Hematology Am Soc Hematol Educ Program***,** 436-41.

DAI, S., HARRIST, R. B., ROSENTHAL, G. L. & LABARTHE, D. R. 2009. Effects of body size and body fatness on left ventricular mass in children and adolescents: Project HeartBeat! *Am J Prev Med,* 37**,** S97-104.

DAMSGAARD, C. T., SCHACK-NIELSEN, L., MICHAELSEN, K. F., FRUEKILDE, M. B., HELS, O. & LAURITZEN, L. 2006. Fish oil affects blood pressure and the plasma lipid profile in healthy Danish infants. *J Nutr,* 136**,** 94-9.

DANIELS, S. R., KIMBALL, T. R., MORRISON, J. A., KHOURY, P., WITT, S. & MEYER, R. A. 1995. Effect of lean body mass, fat mass, blood pressure, and sexual maturation on left ventricular mass in children and adolescents. Statistical, biological, and clinical significance. *Circulation,* 92**,** 3249-54.

DAVEY SMITH, G., STEER, C., LEARY, S. & NESS, A. 2007. Is there an intrauterine influence on obesity? Evidence from parent child associations in the

Avon Longitudinal Study of Parents and Children (ALSPAC). *Arch Dis Child,* 92**,** 876-80.

DAVIS, E. C. 1995. Elastic Lamina Growth in the Developing Mouse Aorta. *J Histochem Cytochem,* 43**,** 1115-1123.

DE JONG, C., BOEHM, G., KIKKERT, H. K. & HADDERS-ALGRA, M. 2011. The Groningen LCPUFA Study: No Effect of Short-Term Postnatal Long-Chain Polyunsaturated Fatty Acids in Healthy Term Infants on Cardiovascular and Anthropometric Development at 9 Years. *Pediatric Research,* 70**,** 411-416.

DE VRIES, W. B., BAL, M. P., HOMOET-VAN DER KRAAK, P., KAMPHUIS, P. J., VAN DER LEIJ, F. R., BAAN, J., STEENDIJK, P., DE WEGER, R. A., VAN BEL, F. & VAN OOSTERHOUT, M. F. 2006. Suppression of physiological cardiomyocyte proliferation in the rat pup after neonatal glucocorticosteroid treatment. *Basic Res Cardiol,* 101**,** 36-42.

DE VRIES, W. B., VAN DER LEIJ, F. R., BAKKER, J. M., KAMPHUIS, P. J., VAN OOSTERHOUT, M. F., SCHIPPER, M. E., SMID, G. B., BARTELDS, B. & VAN BEL, F. 2002. Alterations in adult rat heart after neonatal dexamethasone therapy. *Pediatr Res,* 52**,** 900-6.

DELOACH, S. & TOWNSEND, R. 2008. Vascular Stiffness: Its Measurement and Significance for Epidemiologic and Outcome Studies. *Clin J Am Soc Nephrol,* 3**,** 184-192.

DEMARIA, A. N., NEUMANN, A., LEE, G., FOWLER, W. & MASON, D. T. 1978. Alterations in ventricular mass and performance induced by exercise training in man evaluated by echocardiography. *Circulation,* 57**,** 237-44.

DEY, D., NAKAZATO, R., LI, D. & BERMAN, D. S. 2012. Epicardial and thoracic fat - Noninvasive measurement and clinical implications. *Cardiovasc Diagn Ther,* 2**,** 85-93.

DOGUI, A., KACHENOURA, N., FROUIN, F., LEFORT, M., DE CESARE, A., MOUSSEAUX, E. & HERMENT, A. 2011. Consistency of aortic distensibility and pulse wave velocity estimates with respect to the Bramwell-Hill theoretical model: a cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson,* 13**,** 11.

DONALD, A. E., CHARAKIDA, M., FALASCHETTI, E., LAWLOR, D. A., HALCOX, J. P., GOLDING, J., HINGORANI, A. D., SMITH, G. D. & DEANFIELD, J. E. 2010. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). *Eur Heart J,* 31**,** 1502-10.

DONG, Y., STALLMANN-JORGENSON, I. S., POLLOCK, N. K., HARRIS, R. A., KEETON, D., HUANG, Y., LI, K., BASSALI, R., GUO, D.-H., THOMAS, J., PEIERCE, G. L., WHITE, J., HOLICK, M. F. & ZHU, H. 2010. A 16-Week Randomized Clinical Trial of 2000 International Units Daily Vitamin D3 Supplementation in Black Youth: 25-Hydroxyvitamin D, Adiposity, and Arterial Stiffness. *J Clin Endocrinol Metab,* 95**,** 4584-4591.

DRAKE, A. J. & REYNOLDS, R. M. 2010. Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction,* 140**,** 387-98.

DUGGLEBY, S. L., GODFREY, K. M., INSKIP, H. M. & JACKSON, A. A. 2006. The Southampton Women's Survey Study Group. Differences in body mass index and waist circumference in young women. *Early Human Devel,* 82**,** 558.

EDWARDS, C. R., BENEDIKTSSON, R., LINDSAY, R. S. & SECKL, J. R. 1993. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet,* 341**,** 355-7.

ELDER, S. J., LICHTENSTEIN, A. H., PITTAS, A. G., ROBERTS, S. B., FUSS, P. J., GREENBERG, A. S., MCCRORY, M. A., BOUCHARD, T. J., JR., SALTZMAN, E. & NEALE, M. C. 2009. Genetic and environmental influences on factors associated with cardiovascular disease and the metabolic syndrome. *J Lipid Res,* 50**,** 1917-26.

ERIKSSON, J. G., FORSEN, T., TUOMILEHTO, J., OSMOND, C. & BARKER, D. J. 2001. Early growth and coronary heart disease in later life: longitudinal study. *BMJ,* 322**,** 949-53.

FIENO, D., JAFFE, W., SIMONETTI, O., JUDD, R. & FINN, J. 2002. TrueFISP: assessment of accuracy for measurement of left ventricular mass in an animal model. *J. Magn Reson Imaging,* 15**,** 526-31.

FORSDAHL, A. 1977. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *British Journal of Preventative and Social Medicine,* 31**,** 91-5.

FORSEN, T., ERIKSSON, J. G., TUOMILEHTO, J., TERAMO, K., OSMOND, C. & BARKER, D. J. 1997. Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. *BMJ,* 315**,** 837-40.

FOWDEN, A. L. 1995. Endocrine regulation of fetal growth. *Reprod Fertil Dev,* 7**,** 351-63.

FRANCOIS, J., FIENO, D., SHORS, S. & FINN, J. 2004. Left ventricular mass: manual and automatic segmentation of true FISP and FLASH cine MR images in dogs and pigs. *Radiology,* 230**,** 389-95.

FRASER, A., TILLING, K., MACDONALD-WALLIS, C., SATTAR, N., BRION, M. J., BENFIELD, L., NESS, A., DEANFIELD, J., HINGORANI, A., NELSON, S. M., SMITH, G. D. & LAWLOR, D. A. 2010. Association of maternal weight gain in pregnancy with offspring obesity and metabolic and vascular traits in childhood. *Circulation,* 121**,** 2557-64.

GAILLARD, R., STEEGERS, E. A., DUIJTS, L., FELIX, J. F., HOFMAN, A., FRANCO, O. H. & JADDOE, V. W. 2014. Childhood cardiometabolic outcomes of maternal obesity during pregnancy: the Generation R Study. *Hypertension,* 63**,** 683-91.

GALE, C. R., JIANG, B., ROBINSON, S. M., GODFREY, K. M., LAW, C. M. & MARTYN, C. N. 2006. Maternal diet during pregnancy and carotid intima-media thickness in children. *Arterioscler Thromb Vasc Biol,* 26**,** 1877-82.

GALE, C. R., ROBINSON, S., HARVEY, N., JAVAID, M., JIANG, B., MARTYN, C. N., GODFREY, K. M., COOPER, C. & GROUP, T. P. A. H. S. 2008. Maternal vitamin D status during pregnancy and child outcomes. *European Journal of Clinical Nutrition,* 62**,** 68-77.

GARCIADE-PABLO, N., MALPICA, N., LEDESMA-CARBAYO, M., JIMINEZ-BORREGUERO, L. & SANTOS, A. 2005. Semiautomatic estimation and visualisation of left ventricle volumes in cardiac MRI. *Computers in Cardiology,* 32**,** 399-402.

GIALLAURIA, F., MILANESCHI, Y., TANAKA, T., MAGGIO, M., CANEPA, M., ELANGO, P., VIGORITO, C., LAKATTA, E. G., FERRUCCI, L. & STRAIT, J. 2012. Arterial stiffness and vitamin d levels: the Baltimore longitudinal study of aging. *J Clin Endocrinol Metab,* 97**,** 3717-23.

GLUCKMAN, P. D. & HANSON, M. A. 2004a. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatr Res,* 56**,** 311-7.

GLUCKMAN, P. D. & HANSON, M. A. 2004b. Living with the past: evolution, development, and patterns of disease. *Science,* 305**,** 1733-6.

GLUCKMAN, P. D. & HANSON, M. A. 2006. *Mismatch: why our world no longer fits our bodies,* Oxford, OUP.

GLUCKMAN, P. D., HANSON, M. A., COOPER, C. & THORNBURG, K. L. 2008. Effect of In Utero and Early-Life Conditions on Adult Health and Disease. *The New England Journal of Medicine,* 359**,** 61-73.

GODFREY, K. M. 1998. Maternal regulation of fetal development and health in adult life. *Eur J Obstet Gynecol Reprod Biol,* 78**,** 141-50.

GODFREY, K. M. 2006. The "Developmental Origins" hypothesis: epidemiology. *Developmental Origins of Health and Disease - a Biomedical Perspective.* Cambridge: Cambridge University Press.

GODFREY, K. M., COSTELLO, P. M. & LILLYCROP, K. A. 2015. The developmental environment, epigenetic biomarkers and long-term health. *Journal of Developmental Origins of Health and Disease***,** 1-8.

GODFREY, K. M., SHEPPARD, A., GLUCKMAN, P. D., LILLYCROP, K. A., BURDGE, G. C., MCLEAN, C., RODFORD, J., SLATER-JEFFERIES, J. L., GARRATT, E., CROZIER, S. R., EMERALD, B. S., GALE, C. R., INSKIP, H. M., COOPER, C. & HANSON, M. A. 2011. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes,* 60**,** 1528-34.

GOLDBERG, D. & WILLIAMS, P. 1988. *A user's guide to the General Health Questionnaire,* Windsor, NFER-Nelson.

GOLDING, J., PEMBREY, M., JONES, R. & TEAM, A. S. 2001. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol,* 15**,** 74-87.

GROENINK, M., DE ROOS, A., MULDER, B. J., SPAAN, J. A. & VAN DER WALL, E. E. 1998. Changes in aortic distensibility and pulse wave velocity assessed with magnetic resonance imaging following beta-blocker therapy in the Marfan syndrome. *Am J Cardiol,* 82**,** 203-8.

GROENINK, M., DE ROOS, A., MULDER, B. J., VERBEETEN, B., JR., TIMMERMANS, J., ZWINDERMAN, A. H., SPAAN, J. A. & VAN DER WALL, E. E. 2001. Biophysical properties of the normal-sized aorta in patients with Marfan syndrome: evaluation with MR flow mapping. *Radiology,* 219**,** 535-40.

GROTENHUIS, H. B., WESTENBERG, J. M., STEENDIJK, P., VAN DER GEEST, R. J., OTTENKAMP, J., BAX, J. J., JUKEMA, J. W. & DE ROOS, A. 2009. Validation and Reproducibility of Aortic Pulse Wave Velocity as Assessed With Velocity-Encoded MRI. *Journal of Magnetic Resonance Imaging,* 30**,** 521-526.

HALES, C. N., BARKER, D. J., CLARK, P. M., COX, L. J., FALL, C., OSMOND, C. & WINTER, P. D. 1991. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ,* 303**,** 1019-22.

HALVORSEN, C. P., ANDOLF, E., HU, J., PILO, C., WINBLADH, B. & NORMAN, M. 2006. Discordant twin growth in utero and differences in blood pressure and endothelial function at 8 years of age. *J Intern Med,* 259**,** 155-63.

HANSON, M., GODFREY, K. M., LILLYCROP, K. A., BURDGE, G. C. & GLUCKMAN, P. D. 2011. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. *Prog Biophys Mol Biol,* 106**,** 272-80.

HANSON, M. A. & GLUCKMAN, P. D. 2005. Developmental processes and the induction of cardiovascular function: conceptual aspects. *J Physiol,* 15**,** 27-34.

HARKNESS, M. L., HARKNESS, R. D. & MCDONALD, D. A. 1957. The Collagen and Elastin Content of the Arterial Wall in the Dog. *Proc. R. Soc. Lond. B,* 146**,** 541-551

.

HARRIS, A. & SECKL, J. 2011. Glucocorticoids, prenatal stress and the programming of disease. *Horm Behav,* 59**,** 279-89.

HERRICK, K., PHILLIPS, D. I., HASELDEN, S., SHIELL, A. W., CAMPBELL-BROWN, M. & GODFREY, K. M. 2003. Maternal consumption of a high-meat, low-carbohydrate diet in late pregnancy: relation to adult cortisol concentrations in the offspring. *J Clin Endocrinol Metab,* 88**,** 3554-60.

HOCHNER, H., FRIEDLANDER, Y., CALDERON-MARGALIT, R., MEINER, V., SAGY, Y., AVGIL-TSADOK, M., BURGER, A., SAVITSKY, B., SISCOVICK, D. S. & MANOR, O. 2012. Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors: the Jerusalem Perinatal Family Follow-up Study. *Circulation,* 125**,** 1381-9.

HSE. 2014. *Health Service for England, trend tables: 2013* [Online]. Available: http://www.hscic.gov.uk/pubs/healthsurveyeng133tt [Accessed 14/5/2015 2015].

HUANG, C., LI, Z., WANG, M. & MARTORELL, R. 2010a. Early life exposure to the 1959-1961 Chinese famine has long-term health consequences. *J Nutr,* 140**,** 1874-8.

HUANG, Y., YAN, X., ZHAO, J. X., ZHU, M. J., MCCORMICK, R. J., FORD, S. P., NATHANIELSZ, P. W., REN, J. & DU, M. 2010b. Maternal obesity induces fibrosis in fetal myocardium of sheep. *Am J Physiol Endocrinol Metab,* 299**,** E968-75.

HULT, M., TORNHAMMAR, P., UEDA, P., CHIMA, C., BONAMY, A. K., OZUMBA, B. & NORMAN, M. 2010. Hypertension, diabetes and overweight: looming legacies of the Biafran famine. *PLoS One,* 5**,** e13582.

IACOBELLIS, G., RIBAUDO, M. C., ASSAEL, F., VECCI, E., TIBERTI, C., ZAPPATERRENO, A., DI MARIO, U. & LEONETTI, F. 2003. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab,* 88**,** 5163-8.

IBRAHIM, E., JOHNSON, K., MILLER, A., SHAFFER, J. & WHITE, R. 2010. Research measuring aortic pulse wave velocity using high-field cardiovascular magnetic resonance: comparison of techniques. *Journal of Cardiovascular Magnetic Resonance,* 12**,** 26.

INSKIP, H., GODFREY, K. M., ROBINSON, S. M., LAW, C. M., BARKER, D. J. & COOPER, C. 2006. Cohort profile: The Southampton Women's Survey. *International Journal of Epidemiology,* 35**,** 42-48.

JACKSON, C. E., SHIRODARIA, C. C., LEE, J. M., FRANCIS, J. M., CHOUDHURY, R. P., CHANNON, K. M., NOBLE, J. A., NEUBAUER, S. & ROBSON, M. D. 2009. Reproducibility and accuracy of automated measurement for dynamic arterial lumen area by cardiovascular magnetic resonance. *Int J Cardiovasc Imaging,* 25**,** 797-808.

JADDOE, V. W., DE JONGE, L. L., HOFMAN, A., FRANCO, O. H., STEEGERS, E. A. & GAILLARD, R. 2014. First trimester fetal growth restriction and cardiovascular risk factors in school age children: population based cohort study. *British Medical Journal,* 348**,** g14.

JARVISALO, M. J., HUTRI-KAHONEN, N., JUONALA, M., MIKKILA, V., RASANEN, L., LEHTIMAKI, T., VIIKARI, J. & RAITAKARI, O. T. 2009. Breast feeding in infancy and arterial endothelial function later in life. The Cardiovascular Risk in Young Finns Study. *Eur J Clin Nutr,* 63**,** 640-5.

JIANG, B., GODFREY, K. M., MARTYN, C. N. & GALE, C. R. 2006. Birth Weight and Cardiac Structure in Children. *Pediatrics,* 117**,** 257-261.

KAWEL-BOEHM, N., MACEIRA, A., VALSANGIACOMO-BUECHEL, E. R., VOGEL-CLAUSSEN, J., TURKBEY, E. B., WILLIAMS, R., PLEIN, S., TEE, M., ENG, J. & BLUEMKE, D. A. 2015. Normal values for cardiovascular magnetic resonance in adults and children. *J Cardiovasc Magn Reson,* 17**,** 29.

KELLY, B. A., LEWANDOWSKI, A. J., WORTON, S. A., DAVIS, E. F., LAZDAM, M., FRANCIS, J., NEUBAUER, S., LUCAS, A., SINGHAL, A. & LEESON, P. 2012. Antenatal glucocorticoid exposure and long-term alterations in aortic function and glucose metabolism. *Pediatrics,* 129**,** e1282-90.

KHAN, I., DEKOU, V., HANSON, M., POSTON, L. & TAYLOR, P. 2004. Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. *Circulation,* 110**,** 1097-102.

KHAN, O. A., TORRENS, C., NOAKES, D. E., POSTON, L., HANSON, M. A., GREEN, L. R. & OHRI, S. K. 2005. Effects of pre-natal and early post-natal undernutrition on adult internal thoracic artery function. *European Journal of Cardio-Thoracic Surgery,* 28**,** 811-815.

LANTZ, P. M., HOUSE, J. S., LEPKOWSKI, J. M., WILLIAMS, D. R., MERO, R. P. & CHEN, J. 1998. Socioeconomic factors, health behaviors, and mortality: results from a nationally representative prospective study of US adults. *JAMA,* 279**,** 1703-8.

LARSEN, P. S., KAMPER-JORGENSEN, M., ADAMSON, A., BARROS, H., BONDE, J. P., BRESCIANINI, S., BROPHY, S., CASAS, M., CHARLES, M. A., DEVEREUX, G., EGGESBO, M., FANTINI, M. P., FREY, U., GEHRING, U., GRAZULEVICIENE, R., HENRIKSEN, T. B., HERTZ-PICCIOTTO, I., HEUDE, B., HRYHORCZUK, D. O., INSKIP, H., JADDOE, V. W., LAWLOR, D. A., LUDVIGSSON, J., KELLEHER, C., KIESS, W., KOLETZKO, B., KUEHNI, C. E., KULL, I., KYHL, H. B., MAGNUS, P., MOMAS, I., MURRAY, D., PEKKANEN, J., POLANSKA, K., PORTA, D., POULSEN, G., RICHIARDI, L., ROELEVELD, N., SKOVGAARD, A. M., SRAM, R. J., STRANDBERG-LARSEN, K., THIJS, C., VAN EIJSDEN, M., WRIGHT, J., VRIJHEID, M. & ANDERSEN, A. M. 2013. Pregnancy and birth cohort resources in europe: a large opportunity for aetiological child health research. *Paediatr Perinat Epidemiol,* 27**,** 393-414.

LARSSON, W. J. 2001. *Human Embryology,* London, Churchill Livingstone.

LAURENT, S., COCKCROFT, J., VAN BORTEL, L., BOUTOUYRIE, P., GIANNATTASIO, C., HAYOZ, D., PANNIER, B., VLACHOPOULOS, C., WILKINSON, I. & STRUIJKER-BOUDIER, H. 2006. European network for non-invasive investigation of large arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J,* 27**,** 2588-605.

LEESON, C. P., KATTENHORN, M., DEANFIELD, J. E. & LUCAS, A. 2001a. Duration of breast feeding and arterial distensibility in early adult life: population based study. *BMJ,* 322**,** 643-7.

LEESON, C. P., KATTENHORN, M., MORLEY, R., LUCAS, A. & DEANFIELD, J. E. 2001b. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation,* 103**,** 1264-8.

LEESON, C. P., ROBINSON, M., FRANCIS, J. M., ROBSON, M. D., CHANNON, K. M., NEUBAUER, S. & WIESMANN, F. 2006. Cardiovascular magnetic resonance imaging for non-invasive assessment of vascular function: validation against ultrasound. *J Cardiovasc Magn Reson,* 8**,** 381-7.

LEHMAN, E. 1996. Pulse Wave Velocity as a Marker of Vascular Disease. *Lancet,* 348**,** 744.

LEWANDOWSKI, A. J., LAZDAM, M., DAVIS, E., KYLINTIREAS, I., DIESCH, J., FRANCIS, J., NEUBAUER, S., SINGHAL, A., LUCAS, A., KELLY, B. & LEESON, P. 2011. Short-term exposure to exogenous lipids in premature infants and long-term changes in aortic and cardiac function. *Arterioscler Thromb Vasc Biol,* 31**,** 2125-35.

LI, F., WANG, X., CAPASSO, J. & GERDES, A. 1996. Rapid Transition of Cardiac Myocytes from Hyperplasia to Hypertrophy During Postnatal Development. *Journal of Molecular and Cellular Cardiology,* 28**,** 1737-1746.

LI, S., CHEN, W., SRINIVASAN, S. R. & BERENSON, G. S. 2004. Childhood Blood Pressure as a Predictor of Arterial Stiffness in Young Adults: The Bogalusa Heart Study. *Hypertension,* 43**,** 541-546.

LI, Y., JADDOE, V. W., QI, L., HE, Y., WANG, D., LAI, J., ZHANG, J., FU, P., YANG, X. & HU, F. B. 2011. Exposure to the chinese famine in early life and the risk of metabolic syndrome in adulthood. *Diabetes Care,* 34**,** 1014-8.

LI, Y., KONG, J., WEI, M., CHEN, Z., LIU, S. & CAO, L. 2002. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest,* 110**,** 229-38.

LIM, K., ZIMANYI, M. A. & BLACK, M. J. 2006. Effect of maternal protein restriction in rats on cardiac fibrosis and capillarization in adulthood. *Pediatr Res,* 60**,** 83-7.

LONDON, G. M. & GUERIN, A. P. 1999. Influences of Arterial Pulse and Reflected Waves on Blood Pressure and Cardiac Function. *Am Heart J,* 138**,** 220-224.

LOUEY, S. & THORNBURG, K. L. 2005. The prenatal environment and later cardiovascular disease. *Early Hum Dev,* 81**,** 745-51.

LOW, F. M., GLUCKMAN, P. D. & HANSON, M. A. 2011. Developmental plasticity and epigenetic mechanisms underpinning metabolic and cardiovascular diseases. *Epigenomics,* 3**,** 279-94.

LUBCHENCO, L. O., HANSMAN, C., DRESSLER, M. & BOYD, E. 1963. Intrauterine Growth as Estimated from Liveborn Birth-Weight Data at 24 to 42 Weeks of Gestation. *Pediatrics,* 32**,** 793-800.

LUMEY, L. H., STEIN, A. D., KAHN, H. S., VAN DER PAL-DE BRUIN, K. M., BLAUW, G. J., ZYBERT, P. A. & SUSSER, E. S. 2007. Cohort profile: the Dutch Hunger Winter families study. *Int J Epidemiol,* 36**,** 1196-204.

MAHABADI, A. A., BERG, M. H., LEHMANN, N., KALSCH, H., BAUER, M., KARA, K., DRAGANO, N., MOEBUS, S., JOCKEL, K. H., ERBEL, R. & MOHLENKAMP, S. 2013. Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study. *J Am Coll Cardiol,* 61**,** 1388-95.

MALCOLM, D. D., BURNS, T. L., MAHONEY, L. T. & LAUER, R. M. 1993. Factors affecting left ventricular mass in childhood: the Muscatine Study. *Pediatrics,* 92**,** 703-9.

MANGONI, A., MIRCOLI, L., GIANNATTASIO, C., FERRARI, A. & MANCIA, G. 1996. Heart rate-dependence of arterial distensibility in vivo. *Journal of Hypertension,* 14**,** 897-901.

MANNING, J. & VEHASKARI, V. M. 2001. Low birth weight-associated adult hypertension in the rat. *Pediatr Nephrol,* 16**,** 417-22.

MARTIN, H., HU, J., GENNSER, G. & NORMAN, M. 2000. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. *Circulation,* 102**,** 2739-44.

MARTIN, R., NESS, A., GUNNELL, D., EMMETT, P., DAVEY SMITH, G. & TEAM, A. S. 2004. Does Breast-Feeding in Infancy Lower Blood Pressure in

Childhood? The Avon Longitudinal Study of Parents and Children (ALSPAC). *Circulation,* 109**,** 1259-1266.

MARTIN, R. M., GUNNELL, D. & SMITH, G. D. 2005. Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis. *Am J Epidemiol,* 161**,** 15-26.

MARTYN, C. N. & GREENWALD, S. E. 1997. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. *Lancet,* 350**,** 953-955.

MATTACE-RASO, F. U., VAN DER CAMMEN, T. J., HOFMAN, A., VAN POPELE, N. M., BOS, M. L., SCHALEKAMP, M. A., ASMAR, R., RENEMAN, R. S., HOEKS, A. P., BRETELER, M. M. & WITTEMAN, J. C. 2006. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation,* 113**,** 657-63.

MCDONALD, D. A. 1960. *Blood Flow in Arteries,* London, Arnold.

MCMULLEN, J. R. & JENNINGS, G. L. 2007. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clin Exp Pharmacol Physiol,* 34**,** 255-62.

MEANEY, M. J., SZYF, M. & SECKL, J. R. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med,* 13**,** 269-77.

MELAMED, N., MEIZNER, I., MASHIACH, R., WIZNITZER, A., GLEZERMAN, M. & YOGEV, Y. 2013. Fetal sex and intrauterine growth patterns. *J Ultrasound Med,* 32**,** 35-43.

MESSALI, G., PALUMBO, A., MAFFEI, E., MARTINI, C., SEITUN, S., ALDROVANDI, A., IMBRIACO, M., SALVATORE, M., WEUSTINK, A., MOLLET, N. & CADEMARTIRI, F. 2009. Assessmant of left ventricular volumes with cardiac MRI: comparison between two semi-automated quantitative software packages. *La Radiologica Medica,* 114**,** 718-727.

METAFRATZI, Z. M., EFREMIDIS, S. C., SKOPELITOU, A. S. & DE ROOS, A. 2002. The clinical significance of aortic compliance and its assessment with magnetic resonance imaging. *J Cardiovasc Magn Reson,* 4**,** 481-91.

MI, J., LAW, C. M., ZHANG, K.-L., OSMOND, C., STEIN, C. & BARKER, D. J. P. 2000. Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. *Ann Intern Med,* 132**,** 253-260.

MILES, E. A., NOAKES, P. S., KREMMYDA, L. S., VLACHAVA, M., DIAPER, N. D., ROSENLUND, G., URWIN, H., YAQOOB, P., ROSSARY, A., FARGES, M. C., VASSON, M. P., LIASET, B., FROYLAND, L., HELMERSSON, J., BASU, S., GARCIA, E., OLZA, J., MESA, M. D., AGUILERA, C. M., GIL, A., ROBINSON, S. M., INSKIP, H. M., GODFREY, K. M. & CALDER, P. C. 2011. The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n-3 fatty acid intake, and marine n-3 fatty acid status in maternal and umbilical cord blood. *Am J Clin Nutr,* 94**,** 1986S-1992S.

MITCHELL, B. & SHARMA, R. 2005. *Embryology,* London, Elsevier, Churchill Livingstone.

MITCHELL, G. F., HWANG, S. J., VASAN, R. S., LARSON, M. G., PENCINA, M. J., HAMBURG, N. M., VITA, J. A., LEVY, D. & BENJAMIN, E. J. 2010. Arterial Stiffness and Cardiovascular Events The Framingham Heart Study. *Circulation,* 121**,** 505-511.

MOOKADAM, F., GOEL, R., ALHARTHI, M. S., JIAMSRIPONG, P. & CHA, S. 2010. Epicardial fat and its association with cardiovascular risk: a cross-sectional observational study. *Heart Views,* 11**,** 103-8.

MORRIS, G. S., ZHOU, Q., HEGSTED, M. & KEENAN, M. J. 1995. Maternal Consumption of a Low Vitamin D Diet Retards Metabolic and Contractile Development in the Neonatal Rat Heart. *J Mol Cell Cardiol,* 27**,** 1245-1250.

NAPOLI, C., D'ARMIENTO, F. P., MANCINI, F. P., POSTIGLIONE, A., WITZTUM, J. L., PALUMBO, G. & PALINSKI, W. 1997. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest,* 100**,** 2680-90.

NICHOLS, W. & O'ROURKE, M. 1998. *McDonald's Blood Flow in Arteries,* London, Arnold.

NICHOLS, W. & O'ROURKE, M. F. 2005. Theoretical, Experimental and Clinical Principles. *In:* PRESS, O. U. (ed.) *McDonald's Blood Flow in Arteries.* 5th ed.

NUTTER, D. O., MURRAY, T. G., HEYMSFIELD, S. B. & FULLER, E. O. 1979. The effect of chronic protein-calorie undernutrition in the rat on myocardial function and cardiac function. *Circ Res,* 45**,** 144-52.

O'CONNELL, T., BERRY, J., JARVIS, A., SOMERMAN, M. & SIMPSON, R. 1997. 1,25-Dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy. *Am J Physiol* 272**,** H1751-H1758.

O'CONNOR, T. G., BEN-SHLOMO, Y., HERON, J., GOLDING, J., ADAMS, D. & GLOVER, V. 2005. Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biol Psychiatry,* 58**,** 211-7.

O'ROURKE, M. 1990. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension,* 15**,** 339-47.

O'ROURKE, M. & STAESSON, J. 2002. Clinical Applications of Arterial Stiffness; Definitions and Reference Values. *AJH,* 15**,** 426-444.

OKUBO, H., CROZIER, S. R., HARVEY, N. C., GODFREY, K. M., INSKIP, H. M., COOPER, C. & ROBINSON, S. M. 2015. Diet quality across early childhood and adiposity at 6 years: the Southampton Women's Survey. *Int J Obes (Lond).*

OSMOND, C., BARKER, D. J. P., WINTER, P. D., FALL, C. & SIMMONDS, S. J. 1993. Early growth and death from cardiovascular disease in women. *British Medical Journal,* 307**,** 1519-24.

OWEN, C. G., WHINCUP, P. H., GILG, J. & COOK, D. G. 2003. Effect of Breast Feeding in Infancy on Blood Pressure in Later Life: Systematic Review. *BMJ,* 327**,** 1189-1195.

PAINTER, R. C., DE ROOIJ, S. R., BOSSUYT, P. M., SIMMERS, T. A., OSMOND, C., BARKER, D. J., BLEKER, O. P. & ROSEBOOM, T. J. 2006. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. *Am J Clin Nutr,* 84**,** 322-7; quiz 466-7.

PAINTER, R. C., ROSEBOOM, T. J. & BLEKER, O. P. 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reprod Toxicol,* 20**,** 345-52.

PAMPEL, F. C., KRUEGER, P. M. & DENNEY, J. T. 2010. Socioeconomic Disparities in Health Behaviors. *Annu Rev Sociol,* 36**,** 349-370.

PAPAVASSILIU, T., KUHL, H. P., SCHRODER, M., SUSELBECK, T., BONDARENKO, O., BOHM, C. K., BEEK, A., HOFMAN, M. M. B. & VAN ROSSUM, A. C. 2005. Effect of Endocardial Trabeculae on Left Ventricular Measurements and Measurement Reproducibility at Cardiovascular MR Imaging. *Radiology,* 236**,** 57-64.

PASE, M. P., GRIMA, N. A. & SARRIS, J. 2011. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *American Journal of Clinical Nutrition,* 93**,** 446-454.

PEDNEKAR, A. S., MUTHUPILLAI, R., CHEONG, B. & FLAMM, S. D. 2006. Contribution of Endocardial Trabeculae and Papillary Muscles to Left Ventricular Volumes. *Pr. Intl. Soc. Mag. Reson. Med.***,** 790.

PELLICCIA, A., CULASSO, F., DI PAOLO, F. M. & MARON, B. J. 1999. Physiologic left ventricular cavity dilatation in elite athletes. *Ann Intern Med,* 130**,** 23-31.

PENNELL, D. J. 2003. Cardiovascular magnetic resonance: twenty-first century solutions in cardiology. *Clin Med,* 3**,** 273-278.

PENROSE, L. S. 1954. Some recent trends in human genetics. *Caryologia,* 6**,** 521-30.

PIVIK, R., DYKMAN, R., HJING, H., GILCHRIST, J. & BADGER, T. 2009. Early Infant Diet and the Omega 3 Fatty Acid DHA: Effects on Resting Cardiovascular Activity and Behavioural Development During the First Half-Year of Life. *Developmental Neuropsychology,* 34**,** 139-158.

PORRELLO, E. R., BELL, J. R., SCHERTZER, J. D., CURL, C. L., MCMULLEN, J. R., MELLOR, K. M., RITCHIE, R. H., LYNCH, G. S., HARRAP, S., THOMAS, W. G. & DELBRIDGE, L. M. D. 2009. Heritable pathologic cardiac hypertophy in adulthood is preceded by neonatal growth restriction. *American Journal of Physiology -,* 296**,** R672-R680.

PORRELLO, E. R., WIDDOP, R. E. & DELBRIDGE, L. M. 2008. Early origins of cardiac hypertrophy: does cardiomyocyte attrition programme for pathological 'catch-up' growth of the heart? *Clin Exp Pharmacol Physiol,* 35**,** 1358-64.

RAVELLI, A. C., VAN DER MEULEN, J. H., OSMOND, C., BARKER, D. J. & BLEKER, O. P. 2000. Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Arch Dis Child,* 82**,** 248-52.

REDHEUIL, A., YU, W. C., WU, C. O., MOUSSEAUX, E., DE CESARE, A., YAN, R., KACHENOURA, N., BLUEMKE, D. & LIMA, J. A. 2010. Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. *Hypertension,* 55**,** 319-26.

REILLY, J. J., ARMSTRONG, J., DOROSTY, A. R., EMMETT, P. M., NESS, A., ROGERS, I., STEER, C., SHERRIFF, A., AVON LONGITUDINAL STUDY OF, P. & CHILDREN STUDY, T. 2005. Early life risk factors for obesity in childhood: cohort study. *BMJ,* 330**,** 1357.

REILLY, J. J. & KELLY, J. 2011. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes (Lond),* 35**,** 891-8.

REUSZ, G. S., CSEPREKAL, O., TEMMAR, M., KIS, E., CHERIF, A. B., THALEB, A., FEKETE, A., SZABO, A. J., BENETOS, A. & SALVI, P. 2010. Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension,* 56**,** 217-24.

REYNOLDS, R. M., GODFREY, K. M., BARKER, M., OSMOND, C. & PHILLIPS, D. I. W. 2997. Maternal consumption of a high-meat, low carbohydrate diet during pregnancy and offspring adrenocortical stress responsiveness in adult life. *J Clin Endocrinol Metab,* 92**,** 2208-10.

ROBBERS-VISSER, D., BOERSMA, E. & HELBING, W. A. 2009. Normal biventricular function, volumes, and mass in children aged 8 to 17 years. *J Magn Reson Imaging,* 29**,** 552-9.

ROBINSON, S. M., CROZIER, S. R., BORLAND, S. E., HAMMOND, J., BARKER, D. J. & INSKIP, H. M. 2004. Impact of educational attainment on the quality of young women's diets. *Eur J Clin Nutr,* 58**,** 1174-80.

RONDO, P. H., LEMOS, J. O., PEREIRA, J. A. & SOUZA, J. M. 2010. The relationship between cortisol concentrations in pregnancy and systemic vascular resistance in childhood. *Early Hum Dev,* 86**,** 127-31.

ROSEBOOM, T., DE ROOIJ, S. & PAINTER, R. 2006. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev,* 82**,** 485-91.

ROSSET, A., SPADOLA, L. & RATIB, O. 2004. OsiriX: An Open-Source Software for Navigating in Multidimensional DICOM Images. *Journal of Digital Imaging,* 17**,** 205-216.

SADLER, T. 2012. *Langman's Medical Embryology,* Baltimore, USA, Lippincott Williams & Wilkins.

SALTON, C. J., CHUANG, M. L., O'DONNELL, C. J., KUPKA, M. J., LARSON, M. G., KISSINGER, K. V., EDELMAN, R. R., LEVY, D. & MANNING, W. J. 2002. Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study Offspring cohort. *J Am Coll Cardiol,* 39**,** 1055-60.

SAMUELSSON, A. M., MATTHEWS, P. A., ARGENTON, M., CHRISTIE, M. R., MCCONNELL, J. M., JANSEN, E. H., PIERSMA, A. H., OZANNE, S. E., TWINN, D. F., REMACLE, C., ROWLERSON, A., POSTON, L. & TAYLOR, P. D. 2008. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension,* 51**,** 383-92.

SAPOLSKY, R. M., ROMERO, L. M. & MUNCK, A. U. 2000. How do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocrine Reviews,* 21**,** 55-89.

SARIKOUCH, S., PETERS, B., GUTBERLET, M., LEISMANN, B., KELTER-KLOEPPING, A., KOERPERICH, H., KUEHNE, T. & BEERBAUM, P. 2010. Sex-specific pediatric percentiles for ventricular size and mass as reference values for cardiac MRI: assessment by steady-state free-precession and phase-contrast MRI flow. *Circ Cardiovasc Imaging,* 3**,** 65-76.

SCHULZ-MENGER, J., BLUEMKE, D., BREMERICH, J., FLAMM, S. D., FOGEL, M. A., FRIEDRICH, M. G., KIM, R. J., VON KNOBELSDORFF-BRENKENHOFF, F., KRAMER, C. M., PENNELL, D. J., PLEIN, S. & NAGEL, E. 2013. Standardized image interpretation and post processing in cardiovascular magnetic resonance: Society for Cardiovascular Magnetic Resonance (SCMR) Board of Trustees Task Force on Standardized Pst Processing. *Journal of Cardiovascular Magnetic Resonance,* 15.

SEKI, M., KURISHIMA, C., SAIKI, H., MASUTANI, S., ARAKAWA, H., TAMURA, M. & SENZAKI, H. 2014. Progressive aortic dilation and aortic stiffness in children with repaired tetralogy of Fallot. *Heart Vessels,* 29**,** 83-7.

SHIELL, A., CAMPBELL-BROWN, M., HASELDEN, S., ROBINSON, S., GODFREY, K. M. & BARKER, D. J. P. 2001. High meat, low carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. *Hypertension,* 38**,** 1282-8.

SINGHAL, A. 2009. The Early Origins of Atherosclerosis. *Early Nutrition Programming and Health Outcomes in Later Life: Obesity and Beyond,* 646**,** 51-58.

SOONPAA, M., KIM, K., PALJAK, L., FRANKLIN, M. & FIELD, L. 1996. Cardiomyocyte DNA synthesis and binucleation during murine development. *Am J Physiol,* 271**,** H2183-H2189.

SRINIVASAN, S. R., BAO, W., WATTIGNEY, W. A. & BERENSON, G. S. 1996. Adolescent overweight is associated with adult overweight and related multiple cardiovascular risk factors: the Bogalusa Heart Study. *Metabolism,* 45**,** 235-40.

STANNER, S. A., BULMER, K., ANDRES, C., LANTSEVA, O. E., BORODINA, V., POTEEN, V. V. & YUDKIN, J. S. 1997. Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *BMJ,* 315**,** 1342-8.

STEIN, A. D., ZYBERT, P. A., VAN DE BOR, M. & LUMEY, L. H. 2004. Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. *Int J Epidemiol,* 33**,** 831-6.

STEIN, C., FALL, C., KUMARAN, K., OSMOND, C., COX, V. & BARKER, D. J. P. 1996. Fetal growth and coronary heart disease in South India. *Lancet,* 348**,** 1269-73.

STENHAMMAR, C., OLSSON, G., BAHMANYAR, S., HULTING, A. L., WETTERGREN, B., EDLUND, B. & MONTGOMERY, S. 2010. Family stress and BMI in young children. *Acta Paediatr,* 99**,** 1205-12.

STEWART, C. P., CHRISTIAN, P., KATZ, J., SCHULZE, K. J., WU, L. S. F., LECLERQ, S. C., SHAKYA, T. R., KHATRY, S. K. & WEST, K. P. 2010. Maternal supplementation with vitamin A or β-carotene and cardiovascular risk factors among pre-adolescent children in rural Nepal. *Journal of Developmental Origins of Health and Disease,* 1**,** 262-270.

SUTTON-TYRELL, K., NAJJAR, S., BOUDREAU, R., VENKITACHALAM, L., KUPELIAN, V., SIMONSICK, E., HANVLIK, R., LAKATTA, E., SPURGEON, H., KRITCHEVSKY, S., PAHOR, M., BAUER, D. & NEWMAN, A. 2005. Elevated Aortic Pulse Wave Velocity, a Marker of Arterial tiffness, Predicts Cardiovascular Events in Well-Functioning Older Adults. *Circulation,* 111**,** 3384-3390.

SYMONDS, M. E., SEBERT, S. P., HYATT, M. A. & BUDGE, H. 2009. Nutritional Programming of the Metabolic Syndrome. *Nat Rev Endocrinol,* 5**,** 604-10.

TAAL, H. R., DE JONGE, L. L., TIEMEIER, H., VAN OSCH-GEVERS, L., HOFMAN, A., VERHULST, F. C., HELBING, W. A., VAN DER HEIJDEN, A. J. & JADDOE, V. W. 2013. Parental psychological distress during pregnancy and childhood cardiovascular development. The Generation R Study. *Early Hum Dev,* 89**,** 547-53.

TALGE, N. M., NEAL, C., GLOVER, V., EARLY STRESS, T. R., PREVENTION SCIENCE NETWORK, F., NEONATAL EXPERIENCE ON, C. & ADOLESCENT MENTAL, H. 2007. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry,* 48**,** 245-61.

TANUMIHARDJO, S. A., KURPAD, A. V. & HUNT, J. R. 2014. Research recommendations for applying vitamin A-labelled isotope dilution techniques to improve human vitamin A nutrition. *Int J Vitam Nutr Res,* 84 Suppl 1**,** 52-9.

TARE, M., EMMETT, S. J., COLEMAN, H. A., SKORDILIS, C., EYLES, D. W., MORLEY, R. & PARKINGTON, H. C. 2011. Vitamin D insufficiency is associated with impaired endothelial and smooth muscle function and hypertension in young rats. *J Physiol,* 589**,** 4777-4786.

TAVERAS, E. M., RIFAS-SHIMAN, S. L., OKEN, E., GUNDERSON, E. P. & GILLMAN, M. W. 2008. Short sleep duration in infancy and risk of childhood overweight. *Arch Pediatr Adolesc Med,* 162**,** 305-11.

TAYLOR, A. M., JHOOTI, P., WIESMANN, F., KEEGAN, J., FIRMIN, D. N. & PENNELL, D. J. 1997. MR navigator-echo monitoring of temporal changes in diaphragm position: implications for MR coronary angiography. *J Magn Reson Imaging,* 7**,** 629-36.

TAYLOR, P. D. & POSTON, L. 2007. Developmental programming of obesity in mammals. *Exp Physiol,* 92**,** 287-98.

THORNBURG, K., JONKER, S., O'TIERNEY, P., CHATTERGOON, N., LOUEY, S., FABER, J. & GIRAUD, G. 2011. Regulation of the cardiomyocyte population in the developing heart. *Prog Biophys Mol Biol,* 106**,** 289-99.

TOIKKA, J. O., NIEMI, P., AHOTUPA, M., NIINIKOSKI, H., RONNEMAA, T., VIIKARI, J. S., HARTIALA, J. J. & RAITAKARI, O. T. 2002. Decreased large artery distensibility in borderline hypertension is related to increased in vivo low-density lipoprotein oxidation. *Scand J Clin Lab Invest,* 62**,** 301-6.

TOMANEK, R. J., HU, N., PHAN, B. & CLARK, E. B. 1999. Rate of coronary vascularization during embryonic chicken development is influenced by the rate of myocardial growth. *Cardiovasc Res,* 41**,** 663-71.

ULBAK, J., LAURITZEN, L., HANSEN, H. S. & MICHAELSEN, K. F. 2004. Diet and blood pressure in 2.5-y-old Danish children. *Am J Clin Nutr,* 79**,** 1095-102.

URBINA, E. M., GIDDING, S. S., BAO, W., PICKOFF, A. S., BERDUSIS, K. & BERENSON, G. S. 1995. Effect of body size, ponderosity, and blood pressure on left ventricular growth in children and young adults in the Bogalusa Heart Study. *Circulation,* 91**,** 2400-6.

VAN DIJK, A. E., VAN EIJSDEN, M., STRONKS, K., GEMKE, R. J. & VRIJKOTTE, T. G. 2012. The association between prenatal psychosocial stress and blood pressure in the child at age 5-7 years. *PLoS One,* 7**,** e43548.

VLACHOPOULOS, C., AZNAOURIDIS, K. & STEFANADIS, C. 2010. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol,* 55**,** 1318-27.

VOGES, I., JEROSCH-HEROLD, M., HEDDERICH, J., PARDUN, E., HART, C., GABBERT, D., HANSEN, J., PETKO, C., KRAMER, H.-H. & RICKERS, C. 2012. Normal values of aortic dimensions, distensibility, and pulse wave velocity in children and young adults: a cross-sectional study. *Journal of Cardiovascular Magnetic Resonance,* 14.

WATANABE, H., OHTSUKA, S., KAKIHANA, M. & SUGISHITA, Y. 1993. Coronary circulation in dogs with an experimental decrease in aortic compliance. *J Am Coll Cardiol,* 21**,** 1497-506.

WESTERHOF, N. & O'ROURKE, M. F. 1995. Haemodynamic basis for the development of left ventricular failure in systolic hypertension and for its logical therapy. *J Hypertens,* 13**,** 943-52.

WHO. 2008. *Causes of death 2008* [Online]. Geneva. Available: http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources_methods.pdf [Accessed January 10 2013].

WHO 2011. Global Atlas on Cardiovascular Disease Prevention and Control. *In:* MENDIS, S., PUSKA, P. & B, N. (eds.). Geneva: World Health Organization.

WHO. 2012. *Cardiovascular Diseases (CVDs) Fact sheet 317* [Online]. Geneva: World Health Organization. Available: http://www.who.int/mediacentre/factsheets/fs317/en/index.html [Accessed December 20 2012].

WHO. 2014. *Global Strategy on Diet, Physical Activity and Health: Childhood overweight and obesity* [Online]. Available: http://www.who.int/dietphysicalactivity/childhood/en/ [Accessed 4 September 2015].

WIESMANN, F., PETERSEN, S. E., LEESON, P. M., FRANCIS, J. M., ROBSON, M. D., WANG, Q., CHOUDHURY, R., CHANNON, K. M. & NEUBAUER, S. 2004. Global impairment of brachial, carotid, and aortic vascular function in young smokers: direct quantification by high-resolution magnetic resonance imaging. *J Am Coll Cardiol,* 44**,** 2056-64.

WILLIAMS, D. M., FRASER, A., FRASER, W. D., HYPPONEN, E., DAVEY SMITH, G., DEANFIELD, J., HINGORANI, A., SATTAR, N. & LAWLOR, D. A. 2013. Associations of maternal 25-hydroxyvitamin D in pregnancy with offspring cardiovascular risk factors in childhood and adolescence: findings from the Avon Longitudinal Study of Parents and Children. *Heart,* 99**,** 1849-56.

WILLUM HANSEN, T., STAESSON, J., TORP-PEDERSEN, C., RASMUSSEN, S., THIJS, L., IBSEN, H. & JEPPESEN, J. 2006. Prognostic Value of Aortic Pulse Wave Velocity as Index of Arterial Stiffness in the General Population. *Circulation,* 113**,** 664-670.

ZIESKE, A. W., MALCOM, G. T. & STRONG, J. P. 2002. Natural history and risk factors of atherosclerosis in children and youth: the PDAY study. *Pediatr Pathol Mol Med,* 21**,** 213-37.

ZILE, M. 1998. Vitamin A and embryonic development: an overview. *Journal of Nutrition,* 128**,** 455S-458S.