



THE IMPACT OF PRETERM BIRTH ON THE CARDIOVASCULAR SYSTEM IN YOUNG ADULTHOOD

by

Adam J. Lewandowski

*Thesis presented for the degree Doctor of Philosophy in Cardiovascular
Medicine at the University of Oxford*

Division of Cardiovascular Medicine

Radcliffe Department of Medicine

University of Oxford

Cardiovascular Clinical Research Facility

Level 1, John Radcliffe Hospital

Oxford OX3 9DU

Supervisor: Professor Paul Leeson

Trinity Term 2013

ACKNOWLEDGEMENTS

There are many people who deserve thanks for enabling me to complete this research. I would firstly like to acknowledge the support of the Commonwealth Scholarship Commission and the British Council who made it possible for me to come to Oxford, and who supported me during this work. Thank you to the British Heart Foundation, the National Institute for Health Research Oxford Biomedical Research Centre, the Oxford British Heart Foundation Centre for Research Excellence, and the Oxford Health Services Research Committee for supporting this research. A special thank you to my academic supervisor, Professor Paul Leeson, who gave me this opportunity and has been a remarkable supervisor and mentor throughout my doctorate. Without your patience, insight, and support this work would not have been possible. I look forward to working with you on new projects in the future.

Thank you to Professor Alan Lucas, who designed the original birth cohort of participants back in the early 1980s from which many of the individuals that took part in my research studies were followed up. My sincerest thanks must also go to all the incredibly generous individuals who gave up their time to take part in this research. To everyone in the Oxford Cardiovascular Clinical Research Facility, the Oxford Centre for Magnetic Resonance Research, and the rest of the Division of Cardiovascular Medicine, it has been an absolute pleasure working with you. Thank you all for your help and kindness. Thank you to all the friends I have made while in Oxford, as well as the friends and family around the world that have supported my journey.

I must make a special mention to Professor Alastair Summerlee, who acted as an academic advisor during the transition from my undergraduate at the University of Guelph to my doctorate here at Oxford, and has now become a very dear friend over these past few

years. You have provided me with exceptional insight into how to manage this emotional and intellectual transition, which has helped me develop and mature as an individual. Thank you for being such a great friend.

To my partner and best friend, Jaco. I never expected that coming to Oxford would change my life in such a way as meeting you. You are such an exceptional person in so many ways - thank you for your love, patience, encouragement, and support over these last couple years.

And finally, to my parents and my sister. I am so grateful for all that you have done to get me here and for your support with every new challenge that I embark on. You are always there to celebrate every success and listen to every problem. I love you, and cannot thank you enough for always believing in me.

DEDICATIONS

For my mum and dad

ABSTRACT

THE IMPACT OF PRETERM BIRTH ON THE CARDIOVASCULAR SYSTEM IN YOUNG ADULTHOOD

THESIS PRESENTED FOR THE DEGREE DOCTOR OF PHILOSOPHY IN CARDIOVASCULAR
MEDICINE AT THE UNIVERSITY OF OXFORD

Adam J. Lewandowski

St Cross College

Trinity Term 2013

Advancements in clinical care have led to a growing cohort of preterm-born individuals now entering adulthood. Before birth, such adults were often exposed to a suboptimal intrauterine environment, and after delivery, key developmental stages that would normally occur *in utero* during the third trimester had to take place under *ex utero* physiological conditions. Through detailed cardiovascular phenotyping, this thesis investigates the cardiovascular changes in preterm-born young adults, utilising a cohort of individuals with data collection since recruitment at birth. The detailed perinatal information was first used to design nested case-control studies to investigate the effects of early lipid and glucocorticoid exposure on long-term cardiovascular physiology in individuals born preterm. It was demonstrated that intravenous lipid administration leads to an artificial elevation of total cholesterol levels in immediate postnatal life, which is associated with long-term changes in aortic and left ventricular function proportional to the degree of cholesterol elevation.

Additionally, exposure to antenatal glucocorticoids relates to a regional increase in aortic arch stiffness in young adulthood, as well as changes in glucose metabolism. It was then shown that young adults born preterm have increased left ventricular mass, out of proportion to blood pressure, and a unique three-dimensional left ventricular geometry, with reduced systolic and diastolic function compared to term-born controls. Similarly, they also show distinct differences in the right ventricle, with increased right ventricular mass and a proportion having clinically impaired right ventricular systolic function. Finally, it was demonstrated that preterm-born individuals have increased circulating levels of antiangiogenic factors in young adulthood, which relate to capillary rarefaction and blood pressure elevation. These findings are of considerable public health relevance given that nearly 10% of births are now preterm. Understanding whether modification of these variations in cardiovascular structure and function prevent the development of cardiovascular disease in this growing subgroup of the population will be of future interest.

LIST OF PUBLICATIONS

Original Manuscripts Contributing to D.Phil. Thesis

Lewandowski AJ, Lazdam M, Davis EF, Kylintireas I, Diesch J, Francis F, Singhal A, Lucas A, Neubauer S, Kelly B, Leeson P. Short-term exposure to exogenous lipids in premature infants and long-term changes in aortic and cardiac function. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2011;31(9):2125-2135.

Lewandowski AJ*, Kelly BA*, Worton SA, Davis EF, Lazdam M, Francis J, Neubauer S, Lucas A, Singhal A, Leeson P. Antenatal glucocorticoid exposure and long-term alterations in aortic function and glucose metabolism. *Pediatrics* 2012;129(5):e1282-e1290. *Joint first authors.

Lewandowski AJ, Augustine D, Lamata P, Davis EF, Lazdam M, Francis J, McCormick K, Wilkinson A, Lucas A, Singhal A, Smith N, Neubauer S, Leeson P. Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry and function. *Circulation* 2013; 127(2):197-206.

Lewandowski AJ, Bradlow WM, Augustine D, Davis EF, Francis J, Singhal A, Lucas A, Neubauer S, McCormick K, Leeson P. Right ventricular systolic dysfunction in young adults born preterm. *Circulation* 2013; 128(7):713-720.

Other Published Manuscripts During D.Phil.

Lewandowski AJ, Davis EF, Lazdam M, Leeson P. From gene to epigene-based therapies targeting the vascular endothelium. *Current Vascular Pharmacology* 2012; 10(1):125-137.

Lewandowski AJ, Pitcher A, Banerjee R, Leeson P. Arterial stiffness: using simple surrogate measures to make sense of a complex phenomenon. *Hypertension Research* 2012;35(2):155-156.

Lazdam M, **Lewandowski AJ**, Kylintireas I, Cunnington C, Diesch J, Francis J, Trevitt C, Neubauer S, Singhal A, Leeson P. Impaired endothelial responses in apparently healthy young people associated with subclinical variation in blood pressure and cardiovascular structure. *American Journal of Hypertension* 2012; 25(1):46-53.

Lazdam M, Davis EF, **Lewandowski AJ**, Worton SA, Kenworthy Y, Kelly B, Leeson P. Prevention of vascular dysfunction after preeclampsia: a potential long-term outcome measure and an emerging goal for treatment. *Journal of Pregnancy* 2012; 2012:704146.

Davis EF, Newton L, **Lewandowski AJ**, Lazdam M, Kelly BA, Kyriakou T, Leeson P. Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. *Clinical Science* 2012; 123(2):53-72.

Davis EF, Lazdam M, **Lewandowski AJ**, Worton SA, Kelly BA, Sargent I, Wilkinson A, McCormick K, Redman C, Leeson P. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. *Pediatrics* 2012;129(6):e1552-e1561.

Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis E, **Lewandowski AJ**, Shore A, MacKillop L, Kharbanda R, Alp N, Redman C, Kelly B, Leeson P. Unique blood pressure charac-

teristics in mother and offspring following early-onset preeclampsia. *Hypertension* 2012; 60(5):1338-1345.

Rider OJ, **Lewandowski AJ**, Nethononda R, Petersen SE, Pitcher A, Holloway CJ, Banerjee R, Byrne JP, Leeson P, Neubauer S. Gender specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. *European Heart Journal* 2013;34(4):292-299.

Davis EF, **Lewandowski AJ**, Leeson P. Cardiac dysfunction and preeclampsia: can imaging give clues to mechanism? *Circulation: Cardiovascular Imaging* 2012;5(6):691-692.

Piechnik SK, Ferreira VM, **Lewandowski AJ**, Ntusi NA, Banerjee R, Holloway C, Hofman MB, SadoDM, Maestrini V, White S, Lazdam M, Karamitsos T, Moon JC, Neubauer S, Leeson P, Robson MD. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5T using ShMOLLI. *Journal of Cardiovascular Magnetic Resonance* 2013; 15(1):13.

Augustine D, **Lewandowski AJ**, Lazdam M, Rai A, Francis J, Myerson S, Noble A, Becher H, Neubauer S, Petersen SE, Leeson P. Global and regional left ventricular myocardial deformation measures by magnetic resonance feature tracking in healthy volunteers: comparison with tagging and relevance of gender. *Journal of Cardiovascular Magnetic Resonance* 2013; 15(1):8.

Conference Abstracts and Presentations During D.Phil.

Lewandowski AJ, Lazdam M, Diesch J, Trevitt C, Leeson P. Subclinical Atherosclerosis and Early Changes in Cardiac Systolic Function in Young Adults: A Cardiovascular Magnetic Resonance Study of Myocardial Systolic Strain. *Circulation* 2010;122:A18034.

Lewandowski AJ, Lazdam M, Kylintireas I, Diesch J, Trevitt C, Singhal A, Lucas A, Leeson P. Impact of Exogenous Cholesterol in Newborns on Arterial Stiffness Decades Later: A 25 Year Prospective Follow-Up Study. *Circulation* 2010;122:A18112.

Lewandowski AJ, Lazdam M, Diesch J, Francis J, Augustine D, Banerjee R, Suttie J, Neubauer S, Leeson P. Myocardial systolic strain assessed by cardiovascular magnetic resonance relates to subclinical atherosclerosis in healthy young adults. *Journal of Cardiovascular Magnetic Resonance* 2011;13(Suppl 1):O95.

Lewandowski AJ, Lazdam M, Davis E, Poole R, Diesch J, Francis J, Neubauer S, Lucas A, Singhal A, Kelly B, Leeson P. Short term elevation of cholesterol level in neonatal life and long term changes in aortic stiffness: insights from use of intravenous lipids. *Heart* 2011;97:A37-A38.

Lewandowski AJ, Lazdam M, Davis E, Poole R, Diesch J, Francis J, Augustine D, Banerjee R, Suttie J, Neubauer S, Leeson P. Myocardial systolic strain and subclinical atherosclerosis in young adult life. *Heart* 2011;97:A63.

Lewandowski AJ, Davis EF, Lazdam M, Diesch J, Francis J, Lucas A, Singhal A, Neubauer S, McCormick K, Kelly B, Leeson P. Impact of perinatal interventions on cardiovascular structure and function in young adulthood: a 25-year prospective follow-up study of preterm infants. *British Heart Foundation Oxford Centre of Research Excellence Meeting 2011*.

Lewandowski AJ, Lazdam M, Davis EF, Banerjee R, Diesch J, Francis J, Neubauer S, Singhal A, Lucas A, Leeson P. Altered cardiovascular phenotype in young adults born premature: a 25-year prospective follow-up study. *Circulation* 2011;124:A15557.

Lewandowski AJ, Davis EF, Lazdam M, Diesch J, Francis J, Lucas A, Singhal A, Neubauer S, Wilkinson AR, McCormick K, Kelly B, Leeson P. Impact of perinatal interventions on cardiovascular structure and function in young adulthood: a 25-year prospective follow-up study of preterm infants. *Neonatal Society Meeting Autumn* 2011.

Lewandowski AJ, Augustine DX, Davis EF, Lazdam M, Banerjee R, Singhal A, Lucas A, Redman CWG, Neubauer S, Leeson P. Unique left ventricular geometry and function of young adults born preterm: impact of prematurity and preeclampsia exposure. *European Heart Journal* 2012;33(Suppl 1):1041.

Lewandowski AJ, Augustine D, Lamata P, Lazdam M, Davis EF, Francis J, McCormick K, Wilkinson A, Singhal A, Lucas A, Smith N, Neubauer S, Leeson P. The preterm heart in adult life: cardiovascular magnetic resonance and a 3D computational atlas reveal unique left ventricular geometry, mass and function. *British Heart Foundation Oxford Centre of Research Excellence Meeting* 2012.

Lewandowski AJ, Lamata P, Augustine D, Davis EF, Lazdam M, Francis J, McCormick K, Wilkinson A, Singhal A, Lucas A, Smith N, Neubauer S, Leeson P. Cardiac atlas of the preterm heart: cardiovascular magnetic resonance reveals unique variation in 3D left ventricular geometry in young adulthood. *Circulation* 2012;126:A10659.

Lewandowski AJ, Augustine D, Davis EF, Francis J, Singhal A, Lucas A, Wilkinson AR, McCormick K, Neubauer S, Leeson P. Right ventricular systolic dysfunction in young adults born preterm. *European Heart Journal*. Forthcoming 2013.

Augustine DX, Basagiannis C, Suttie J, Cox P, Aitzaz R, **Lewandowski AJ**, Lazdam M, Holloway C, Becher H, Leeson P. Left ventricular 3D speckle tracking echocardiography with CMR correlation: agreement between vendor dependent and independent software. *European Journal of Echocardiography* 2011;12(Suppl 2):ii48-ii78.

Banerjee R, Rial B, Suttie JJ, Cox P, **Lewandowski AJ**, Johnson A, Rider O, Holloway C, Francis J, Robson MD, Schneider J, Clarke K, Leeson P, Neubauer S. Cardiac steatosis is associated with excess body weight in otherwise healthy adults. *Journal of Cardiovascular Magnetic Resonance* 2011;13(Suppl 1):O82.

Kelly B, Lazdam M, **Lewandowski A**, Lucas A, Trevitt C, Kylintireas I, Worton S, Leeson P. Long term impact of antenatal glucocorticoid exposure on aortic structure and function: a 20 year prospective follow-up study of premature infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2011;96:Fa16.

Augustine D, Basagiannis C, Cox P, Suttie J, Holloway C, **Lewandowski AJ**, Lazdam M, Davis E, Leeson P. Assessment of left ventricular 3D speckle tracking echocardiography using vendor dependent and vendor independent software with CMR correlation. *European Heart Journal* 2011;32(Suppl 1):52.

Augustine DX, Suttie J, Cox P, **Lewandowski AJ**, Holloway C, Petersen CE, Myerson S, Neubauer S, Leeson P. CMR right ventricular strain assessment using feature tracking cine images: agreement with echocardiography. *Journal of Cardiovascular Magnetic Resonance* 2012;14(Suppl 1):P244.

Piechnik SK, Ferreira VM, **Lewandowski AJ**, Ntusi NAB, Sado D, Maestrini V, White S, Lazdam M, Banerjee R, Hofman MBM, Moon JC, Neubauer S, Leeson P, Robson MD. Age and gender dependence of pre-contrast T1-relaxation times in normal human myocardium at 1.5T using ShMOLLI. *Journal of Cardiovascular Magnetic Resonance* 2012;14(Suppl 1):P221.

Lamata P, **Lewandowski A**, Kerfoot E, Sinclair M, Leeson P, Smith N. Population analysis of shape based on an automatic meshing service. *2012 Cardiac Physiome Workshop*.

Rider OJ, **Lewandowski AJ**, Nethononda R, Petersen SE, Pitcher A, Holloway CJ, Banerjee R, Byrne JP, Leeson P, Neubauer S. Gender specific differences in left ventricular remodelling in obesity may explain differences in obesity related mortality. *Journal of Cardiovascular Magnetic Resonance* 2012;14(Suppl 1):P75.

DX Augustine, **Lewandowski AJ**, Rai A, Lazdam M, Francis J, Becker H, Petersen SE, Myerson S, Neubauer S, Leeson P. LV mass volumes and ejection fraction estimation with magnetic resonance feature tracking: a comparison with traditional short axis LV CMR contouring. *European Heart Journal* 2012;33(Suppl1):1023.

Banerjee R, Rial B, Suttie J, Ntusi N, **Lewandowski AJ**, Rider OJ, Robson MD, Schneider JE, Leeson P, Neubauer S. The effects of excess weight on cardiac strain and steatosis in adults and children. *Journal of Cardiovascular Magnetic Resonance* 2013; 15(Supple 1):O30.

Davis A, Holloway C, **Lewandowski AJ**, Ntusi N, Nethononda RM, Pitcher A, Francis JM, Leeson P, Neubauer S, Rider OJ. Diameters of the normal thoracic aorta measured by cardiovascular magnetic resonance imaging; correlation with gender, body surface area and body mass index. *Journal of Cardiovascular Magnetic Resonance* 2013; 15(Suppl 1):E77.

Davis EF, **Lewandowski AJ**, Kenworthy Y, Wilkinson AR, McCormick K, Singhal A, Lucas A, Leeson P. Preeclampsia and offspring cardiometabolic phenotype: insights from a 25 year prospective follow up study. *European Heart Journal*. Forthcoming 2013.

CONTENTS

Acknowledgements	ii
Dedications	iv
Abstract	v
List of Publications	vii
Original Manuscripts Contributing to D.Phil. Thesis	vii
Other Published Manuscripts During D.Phil.	viii
Conference Abstracts and Presentations During D.Phil.	x
Contents	xiv
List of Figures	xxi
List of Tables	xxiv
1 Introduction	1
1.1 Prevalence of Preterm Birth	2
1.2 Causes of Preterm Birth	2
1.3 The Developmental Origins of Adult Disease and Early Life ‘Programming’ . .	3
1.4 Cardiovascular Risk in Modern Preterm-Born Cohorts	4
1.4.1 Hypertension	5
1.4.2 Metabolic Abnormalities	5
1.5 Mechanisms Underlying Cardiovascular Risk	6

1.5.1	Hypertension	6
1.5.2	Metabolic Abnormalities	7
1.6	Cardiovascular Development and Being Born Early	8
1.6.1	Vascular Development and Preterm Birth	9
1.6.2	Cardiac Development and Preterm Birth	11
1.7	Further Studies	12
1.7.1	Perinatal Interventions	13
1.7.1.1	Intravenous Lipid Infusions	13
1.7.1.2	Antenatal Glucocorticoids	14
1.7.1.3	Mechanical Ventilation	16
1.7.2	Preeclampsia	17
1.8	Thesis Hypotheses and Objectives	18
2	Study Population	20
2.1	Study Cohorts	21
2.1.1	Cohort 1	21
2.1.2	Cohort 2	23
2.1.3	Cohort 3	23
2.2	Study Recruitment	24
2.2.1	Preterm-Born Cohort (Cohort 1) and Older Term-Born Cohort (Cohort 3)	24
2.2.2	Term-Born Young Adult Cohort (Cohort 2)	25
2.3	Inclusion and Exclusion Criteria for Participation	25
2.3.1	Inclusion	25
2.3.1.1	Preterm-Born Young Adults (Cohort 1)	26
2.3.1.2	Term-Born Young Adults and Older Adults (Cohort 2 and Cohort 3)	26
2.3.2	Exclusion	26
3	General Methods	28

3.1	Follow-Up Study Visit Outline	28
3.2	Cardiovascular Magnetic Resonance	30
3.2.1	Aortic Imaging	30
3.2.1.1	Aortic Pulse Wave Velocity	30
3.2.1.2	Aortic Distensibility	31
3.2.2	Cardiac Imaging	34
3.2.2.1	Cine Acquisition	34
3.2.2.2	Ventricle Volumes and Mass	34
3.2.2.3	Left Ventricle Dimensions	36
3.2.2.4	Right Ventricle Dimensions	38
3.2.2.5	Left Ventricle Tagging	38
3.2.2.6	Feature Tracking Analysis	40
3.3	Intravital Capillary Video Microscopy	45
3.4	Anthropometry and Lifestyle Questionnaire	46
3.5	Blood Sample Collection	47
3.6	Enzyme-Linked Immunosorbent Assays	47
3.6.1	Sample Preparation	48
3.6.2	Standard Preparation	48
3.6.3	Assay Procedure	48
3.7	Brachial and Aortic Blood Pressure Measurements	49
3.8	Ambulatory Blood Pressure Monitoring	50
3.9	Statistical Analysis	51
4	Methods Development	52
4.1	Introduction	52
4.2	Methods	53
4.2.1	Study Population	53
4.2.2	Image Acquisition	53
4.2.3	Acetate Analysis Method	53

4.2.4	Image Pro Plus Analysis Method	54
4.2.5	Statistical Analysis	55
4.3	Results	56
4.3.1	Acetate Analysis Method	56
4.3.2	Image Pro Plus Analysis Method	56
4.3.3	Agreement Between Methods	56
4.4	Discussion	57
4.5	Conclusions	58
5	Postnatal Intravenous Lipids and Cardiovascular Development	59
5.1	Introduction	60
5.2	Methods	61
5.2.1	Study Population	61
5.2.2	Study Visit	63
5.2.3	Statistical analysis	64
5.3	Results	64
5.3.1	Population Characteristics in Early Postnatal Life	64
5.3.2	Population Characteristics in Young Adult Life	65
5.3.3	Aortic Function	67
5.3.3.1	Intravenous Lipid Administration	67
5.3.3.2	Association between Intravenous Lipids, Neonatal Biochem- istry, and Aortic Stiffness	68
5.3.3.3	Intravenous Lipid and Cholesterol Levels in Neonatal and Young Adult Life	70
5.3.3.4	Early Cholesterol Levels and Other Predictors of Pulse Wave Velocity in Young Adulthood	72
5.3.4	Left Ventricular Function	74
5.3.4.1	Intravenous Lipid Administration	74
5.3.4.2	Association with Total Cholesterol Level	75

5.3.4.3	Myocardial and Aortic Function	77
5.4	Discussion	78
5.5	Conclusion	83
6	Antenatal Steroids, Aortic Stiffness, and Glucose Metabolism	84
6.1	Introduction	85
6.2	Methods	86
6.2.1	Study population	86
6.2.2	Study Visit	86
6.2.3	Statistical analysis	88
6.3	Results	89
6.3.1	Study population	89
6.3.2	Antenatal Glucocorticoids and Cardiovascular Risk Factors	89
6.3.3	Antenatal Glucocorticoids and Aortic Stiffness	92
6.3.4	Antenatal Glucocorticoids and Aortic Ageing	92
6.3.5	Glucose Homeostasis, Aortic Stiffness, and Antenatal Glucocorticoids	93
6.4	Discussion	96
6.5	Conclusion	100
7	Preterm Birth and the Adult Left Ventricle	101
7.1	Introduction	102
7.2	Methods	102
7.2.1	Study Population	102
7.2.2	Study Visit	103
7.2.3	Cardiac Atlas for Assessment of Left Ventricular Geometry	103
7.2.4	Statistical Analysis	105
7.3	Results	105
7.3.1	Study Population Characteristics	105
7.3.2	Elevated Left Ventricular Mass in Preterm-Born Young Adults	105
7.3.3	Geometric Changes of the Preterm Left Ventricle	109

7.3.4	Altered Left Ventricular Systolic and Diastolic Function	115
7.3.5	Preterm Birth Risk Factors and Left Ventricular Geometry and Function	117
7.4	Discussion	122
7.5	Conclusion	126
8	Right Ventricle Structure and Function	127
8.1	Introduction	127
8.2	Methods	128
8.2.1	Study Population	128
8.2.2	Study Visit	128
8.2.3	Statistical Analysis	129
8.3	Results	130
8.3.1	Study Population	130
8.3.2	Right Ventricular Geometry	130
8.3.3	Right Ventricular Function	133
8.3.4	Comparisons Between the Right and Left Ventricles	136
8.3.5	Perinatal Risk Factors and the Right Ventricle	137
8.4	Discussion	140
8.5	Conclusion	143
9	Antiangiogenic Factors, Capillary Density, and Blood Pressure	144
9.1	Introduction	144
9.2	Methods	145
9.2.1	Study Population	145
9.2.2	Study Visit	146
9.2.3	Statistical Analysis	146
9.3	Results	147
9.3.1	Study Population	147
9.3.2	Increased Levels of Circulating Antiangiogenic Factors	148
9.3.3	Capillary Density and the Rise in Circulating Antioangiogenic Factors .	149

9.4	Antiangiogenesis, Capillary Rarefaction, and Elevated Blood Pressure	152
9.5	Discussion	156
9.6	Conclusion	159
10	Conclusions	160
10.1	Future Work	164
10.1.1	Further Questions Regarding Cardiac Changes	166
10.1.1.1	Original Hypotheses	167
10.1.1.2	Expected Value of Results	168
	List of References	170
	Appendices	201
A	Lifestyle and Medical History Questionnaire	202
B	Parental Smoking Questionnaire	210

LIST OF FIGURES

2.1	The Study Cohort	22
2.2	Study Recruitment Outline	27
3.1	Study Visit Outline	29
3.2	Oblique Saggital Image of the Aorta	31
3.3	Aortic Pulse Wave Velocity Analysis	32
3.4	Aortic Distensibility Analysis	33
3.5	Left Ventricular Volume and Mass Measurements	35
3.6	Right Ventricular Volume and Mass Measurements	35
3.7	Left Ventricular Internal and External Cavity Diameter	37
3.8	Left Ventricular Length	38
3.9	Right Ventricular Dimension Measurements	39
3.10	Left Ventricle Tagging	40
3.11	Left Ventricular Longitudinal Myocardial Deformation Analysis	42
3.12	Right Ventricular Longitudinal Myocardial Deformation Analysis	42
3.13	Left Ventricular Circumferential Myocardial Deformation Analysis	43
3.14	Imaging Fields for Intravital Capillary Video Microscopy	46
4.1	Acetate Sheet Capillary Density Analysis	54
4.2	Image Pro Plus Capillary Density Analysis	55
4.3	Bland-Altman Plot Comparing the Image Pro Plus and Acetate Methods of Capillary Density Analysis	57

5.1	Preterm Study Cohort: Intravenous Lipids	63
5.2	Increased Aortic Pulse Wave Velocity in Young Adulthood in Individuals Given Postnatal Intravenous Lipids	68
5.3	Reduced Abdominal Aortic Distensibility in Young Adulthood in Individuals Given Postnatal Intravenous Lipids	69
5.4	Association between Intravenous Lipid Parameters in the First Nine Weeks of Life with Aortic Pulse Wave Velocity at Age 25 Years	70
5.5	Postnatal Maximum Cholesterol and Aortic Pulse Wave Velocity in Young Adulthood	71
5.6	Postnatal Maximum Cholesterol and Abdominal Aortic Distensibility in Young Adulthood	72
5.7	Total Cholesterol Levels in Postnatal Life, Adolescence, and Young Adulthood . . .	73
5.8	Example Cholesterol Curve with Intravenous Lipid Exposure	74
5.9	Reduced Myocardial Strain in Young Adulthood in Individuals Given Postnatal In- travenous Lipids	76
5.10	Example of Myocardial Strain Altered by Intravenous Lipid Exposure	77
6.1	Preterm Study Cohort: Antenatal Glucocorticoids	87
6.2	Glucose Homeostasis in Preterm-Born Individuals at Age 15 Years and Age 25 Years.	91
6.3	Increased Aortic Stiffness in Preterm-Born Young Adults Exposed to Antenatal Glucocorticoids	93
6.4	Oblique Saggital Image of the Aorta with Regional Flow Profiles	94
6.5	Interpolated Aortic 'Age' of Preterm-Born Young Adults Exposed to Antenatal Glu- cocorticoids and Not Exposed to Antenatal Glucocorticoids	95
7.1	General Overview of the Cardiac Atlas Formation	104
7.2	Example Case of the Statistical Meshing Process	104
7.3	Left Ventricular Mass Indexed to Body Surface Area	106
7.4	Left Ventricular Mass Indexed to Body Surface Area by Equivalent Blood Pressure	108
7.5	Differences in Shape Between Groups by Modes of Variation of the Principal Com- ponent Analysis	111

7.6	Cumulative Variance Explained by Each Mode of Variation	111
7.7	The First Six Independent Modes of Variation Resulting from the Principal Component Analysis	112
7.8	Distribution of All Subjects in the Principal Component Analysis Coordinates . . .	113
7.9	Statistical Average Shape for Each Group	113
7.10	Left Ventricular Volumes	114
7.11	Longitudinal Peak Systolic and Diastolic Strain Rate	120
7.12	Example Longitudinal Strain Rate Curves for Each Group	121
7.13	Longitudinal Peak Systolic Strain for Preterm-Born Young Adults	123
8.1	Elevated Right Ventricular Mass in Preterm-Born Young Adults	132
8.2	Reduced Ejection Fraction in Preterm-Born Young Adults	135
8.3	Reduced Peak Systolic Strain and Strain Rate in Preterm-Born Young Adults	137
8.4	Percent Change per Gestational Week in Cardiac Measures in the Preterm Group .	138
9.1	Elevated Circulating Antiangiogenic Factors in Preterm-Born Young Adults	149
9.2	Elevated Circulating sFlt-1 Levels with Exposure to Maternal Preeclampsia	151
9.3	Reduced Capillary Density in Preterm-Born Young Adults	153
9.4	Relationship Between Circulating Antiangiogenic Factors, Capillary Density, and Blood Pressure in Preterm-Born Young Adults	159
10.1	Summary of Key Perinatal Factors Related to Cardiovascular Changes in Preterm-Born Young Adults	165
10.2	Study Overview of Cardiovascular Mechanisms in the Preterm-Born Cohort	169

LIST OF TABLES

1.1	Preterm Birth Rates by Geographical Region	2
1.2	Risk Factors for Iatrogenic and Spontaneous Preterm Birth	3
2.1	Comparison of Key Perinatal Characteristics Between the Original Birth Cohort and Follow-Up Cohort	22
2.2	Gestational Age Distribution of Preterm-Born Young Adult Cohort	23
3.1	Intra- and Inter-Observer Coefficients of Variation for Aortic Pulse Wave Velocity and Aortic Distensibility	33
3.2	Aortic Arch Pulse Wave Velocity Data for Calculation of Intra-Observer Coefficient of Variation	34
3.3	Intra- and Inter-Observer Coefficients of Variation for Left and Right Ventricular Mass and Volume Measurements	37
3.4	Intra- and Inter-Observer Coefficients of Variation for Left and Right Ventricle Di- mension Measurements	39
3.5	Intra- and Inter-Observer Coefficients of Variation for Left Ventricle Tagging . . .	41
3.6	Intra- and Inter-Observer Coefficients of Variation for Left Ventricle Feature Track- ing Analysis	44
3.7	Intra- and Inter-Observer Coefficients of Variation for Right Ventricle Feature Track- ing Analysis	45
4.1	Intra- and Inter-Observer Coefficients of Variation for Capillary Density Analysis using the Acetate Method and Image Pro Plus Method	56

5.1	Perinatal Characteristics and Maternal Conditions	62
5.2	Percentage of Individuals at the Five UK Centres from the Original Birth Cohort that Received Intravenous Lipids	65
5.3	Number of Individuals Randomised to Banked Breast Milk or Formula Milk in the Preterm-Born Young Adult Groups	65
5.4	Thyroid Function and Cortisol Levels (Age 0 to 9 Weeks) in the Preterm-Born Young Adult Groups	66
5.5	Characteristics in Young Adult Life of Preterm-Born Individuals	66
5.6	Paternal and Grandparent Family History of Preterm-Born Individuals	67
5.7	Bivariable Regression Associations with Log Transformed Aortic Pulse Wave Ve- locity for the Full Group of Young Adults Born Preterm	75
6.1	Percentage of Individuals at the Five UK Centres from the Original Birth Cohort Exposed to Antenatal Glucocorticoids	86
6.2	Birth, Parental and Neonatal Characteristics of Preterm-Born Young Adults Ex- posed and Not Exposed to Antenatal Glucocorticoids	88
6.3	Number of Individuals Randomised to Banked Breast Milk or Formula Milk in the Preterm-Born Young Adult Groups	89
6.4	Baseline Characteristics of Preterm-Born Individuals in Young Adulthood	90
6.5	Bivariable Regression Associations with Ascending Aorta Distensibility	96
6.6	Bivariable Regression Associations with Aortic Pulse Wave Velocity	97
7.1	Characteristics of Cohorts	106
7.2	Biochemistry and Blood Pressure	107
7.3	Relationship of Variables with Left Ventricular Mass Index	110
7.4	Relationship of Variables with Left Ventricular End-Diastolic Volume Index	116
7.5	Relationship of Variables with Left Ventricular Mass / End-Diastolic Volume	117
7.6	Left Ventricular Volumes and Dimensions	118
7.7	Left Ventricular Systolic and Diastolic Function	119
7.8	Relationship of Variables with Left Ventricular Peak Longitudinal Systolic Strain	121

7.9	Multivariable Regression Coefficient for Gestational Age in Multiple Regression Models of Key Left Ventricular Parameters	122
8.1	Characteristics of Cohorts	131
8.2	Right Ventricular Volumes and Dimensions	132
8.3	Relationship of Variables with Right Ventricular End-Diastolic Volume Index . . .	133
8.4	Relationship of Variables with Right Ventricular Mass Index	134
8.5	Relationship of Variables with Right Ventricular Ejection Fraction	136
8.6	Bivariable Regression Coefficient for Perinatal and Adult Risk Factors and Key Right Ventricular Parameters	139
8.7	Multivariable Regression Coefficient for Gestational Age in Multiple Regression Models of Key Right Ventricular Parameters	140
9.1	Comparison of Key Perinatal Characteristics Between the Original Birth Cohort and Follow-Up Cohort and Subgroup	147
9.2	Characteristics of Cohorts	148
9.3	Relationship of Variables with Soluble Endothelial Growth Factor Receptor 1 . . .	150
9.4	Relationship of Variables with Soluble Endoglin	150
9.5	Characteristics of Cohorts	152
9.6	24 Hour Ambulatory Blood Pressure Levels	154
9.7	Bivariable Correlations Between Functional Capillary Density and Blood Pressure	154
9.8	Bivariable Correlations Between Structural Capillary Density and Blood Pressure .	155
9.9	Bivariable Correlations Between sENG and Blood Pressure	155
9.10	Bivariable Correlations Between sFlt-1 and Blood Pressure	156

1

INTRODUCTION

Cardiovascular diseases remain the single largest cause of mortality and premature mortality in the United Kingdom, despite the reductions in incidence achieved through major initiatives to treat classical risk factors [1]. Identification of novel biological pathways that underlie disease susceptibility raise the potential for new early primary prevention strategies to complement classical management. Investigations into how perinatal growth and *in utero* environment may ‘programme’ risk of later cardiovascular disease have been ongoing for over two decades [2; 3; 4; 5]. One of the recent outcomes of these studies is the observation that certain pregnancy-related conditions, such as preterm birth (less than 37 weeks gestation), have an unusually large impact on the long-term cardiovascular health of the offspring. As such, preterm birth is now emerging as a risk factor for cardiovascular disease [6; 7; 8; 9]. Public health interest in preterm birth has grown as the incidence over recent decades has risen and the number of preterm-born survivors into young adulthood has gradually increased [10; 11]. This has been due in large part to advancements in prenatal and postnatal care .

1.1 Prevalence of Preterm Birth

Preterm birth is now the leading cause of perinatal morbidity and mortality in the developed world [10]. In 2005, it was estimated that preterm birth affected 9.6% of births worldwide, or 12.9 million births per year, with the highest rates in North America and Africa (10.6% and 11.9% of all births, respectively). The lowest rate of preterm birth was seen in Europe, affecting 6.2% of all births (Table 1.1) [11].

Table 1.1: Preterm Birth Rates by Geographical Region

Region	Number in 1000s	Preterm Birth Rate (%)
Europe	466	6.2
North America	480	10.6
Oceania	36	6.4
Africa	4,047	11.9
Asia	6,907	9.1
Latin America and the Carribean	933	8.1
World Total	12,870	9.6

Approximately 5% of preterm births occur at less than 28 weeks gestation (extremely preterm), approximately 15% occur at 28 to 31 weeks gestation (very preterm), and approximately 80% occur at 32 to 36 weeks gestation (moderate to late preterm) [12].

1.2 Causes of Preterm Birth

Preterm birth should not simply be viewed as an abrupt termination of pregnancy, and rather, is a pathophysiological event [13]. Approximately 30 to 35% of preterm births are delivered by prelabour caesarean section or induced (iatrogenic), while 65 to 75% of preterm births are spontaneous (idiopathic). Spontaneous births include both preterm premature rupture of the membranes (25 to 30% of preterm births) and spontaneous preterm labour with intact membranes (40 to 45% of preterm births) [14]. Though the exact mechanism leading to preterm birth is not known, it is believed to result from the interplay of parental factors as well as fetal characteristics and intrauterine conditions [10; 15; 16; 17; 18; 19; 20;

Table 1.2: Risk Factors for Iatrogenic and Spontaneous Preterm Birth

Risk Factor Categories	Examples
<i>Maternal Lifestyle</i>	- Nutritional status (including body size, nutritional intake, vitamin deficiencies) - Smoking or taking drugs - Psychological or social stress - Young age (<18 years) or advanced age (>40 years) - Low socioeconomic status - Multiple partners
<i>Paternal Lifestyle</i>	- Low socioeconomic status - Multiple partners
<i>Pregnancy History</i>	- Decreased time between pregnancies - Previous preterm delivery - Multiple gestations
<i>Genetic Predisposition</i>	- Family history of preterm delivery - Modified fetal, maternal and paternal genotypes
<i>Pre-Existing Maternal Medical Disorders</i>	- Thyroid disorders, asthma, diabetes, hypertension, and obesity
<i>Pregnancy/Intrauterine Complications</i>	- Preeclampsia and hypertension - Infection or inflammation - Fetal growth restriction - Uterine distension - Uteroplacental ischaemia or haemorrhage

21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 31]. Table 1.2 summarises major risk factors for preterm birth.

1.3 The Developmental Origins of Adult Disease and Early Life ‘Programming’

The ‘Barker hypothesis’, or ‘developmental origins of adult disease hypothesis’, stated that adverse influences early in development, particularly *in utero*, could result in permanent physiological and metabolic changes that increase disease risk. The hypothesis originally derived from the observation in the early 1900s that the regions in England with the highest infant mortality rates had the highest rates of coronary heart disease decades later [32]. Since low birth weight was the greatest recorded cause of infant mortality during this time, the hypothesis was generated that low birth weight infants who survived into adulthood were more likely to develop coronary heart disease. Barker *et al.* [3, 33] went on to show a direct rela-

tionship between decreasing birth weight and size with death from coronary heart disease in two large studies of men born in Hertfordshire and Sheffield in the early 1900s. However, preterm birth *per se* did not seem to have an effect, and rather it was growth restriction that was concluded to mediate risk.

A limitation of these large epidemiological studies was the limited data available on gestational age of the subjects. Furthermore, the findings from these studies may be due in part to selective survival, particularly among preterm-born individuals, as the majority of preterm-born individuals in this era were above average birth weight for gestational age. This is in contrast to the fetal growth pattern of preterm-born infants in recent decades. Moreover, Lucas [34, 35] argued that the human epidemiological data presented by Barker *et al.* suggests a link between early nutrition and cardiovascular disease, though it could not actually prove nutritional cause. A large body of evidence in animals has confirmed that early life nutrition affects long-term cardiovascular health outcomes [36]. Lucas proposed the idea of nutritional ‘programming’; the concept that a stimulus or insult can have a long-term influence on an organism if it occurs during a critical period of development. To investigate this hypothesis, Lucas *et al.* [37] developed an experimental approach in human preterm-born infants. Preterm-born infants provided an ideal human model to investigate ‘programming’ as they are born during a stage of critical development and are thus likely to be particularly sensitive to nutritional stimuli. In addition, intensive nutritional postnatal clinical monitoring is possible in this population. Lucas *et al.* [34; 37] went on to show that early feeding diets influence cognitive development and cardiovascular disease risk in childhood and adolescence.

1.4 Cardiovascular Risk in Modern Preterm-Born Cohorts

Preterm birth is now the most common cause of low birth weight (less than 2,500 grams) for the majority of countries worldwide, accounting for over 80% of preterm-born infants [10; 38]. The survival of large numbers of preterm-born infants into adulthood is a relatively recent phenomenon and thus long-term outcome studies are limited but of increasing pub-

lic health interest. Knowledge is growing specific to the preterm infant that they are at risk of cardiovascular disease, irrespective of size at birth, demonstrating similar development of hypertension and reduced insulin sensitivity as seen in individuals who were growth restricted *in utero* [6; 7; 8; 9].

1.4.1 Hypertension

The two largest studies investigating blood pressure in modern cohorts of preterm-born young adults are population-based studies from Sweden [39; 40]. The first study was published in 2005 by Johansson *et al.* [39] on a cohort of 329,477 men with a mean age of 18 years. The investigators reported an inverse relationship between brachial systolic blood pressure and gestational age, and demonstrated that their findings could not be explained by *in utero* growth restriction. Preterm-born individuals had a mean brachial systolic blood pressure increase of 2 to 3 mmHg compared to term-born controls. In the second study from 2011, Crump *et al.* [40] showed in a cohort of 636,552 individuals followed to age 25 to 37 years that young adults born preterm have an increased relative rate of antihypertensive medication prescription, which increased monotonically with the degree of prematurity and was independent of fetal growth restriction. Other recent follow-up studies in adolescents and young adults have shown similar or greater magnitudes of increase between individuals born preterm and term-born controls (3 to 15 mmHg) [8; 41; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52]. These differences in blood pressure are of clinical concern as blood pressure is known to track into later life [53], and it has been demonstrated on a population level that a 5 mmHg increase in brachial systolic blood pressure corresponds to a 21% increase in cardiovascular death and a 34% increased incidence of stroke [54].

1.4.2 Metabolic Abnormalities

The metabolic syndrome begins with early insulin resistance, followed by the development of hyperinsulinaemia, hypertension, obesity, and dyslipidaemia [55]. Children born preterm, regardless of birth weight, have a reduction in insulin sensitivity similar to growth restricted

term-born individuals, which may be a risk factor for type 2 diabetes [56]. Individuals born preterm often suffer metabolic problems such as transient hyperglycaemia despite high insulin levels [57], and it has thus been suggested that the elevation in postnatal insulin levels may cause long-term changes in lipid and glucose metabolism [58]. Such changes may also predispose preterm-born individuals to cardiovascular disease in later life.

1.5 Mechanisms Underlying Cardiovascular Risk

1.5.1 Hypertension

Determining why blood pressure changes occur in preterm-born individuals has encompassed a great body of work, with several potential mechanisms being proposed. Gournay *et al.* [59] have shown that *ex utero* baroreflex maturation occurs at a much slower rate than *in utero* maturation. If arterial baroreflex changes persist, they could lead to long-term alterations in blood pressure [59]. Similarly, preterm birth also interrupts renal development [38]. Sixty percent of nephrons are formed during the last trimester, reaching completion by 36 weeks gestation. Studies of preterm-born individuals have demonstrated a development of proteinuria in adolescence and adulthood, which may result from a loss of nephron mass and subsequent hyperfiltration of remaining nephrons [38]. This could contribute to blood pressure elevation.

Others have demonstrated that nutrition and growth in early life alter blood pressure in later life, highlighting the sensitivity of this period of development and the potential for early intervention to modify risk. The concept that undernutrition ‘programmes’ later cardiovascular outcomes such as blood pressure [60; 61] has resulted in increased interest in the potential implications of feeding diets, specifically those for individuals born small due to being born preterm. Singhal, Lucas *et al.* [62] have carried out the only randomised control trial of this nature in preterm-born infants with follow-up data into adolescence. Preterm-born neonates were randomised to banked breast milk or formula feed diets, and it was shown that, in adolescence, individuals randomised to banked breast milk in neona-

tal life had on average a 4 mmHg lower mean arterial pressure in adolescence than those given formula feed. Though further randomised studies are required, this finding is of potential public health relevance for the development of novel primary prevention strategies in preterm-born neonates.

Although follow-up studies report that adolescents and young adults born preterm show no difference in body stature and may even be shorter and lighter than term-born controls [63; 64; 65], a close relationship between body mass index and systolic blood pressure in preterm-born individuals is apparent [42; 46; 47; 48; 49; 50]. It has been proposed that this relationship is actually a reflection of the rate of weight gain or postnatal catch-up growth [50]. Lucas *et al.* [66] suggest that change in size between birth and later life, or postnatal centile crossing, needs to be considered to try to separate prenatal from postnatal factors contributing to disease risk.

1.5.2 Metabolic Abnormalities

Postnatal weight gain has been demonstrated to contribute to the development of insulin resistance in individuals born preterm. Regan *et al.* [67] found that preterm-born children who had increased postnatal weight gain had an additional reduction in insulin sensitivity, which appeared to be related to nutritional intake during this period. Further metabolic derangements in the preterm-born infant have been identified using magnetic resonance spectroscopy [68]. Magnetic resonance spectroscopy allows for the non-invasive assessment of intrahepatocellular lipids, which are an important reserve of essential fatty acids. Rapid liver growth normally occurs during the third trimester, and thus early *ex utero* exposure of the preterm-born infant alters this pattern of development. Infants born preterm were shown to have an elevation in intrahepatocellular lipids at term equivalent age, which is known to progress from simple steatosis and steatohepatitis through to cirrhosis [68].

Adipose tissue quantity and distribution is also altered in preterm-born individuals, with an increased deposition in the deep subcutaneous and visceral abdominal compartments [69]. Subcutaneous adiposity is primarily related to generalised obesity and circulating levels

of leptin, while visceral adiposity is associated with insulin resistance and dyslipidaemia [70]. The development of obesity in childhood appears to be modifiable by common postnatal feeding regimes, with preterm-born infants breastfed in the newborn period being less likely to develop obesity than infants born preterm fed fortified formulas [35; 71].

The association between preterm birth and cardiovascular risk in adult life is also likely to have genetic links. Inherited genetic polymorphisms and changes in gene expression as a result of epigenetic alterations are seen in the fetal genome, which may manifest *in utero*. During early embryogenesis, DNA undergoes demethylation and remethylation [72]. This epigenetic reprogramming of DNA methylation has been shown to be particularly relevant for genes regulating placental and fetal growth. Furthermore, early nutrition can influence DNA methylation because dietary methyl donors and cofactors are of central importance in providing methyl groups for biological methylation reactions [73]. Such methylation changes may be particularly relevant to metabolic imprinting; the concept that specific environmental events early in life ‘programme’ long-term metabolic alterations throughout the course of development and adulthood [74]. Therefore, placental dysfunction or other perinatal insults may lead to changes in DNA methylation and other epigenetic alterations, which may in turn increase long-term cardiovascular disease susceptibility [75].

1.6 Cardiovascular Development and Being Born Early

Preterm birth occurs during a very sensitive period of cardiovascular development, corresponding to the third trimester of labour normally occurring *in utero*. Thus, it is plausible that the interruption of this normal pattern of cardiovascular development may result in physiological changes and adaptations that predispose these individuals to cardiovascular disease. In order to better study mechanisms driving risk and quantify distinct differences in cardiovascular structure and function in those born preterm, it is important to consider the key fetal and neonatal stages of cardiac and vascular development, and during which window of this developmental process preterm birth occurs.

1.6.1 Vascular Development and Preterm Birth

The vascular system forms in the developing embryo through a combination of vasculogenesis and angiogenesis [76]. Vasculogenesis is the process of new vessel formation as a result of the assembly of endothelial precursors known as angioblasts, while angiogenesis is the process of new vessel formation through the migration and proliferation of endothelial cells from preexisting vessels [76]. Specific circulating molecules, such as the proangiogenic peptide vascular endothelial growth factor, regulate this process [76]. It has been demonstrated that preterm birth is associated with an altered angiogenic state [77]. Preterm birth has also been associated with abnormal retinal vascularisation [78; 79] and a reduction in dermal capillary density [80], which may all be interrelated.

As part of the normal vascular development process, newly formed vessels undergo vascular remodeling, which involves the formation of new vessels and regression of others. Furthermore, vascular remodeling results in the growth of already formed vessels whereby both luminal area and wall thickness increase [76]. As vessels mature, such as in the larger arteries, veins and arterioles, vascular smooth muscle cells form in response to soluble factors secreted by endothelial cells [81]. Unlike in smaller vessels, in medium and large vessels, the smooth muscle cells contribute very little to the static mechanical properties of the vessel, and rather are responsible for synthesising and maintaining the functional extracellular matrix [81].

The organisation of the extracellular matrix changes throughout the period of vessel wall development during gestation [82]. Collagen and elastin are structural matrix proteins of central importance to the mechanical properties of the extracellular matrix [83; 84; 85]. The collagen fibres have a high tensile strength and modulus of elasticity, such that they bear most of the increase in physical force. Elastin fibres, however, have a low tensile strength and are highly extensible and resilient, and thus their role is to evenly distribute force onto collagen fibres and throughout the vessel wall, acting as an elastic reservoir [83; 84; 85]. Mechanistically, the elastic fibres are essential to aortic Windkessel function, whereby the aorta acts as an elastic buffering chamber for the heart, distending during systole in response to the in-

crease in blood pressure, so as to reduce the pressure within the aorta and left ventricular workload [86]. During diastole, the aorta recoils when blood pressure falls. However, due to peripheral vascular resistance, blood enters the aorta from the heart faster than it leaves it, and as a result, approximately 50% of the left ventricular stroke volume in systole is stored in the aorta. This stored blood volume is ejected during diastole due to the elastic nature of the vessel, resulting in a smooth pulsatile output from the heart, reducing cardiac afterload and maintaining organ perfusion [86].

Preterm birth occurs during a very important phase of arterial growth. Wells and Walter [82] showed in bovine aortas that the third trimester is the period of most rapid aortic remodeling. It is also the period of greatest accumulation of elastin, and is thus the most influential period for ensuring long-term vessel structure and function. Approximately 60% of aortic growth occurs during the third trimester [87]. Elastin synthesis falls rapidly following the perinatal period, with little to no synthesis or turnover in adult tissue [81; 88; 89]. The early birth transition from the *in utero* to *ex utero* environment in preterm-born infants results in an abrupt haemodynamic insult on the underdeveloped vascular system, and it is forced to compensate in response to this early transition. The structural composition of major arteries is heavily driven by local haemodynamic conditions during the perinatal period, which directly influence their functional properties [90]. The steady increase in luminal pressure that the fetal aorta is normally exposed to provides a normal environment for aortic development, rising from approximately 28 mmHg at 20 weeks gestation to around 45 mmHg at 40 weeks gestation [91]. In animal models, abnormal haemodynamic changes alter normal patterns of arterial development, including the accumulation of specific wall constituents such as the ratio of elastin-to-collagen [92; 93; 94]. A few studies have now shown that arterial stiffness is increased in preterm-born individuals, which may contribute to the observed blood pressure elevation in this subpopulation [51; 95; 96; 97]. It remains to be explored in great detail what specific factors related to preterm birth are the greatest determinants of long-term alterations in arterial stiffness.

1.6.2 Cardiac Development and Preterm Birth

Normally during embryonic and fetal life, myocardial cells undergo hyperplasia [98]. During this period of development, cardiac size increases primarily via an increase in the number of mononucleated myocardial cells. At birth, there is a switch in cardiomyocyte phenotype from a fetal hyperplastic pattern to neonatal hypertrophic response. While hyperplasia continues to an extent following birth, there is little evidence of continued hyperplasia beyond three weeks postnatally [98]. In early postnatal life, the differentiated mononucleated cells can synthesise DNA and undergo mitosis. Continued karyokinesis without cytokinesis (mitotic division of the cell nucleus without cytoplasmic separation) results in formation of multinucleated myocardial cells [99; 100]. As a result, in humans, adult myocardial cells are primarily binucleated (approximately 20%) [101] and, as individuals age and hypertrophy progresses, polyploidy of myocardial cells increases [102]. DNA replication and myocardial cell mitosis cease early in the first month of postnatal life, and following this phase, any increase in myocardial mass is almost exclusively driven by hypertrophy of myocardial cells [101]. As such, in the first few months of postnatal life, myocardial cell volume increases approximately 30-fold in humans. In addition, during the hypertrophic growth phase, hyperplasia of non-muscular components of the myocardium occurs, which also contributes to the increase in cardiac growth [101]. In line with this, exposure of the neonatal heart to stress has been shown to alter the pattern of normal development in animal models [101; 103; 104; 105]. In rats, exposure to stress during the transition phase of hyperplastic to hypertrophic growth, including an increased haemodynamic load, results in heightened activation of DNA synthesis, which alters myocardial cell hyperplasia. More significantly, this changes the profile of binucleated myocardial cells and results in an increase in myocardial cell polyploidy. This indication that polyploidy can be altered and programmed by events early in development is important because polyploidy is considered to be irreversible and is linked to cardiac dysfunction [106; 107; 108].

The most dynamic change in circulation in humans occurs during the transition from fetal to neonatal life. Significant flow changes take place as the low resistance placental cir-

culuation transforms into a high resistance arterial system [109]. The haemodynamic shift of preterm birth is particularly dramatic, as the abrupt and premature shift to *ex utero* life takes place during a period of development that would normally take place *in utero*. As such, the heart is insufficiently developed to cope with the sudden, sustained increase in afterload and fluctuation in preload that occurs after birth [110]. In lambs, Bensley *et al.* [111] demonstrated for the first time that the early transition of preterm birth leads to cardiac remodeling. The investigators demonstrated that the myocardium of preterm-born lambs showed an increase in collagen content (five- to seven-fold increase), an increase in myocardial cell volume indicative of hypertrophy, and an increase in myocardial cell ploidy, suggestive of accelerated maturation. Furthermore, the differentiated mononucleated myocardial cells of the heart of preterm-born lambs appeared to undergo endomitosis rather than karyokinesis. Endomitosis is a mitotic process that does not involve the breakdown of the nuclear membrane or cytoplasmic division, but rather is defined by a doubling of the number of chromosomes within the nucleus [105]. This is indicative of abnormal cardiac maturation. Therefore, based on these findings, it would seem that this haemodynamic transition and the early exposure to *ex utero* life alters development of the myocardium and may thus result in long-term cardiac vulnerability. Cardiac structural and functional changes remain to be explored in preterm-born humans. Understanding whether cardiac structure and function is altered long-term in individuals born preterm, and whether specific factors during the perinatal period are influencing these alterations, is of importance in the understanding of their potential risk of cardiac disease.

1.7 Further Studies

While the understanding of long-term cardiovascular risk of preterm-born individuals continues, and studies are now investigating cardiovascular structure and function in greater detail, there remains limited data defining specific perinatal events of preterm birth that alter cardiovascular development and risk. Furthermore, large knowledge gaps in our understanding still exist in terms of what changes are seen in adulthood in these individuals,

such as whether preterm birth is related to changes in cardiac structure and function. The highly sensitive period of growth and development in preterm-born individuals corresponds with a time where they are exposed to a range of environmental stressors and interventions to the cardiovascular system, both *in utero* and *ex utero*, which may alter development and result in physiological changes that increase disease susceptibility. For instance, in addition to how preterm an individual is at birth, postnatal weight gain, and *in utero* growth restriction, which have already been discussed as perinatal factors that may moderate risk, clinical perinatal interventions and pregnancy complications such as preeclampsia may further complicate risk in this growing subgroup of the population. Further investigation of changes in vascular and cardiac structure and function, as well as determining the relevance of perinatal factors associated with preterm birth to these changes, is important for understanding cardiovascular disease risk and for longer term development of potential targets for prevention.

1.7.1 Perinatal Interventions

The more methodical introduction of perinatal interventions in the late 1970s and early 1980s increased preterm neonatal survival [9]. Some of the most significant interventions included intravenous lipid infusions, antenatal glucocorticoids, and mechanical ventilation, which are relevant to growth and development during the perinatal period. Given the sensitivity of this period of development, the potential cardiovascular implications of these interventions are worth consideration.

1.7.1.1 Intravenous Lipid Infusions

Intravenous lipids, such as Intralipid, are widely used in parenteral nutrition of preterm-born individuals as a source of energy and essential fatty acids. Infants born preterm are at risk of significant morbidity, and intravenous lipids are proven to be beneficial for neonatal brain and central nervous system development [112]. Intralipid consists of triglycerides in the form of soybean oil emulsified by egg phospholipid and glycerol [113]. Intralipid ad-

ministration results in an elevation of free cholesterol as a result of an exceeded capacity to clear phospholipids, likely due in part to saturation of the lecithin cholesterol acyltransferase pathway. This leads to a rise in cholesteryl esters, but greater proportional rise in phospholipid and free cholesterol in the form of lipoprotein X in the low density lipoprotein fraction [113; 114; 115]. Hypercholesterolaemia is an important mediator of atherogenesis, which begins with lipid rich fatty streaks [116; 117; 118]. Fatty streaks first emerge in the abdominal aorta during fetal life [119; 120], and exposure to abnormal circulating cholesterol levels of the mother during pregnancy has been shown to increase aortic fatty streaks in the offspring [121]. Therefore, it is of interest to determine whether intravenous lipid exposure in early postnatal life, which is a critical period of aortic development, also relates to long-term changes in aortic physiology. Furthermore, it has been shown in animal models that a diet induced hyperlipidaemia is directly related to myocardial dysfunction [122; 123], and thus investigation of whether intravenous lipid infusions relate to long-term changes in myocardial structure and function is of interest.

1.7.1.2 Antenatal Glucocorticoids

Antenatal glucocorticoids, such as betamethasone and dexamethasone, are commonly given to women who show evidence of preterm labour as they are beneficial for the prevention of neonatal respiratory distress syndrome in preterm-born infants, and thus substantially reduce neonatal morbidity and mortality [124]. Glucocorticoids are potent regulators of growth and development, thus concerns of possible adverse effects of antenatal glucocorticoid exposure on long-term cognitive, respiratory, metabolic, and cardiovascular function in the offspring have been raised [125]. While it has been shown that a single course of antenatal glucocorticoid treatment is beneficial for lung maturation and reduces complications such as neonatal respiratory distress syndrome [126], concerns have increased over whether multiple doses have a greater influence on fetal development and later risk of adverse health [127]. A large randomised trial of multiple doses of antenatal glucocorticoids published in *The Lancet* in 2008 showed that this method of treatment did not improve preterm birth outcomes, and in fact, reduced normal development, resulting in reduced size and weight

of the offspring at birth [128]. However, the literature remains conflicting as Crowther *et al.* [129] have published a Cochrane review reporting that a repeat dose of antenatal glucocorticoids reduces the risk of respiratory complications and other serious health problems in the offspring in early postnatal life.

A consistent finding from animal studies, both in sheep and in rats, is that even transient exposure to betamethasone and dexamethasone glucocorticoids during specific windows of fetal life may have lasting effects on vascular function into adulthood [130]. This has led to concerns that antenatal glucocorticoids given to pregnant women at risk of preterm delivery may influence early life programming of cardiovascular risk in the offspring. There is a surge in elastin and collagen connective tissue accumulation in late gestation and early neonatal life, partly due to a surge in corticosteroid levels, which may be further influenced by the regulatory actions of antenatal glucocorticoids [92]. In the only double-blind, placebo-controlled, randomised trial of antenatal betamethasone investigating vascular and metabolic changes in humans, there was no evidence of any differences in brachial blood pressure parameters in the offspring [131]. These findings suggest no clear signs of glucocorticoid induced hypertension, contrary to findings from animal models. However, after a 75 gram oral glucose tolerance test, insulin concentration was increased at 30 minutes and glucose concentration was decreased at 120 minutes in those exposed to antenatal glucocorticoids. This suggests that antenatal exposure to betamethasone may result in insulin resistance in adult offspring. It remains to be explored, however, whether exposure to antenatal glucocorticoids impacts the aorta. This is of particular interest given that connective tissue accumulation and arrangement within the aortic wall is critically altered in animal models in late gestation in response to antenatal glucocorticoid exposure [132], and, therefore, *in utero* glucocorticoid exposure may induce a localised aortic rather than systemic arterial effect on the offspring.

Furthermore, cortisol levels were shown in fetal lambs to be a contributor to the postnatal change in the myocardial growth pattern [133]. During the switch from the myocyte hyperplastic to hypertrophic growth phase, myocytes largely lose the ability to replicate, which is characterised by a decrease in myocardial DNA concentration and an increase in protein-

to-DNA concentration ratio [134]. Rudolph *et al.* [133] infused cortisol into the left coronary arteries of preterm-born lambs, which only supplies blood flow to the free wall of the left ventricle and the ventricular septum, but not the right ventricle free wall. Lambs given cortisol intravenously into their left coronary arteries showed altered left ventricular myocardial DNA concentrations with no changes in right ventricular myocardial DNA concentrations, suggesting an early switch from a hyperplastic to hypertrophic growth phase *in utero*. It is therefore of interest to see whether, in humans, exposure to antenatal glucocorticoids has long-term developmental impacts on myocardial growth.

1.7.1.3 Mechanical Ventilation

Respiratory disease and the need for ventilation are major complications of preterm birth [135; 136]. While respiratory distress or infection may lead to changes in the pulmonary vasculature sufficient to have a haemodynamic impact on the heart, mechanical ventilation may in itself negatively affect cardiac remodeling. The use of mechanical ventilation in neonates is known to increase survival and is considered an essential clinical intervention for managing neonates born preterm with respiratory distress syndrome [135]. Despite these advantages, it is recognised that changes in pulmonary physiology induced by mechanical ventilation may have negative influences on normal cardiac function and lead to distinct changes in cardiac remodeling [136]. For instance, mechanical ventilation induces extreme changes in intrathoracic pressure and lung volume [136]. This change in pressure may lead to increased right atrial pressure, followed by a reduction in venous return and subsequent reduction in right ventricle preload and cardiac output [137]. In addition, hyperinflation induced by mechanical ventilation may result in compression of alveolar vessels as a result of alveolar distension, causing an increase in pulmonary vascular resistance and leading to an increase in right ventricular afterload [137]. According to the Laplace relationship, an early adaptation to an increase in intraluminal pressure caused by an increase in right ventricle afterload is compensated by an increase in right ventricle wall thickness [138]. The left ventricle may also be affected by the induced intrathoracic pressure changes, as this affects transmural pressure of the aorta and could therefore increase left ventricular afterload

[136]. Therefore, the relationship between postnatal ventilation and long-term alterations in left and right ventricular structure and function in preterm-born individuals needs to be considered.

1.7.2 Preeclampsia

The International Society for the Study of Hypertension in Pregnancy defines preeclampsia as the new onset of hypertension (brachial systolic blood pressure ≥ 140 mmHg and/or brachial diastolic blood pressure ≥ 90 mmHg) after 20 weeks of gestation, returning to normal postpartum, and proteinuria (≥ 300 mg/day or a spot urine protein/creatinine ratio of ≥ 30 mg/mmol) [139]. Preeclampsia is one of the leading causes of preterm birth and is recognised as an antecedent for around 20 to 30% of all preterm births [10; 140; 141]. It is increasingly being recognised as more than an isolated disease of pregnancy that resolves itself completely upon delivery of the placenta [142]. Both in animal experimental models and human epidemiological studies, the offspring of preeclampsia have been shown to be at an increased risk of developing high blood pressure, with almost double the risk of stroke in later life [142]. The risk is greatest in early onset preeclampsia, diagnosed before 34 weeks gestation, which generally results in preterm birth [143].

Preeclampsia is characterised by abnormal placental development [142]. In addition, reactive oxygen species released from the ischaemic placenta trigger a systemic oxidative and inflammatory state [144; 145; 146]. As such, the offspring of preeclamptic pregnancies develop in an environment of placental insufficiency and restricted oxygen supply [147]. Furthermore, preeclampsia is characterised by the development of altered endothelial function [147] and abnormal levels of circulating antiangiogenic factors in the mother [148], predominantly during the second trimester. As such, the fetus is exposed to a multitude of *in utero* risk factors that may influence cardiovascular development. Though preeclampsia often occurs in combination with *in utero* growth restriction and preterm birth, it has been demonstrated that exposure to maternal preeclampsia results in an independent risk of stroke [149] and endothelial dysfunction [150] in normal birth weight, term-born offspring. Endothelial

dysfunction and increased intima media thickness, a subclinical marker of atherosclerosis, are the predominant changes seen in the vasculature in preterm-born individuals exposed to preeclampsia compared to individuals born preterm to a normotensive pregnancy [51].

It has been demonstrated in rats that exposure to maternal hypoxia results in elevated levels of collagen within myocardial tissue [151; 152]. Furthermore, rats exposed to hypoxia during late fetal development have altered cardiac proton production and during reperfusion, increased production of myocardial acetyl coenzyme A [153; 154]. In addition, protein kinase C levels, relevant to ischaemic cardioprotection, are also significantly reduced [150]. In a follow-up study of individuals born to hypertensive pregnancies, Himmelmann *et al.* [155] found no changes in left ventricular mass by echocardiography in adolescence. Fugelseth *et al.* [156], however, found that children exposed to maternal preeclampsia had reduced diastolic function, along with shorter left ventricles and increased heart rates. However, in this study, the group of preeclamptic offspring were born about six weeks earlier and weighed on average nearly 2,000 grams lighter at birth. Therefore, it is not possible to determine from this whether these differences were due to preeclampsia *per se* or whether they were a consequence of being born early. Further investigation into the specific impacts of maternal preeclampsia on the offspring cardiac structure and function within a preterm-born cohort is required.

1.8 Thesis Hypotheses and Objectives

It is predicted that being born preterm will be related to distinct changes in cardiovascular structure and function in young adulthood, and that specific perinatal events modify the degree of changes seen in later life. More specifically, it is predicted that:

1. Postnatal intravenous lipid and antenatal glucocorticoid exposure in preterm-born individuals will be related to distinct changes in aortic function and left ventricular structure and function in young adulthood;

2. Preterm-born individuals will demonstrate a distinct left ventricular and right ventricular structure and function in young adulthood compared to individuals that were born term;
3. Preterm birth will be related to a heightened antiangiogenic state in young adulthood, which will be associated with elevated blood pressure levels and lower capillary density in young adulthood in those born preterm.

In order to investigate these hypotheses, this thesis therefore aims to:

1. Determine methods to be used for detailed cardiovascular phenotyping of preterm-born and term-born individuals;
2. Establish the long-term impact of perinatal interventions relevant to growth and development on the aorta and heart in preterm-born individuals;
3. Quantify changes in left ventricular structure and function between preterm-born and term-born individuals, and perinatal factors related to these changes;
4. Quantify changes in right ventricular structure and function between preterm-born and term-born individuals, and perinatal factors related to these changes;
5. Establish changes in circulating antiangiogenic and angiogenic factors in preterm-born individuals, and their relationship with changes in functional and structural capillary density and blood pressure;
6. Discuss and propose future work to further explore the underlying pathophysiology of the findings from this thesis.

2

STUDY POPULATION

The work in this thesis is based on a study my supervisor and I designed. I then carried out the recruitment and data collection for the study, which included 234 participants from three different cohorts. This chapter examines the cohorts in detail and how participants were recruited for the study. The first of these three cohorts is a group of preterm-born young adults who were originally recruited at birth (*cohort 1*). I followed up these individuals in young adulthood to perform detailed cardiovascular phenotyping to allow for more precise quantification of early disease development, as well as a more holistic understanding of the factors driving these changes. A significant strength of this longitudinal study of preterm-born individuals is the prospective collection of parental and perinatal data in this cohort. As comparison groups, I recruited a group of young adults born term to uncomplicated pregnancies, with similar age and sex distributions to the young adults born preterm, to undergo identical investigations (*cohort 2*), as well as a group of term-born individuals a decade older with a similar sex distribution to characterise normal ageing related cardiovascular changes (*cohort 3*).

2.1 Study Cohorts

2.1.1 Cohort 1

A cohort of 926 preterm-born infants were enrolled, at birth, from five centres in England (Cambridge: $n = 335$, 36.2%; Ipswich: $n = 101$, 10.9%; King's Lynn: $n = 66$, 7.1%; Norfolk and Norwich: $n = 233$, 25.2%; Sheffield: $n = 191$, 20.6%) between 1982 and 1985 into a randomised trial of milk feeding regimes to study the influence of early diet on later cognitive function and cardiovascular disease. Participants were born low birth weight ($<1850\text{g}$) and obstetric and neonatal details recorded at birth. Details included pregnancy complications of the mother, information on interventions during their hospital stay, and biochemical analysis of blood samples during the first nine weeks of life [157]. Follow-up studies of the children had been performed at ages 18 months [37] and seven years [158], with a substudy at age 15 years [157]. I recontacted 240 individuals from the preterm-born cohort who were now young adults and who had agreed to be contacted about future studies, of whom 102 took part in the follow-up study (Figure 2.1) [159; 160; 161; 162]. The number of individuals studied from this cohort was based on the number interested in participating. Compared to the original cohort of preterm-born infants recruited at birth, those followed up in young adulthood were similar in perinatal characteristics (Table 2.1), apart from a small 70 gram (5%) difference in birth weight. This is accounted for by a marginally more preterm cohort such that birth weight Z score (birth weight adjusted for gestational age and sex) is identical between groups. Of the 102 individuals in the cohort, 14 (13.7%) were born extremely preterm (less than 28 weeks gestation), 56 (54.9%) were born very preterm (28 to 31 weeks gestation), and 32 (31.4%) were born moderate to late preterm (32 to 36 weeks gestation) (Table 2.2). The proportion of young adults born preterm to a pregnancy complicated by preeclampsia was the same as in the original cohort and similar to that reported in other studies of preterm birth. Preeclampsia is recognised as an antecedent for around 20 to 30% of all preterm births [10; 140; 141].

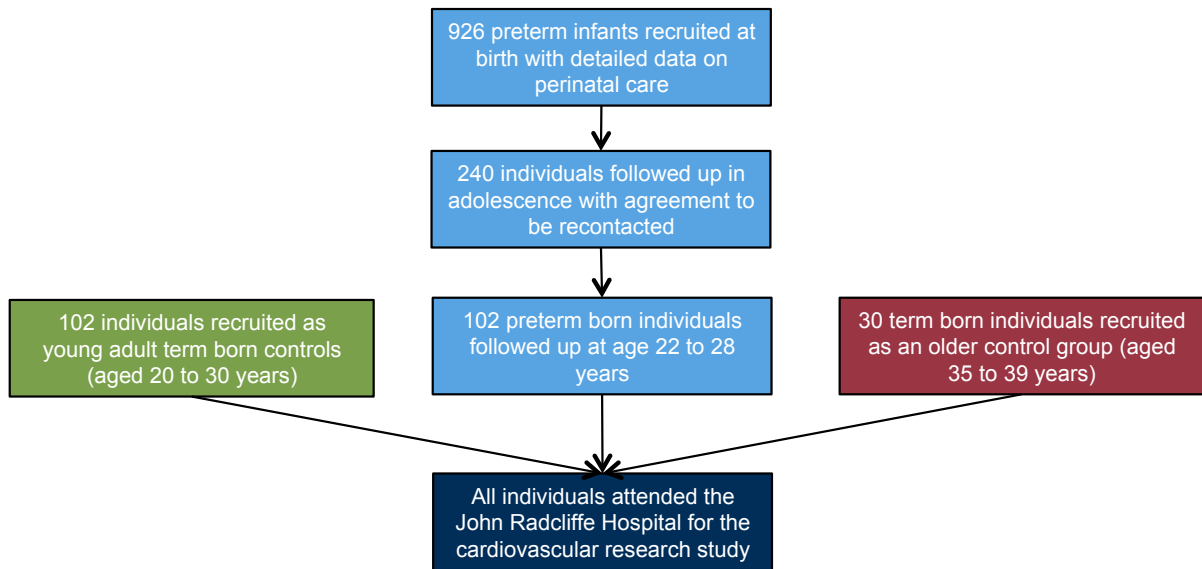


Figure 2.1: The study cohort. Two hundred and forty individuals with agreement to be recontacted from a cohort of 926 preterm-born individuals (blue) were followed up in adolescence. One hundred and two of these individuals were seen in young adulthood to undergo cardiovascular phenotyping. A group of 102 term-born young adults (green) frequency matched for age and sex and a group of 30 term-born adults a decade older (red) frequency matched for sex were recruited to undergo identical investigations.

Table 2.1: Comparison of Key Perinatal Characteristics Between Birth Cohort and Follow-Up Cohort

	Original cohort (n = 926)	Follow-up cohort (n = 102)	P Value
Males, n (%)	463 (50.0)	47 (46.1)	0.48
Gestational age (weeks)	30.8 ± 2.8	30.3 ± 2.5	0.30
Birth weight (grams)	1369.8 ± 315.5	1297.0 ± 286.8	0.03
Birth weight Z score	-0.81 ± 1.32	-0.84 ± 1.21	0.83
Small for gestational age, n (%)	310 (33.5)	32 (31.4)	0.67
Maternal smoking during pregnancy, n (%)	299 (32.3)	25 (24.5)	0.11
Paternal smoking during pregnancy, n (%)	224 (24.2)	31 (30.4)	0.17
Maternal preeclampsia, n (%)	259 (28.0)	29 (28.4)	0.92
Mechanical ventilation (days)	4.65 ± 9.84	4.14 ± 7.43	0.61
Number of individuals ventilated, n (%)	476 (51.4)	55 (53.9)	0.63

Values as Mean ± Standard Deviation unless stated otherwise.

Table 2.2: Gestational Age Distribution of Preterm-Born Young Adult Cohort

Gestational Age (weeks)	Number of Individuals, n (%)
26	7 (6.86)
27	7 (6.86)
28	13 (12.7)
29	16 (15.7)
30	9 (8.82)
31	18 (17.6)
32	13 (12.7)
33	10 (9.80)
34	4 (3.92)
35	3 (2.94)
36	2 (1.96)

2.1.2 Cohort 2

A cohort of 102 controls born to uncomplicated pregnancies, including being born normal birth weight for gestational age, being born between 38 and 42 weeks gestation, and who were not exposed to gestational hypertension or preeclampsia, were recruited from the local community (Figure 2.1). Information on family history and pregnancy history was ascertained by questionnaire (see Appendix A). These individuals were frequency matched for age and sex to the young adults born preterm. The number of young adult term-born controls recruited was based on the number of preterm-born young adults recruited for the study.

2.1.3 Cohort 3

A cross-sectional cohort of 348 subjects born in Mill Road Maternity Hospital, Cambridge, United Kingdom between 1969 and 1975 for whom birth and early growth measures were available from birth records. This cohort was recruited around ten years ago to attend clinics at the Vascular Physiology Unit in Great Ormond Street Hospital to gather detailed information on vascular function, classical cardiovascular risk factors, body size, lifestyle, diet, fitness, and birth measures [163]. Thirty individuals from this cohort were recruited to take part in the follow-up study (Figure 2.1). The purpose of recruiting this group was to characterise normal ageing related cardiovascular changes. The number of individuals studied from this cohort was based on the number interested in participating.

2.2 Study Recruitment

2.2.1 Preterm-Born Cohort (Cohort 1) and Older Term-Born Cohort (Cohort 3)

Follow-up of the preterm-born cohort (*cohort 1*) and cohort of term-born adults a decade older (*cohort 3*) followed a standardised procedure as outlined by the blue/red boxes in Figure 2.2. As the last follow-up studies took place at the MRC Childhood Nutrition Research Centre at University College London about ten years prior to the follow-up study I carried out, birth dates, subject names, previous known addresses, and their last registered general physician information was provided by them in three coded databases. Following decoding of the databases, cover letters with a return form to send back to me were then sent directly to the individual at the previous address to enquire whether they would like further information regarding the follow-up study at the John Radcliffe Hospital in Oxford. If the letter was returned due to a change of address, the individual's current address was traced via the National Health Service Strategic Tracing Service and they were then sent the cover letter and return form as above. If the individual did not respond to the first letter sent out and there was no return of the letter indicating a change of address, the letter was sent out again up to a maximum of three times.

Where the individual expressed an interest in the study, they were then provided with the full study information sheet through the post and/or by email. After at least a week, the individual was contacted (according to the individual's wishes expressed in the return form) by telephone or email and given the opportunity to talk through any issues and discuss what exactly would be required of them as a participant in the study. If the individual expressed continued interest in taking part, they were then invited to be a participant in the study and to attend the study visit in Oxford at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) and the Oxford Cardiovascular Clinical Research Facility (CCRF) at the John Radcliffe Hospital.

2.2.2 Term-Born Young Adult Cohort (Cohort 2)

Recruitment of young adult term-born controls followed the procedure outlined by the green boxes in Figure 2.2. Emails were sent to Oxford University Colleges and Departments with information to be sent out to students and staff regarding the study. Posters were also put up around the John Radcliffe Hospital and Churchill Hospital, and all Graduate Common Rooms of the Colleges of the University of Oxford. Where an individual expressed interest in the study, they were provided with the study information sheet by letter or email as requested and asked to get in touch by email or telephone if still interested in taking part. If interested, they were asked questions about gestational age, birth weight, any medications they were on and overall health. This information was confirmed by questionnaire at the time of the study visit (see Appendix A). If the individual was born term, at normal size for gestational age, their mother did not have any pregnancy complications with them such as preeclampsia or gestational hypertension, and had no history of health complications or medications that may affect cardiovascular function, they were considered eligible for the study. The individual was then contacted by phone or email as per requested and given the opportunity to talk through any issues. If the individual expressed continued interest in taking part, they were then invited to be a participant in the study and to attend the study visit in Oxford at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) and the Oxford Cardiovascular Clinical Research Facility (CCRF) at the John Radcliffe Hospital.

2.3 Inclusion and Exclusion Criteria for Participation

2.3.1 Inclusion

- Participant was willing and able to give informed consent for participation in the study;
- Able (in my opinion and that of other researchers if in question) and willing to comply with all study requirements.

2.3.1.1 Preterm-Born Young Adults (Cohort 1)

- Born preterm (<37 weeks completed gestation).

2.3.1.2 Term-Born Young Adults and Older Adults (Cohort 2 and Cohort 3)

- Born at term (>37 weeks completed gestation);
- Born normal birth weight for gestational age;
- Not exposed to pregnancy complications such as preeclampsia or gestational hypertension.

2.3.2 Exclusion

The participant could not enter the study if ANY of the following applied:

- Unwilling or unable to give informed consent for participation in the study;
- Pregnant or lactating during the course of the study;
- Donated blood within four months of study commencement;
- Suffered from known cardiovascular disease, diabetes mellitus or hypertension requiring medication;
- Scheduled for elective surgery or other procedures requiring general anaesthesia within a month of study commencement;
- Any other significant disease or disorder which, in the opinion of the investigators, might have influenced the participant's ability to participate in the study.

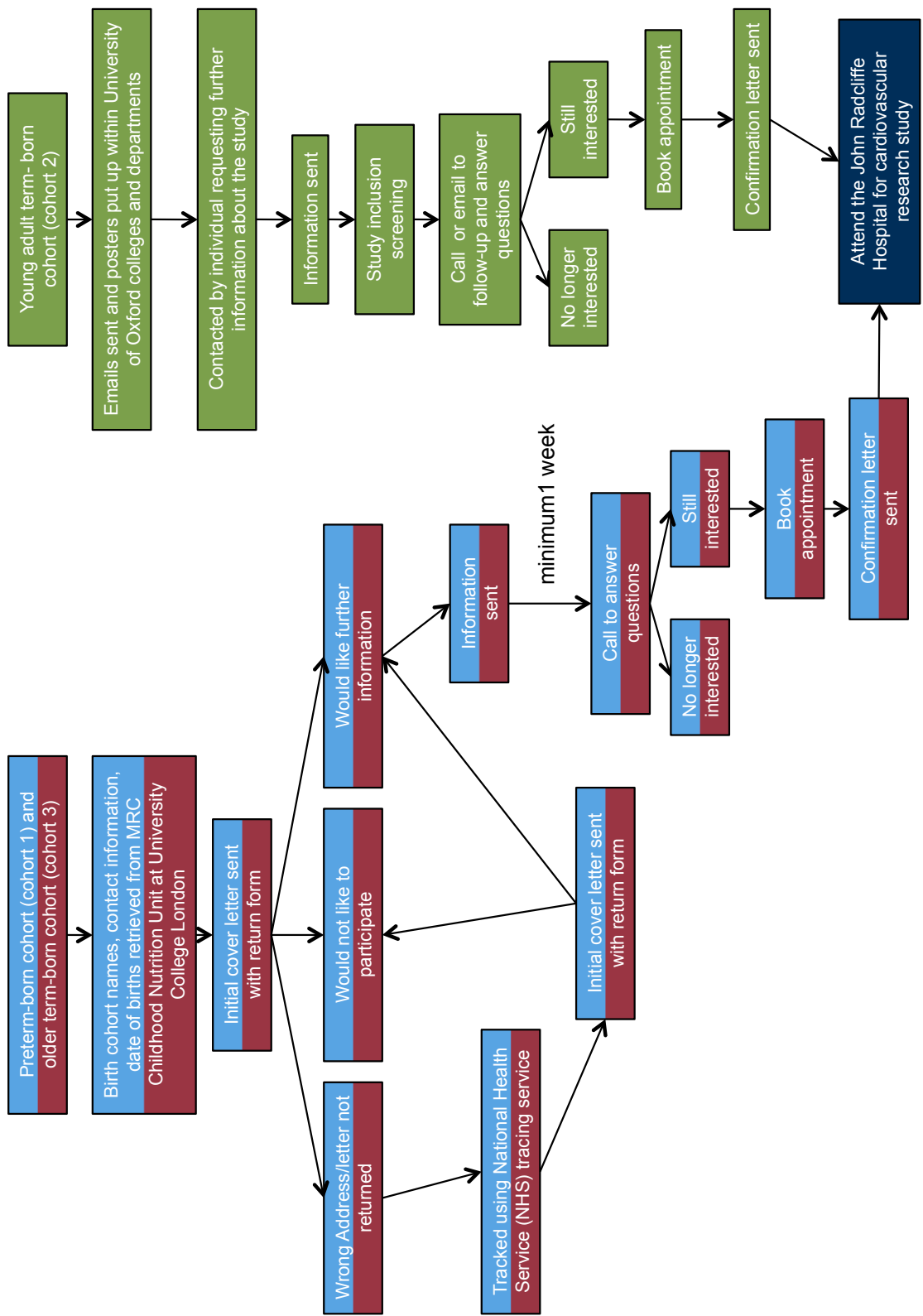


Figure 2.2: Study recruitment outline. The blue/red boxes demonstrates the recruitment procedure for the preterm-born cohort and the older term-born cohort. The green boxes demonstrates the recruitment procedure for the young adult term-born controls.

3

GENERAL METHODS

The following chapter examines the methods that were used to investigate the cardiovascular system of preterm-born young adults compared to term-born controls. First, I present a general overview of the study visit that all participants underwent. I then present details of the imaging modalities used as well as blood sample collection, acquired lifestyle information, and basic cardiovascular risk factor assessment. Finally, for each of the measurements done in the study, I discuss how the data were analysed, along with their respective intra- and inter-observer coefficients of variation. Intra- and inter-observer coefficients of variation for all measurements were determined based on 12 randomly selected scans from the study groups described in Chapter 2.

3.1 Follow-Up Study Visit Outline

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity and blinded analysis. Preterm-born young adults (*cohort 1*) and the older group of adults born term (*cohort 3*) travelled to Oxford the day before the study and stayed in a hotel near the John Radcliffe Hospital. Details of the study procedures and what to expect were once

again described to all participants prior to providing written informed consent, with the opportunity to raise and discuss any further questions and concerns. Following consent, all participants underwent a cardiovascular magnetic resonance scan of their heart and aorta, non-invasive brachial and aortic blood pressure measurements, venepuncture, anthropometric measurements, and completed detailed lifestyle and medical history questionnaires (Figure 3.1). A subgroup of individuals from the preterm-born ($n = 30$) and term-born young adult groups ($n = 102$) (*cohort 1* and *cohort 2*, respectively) underwent microvascular structural and functional imaging and 24 hour brachial blood pressure measurements.

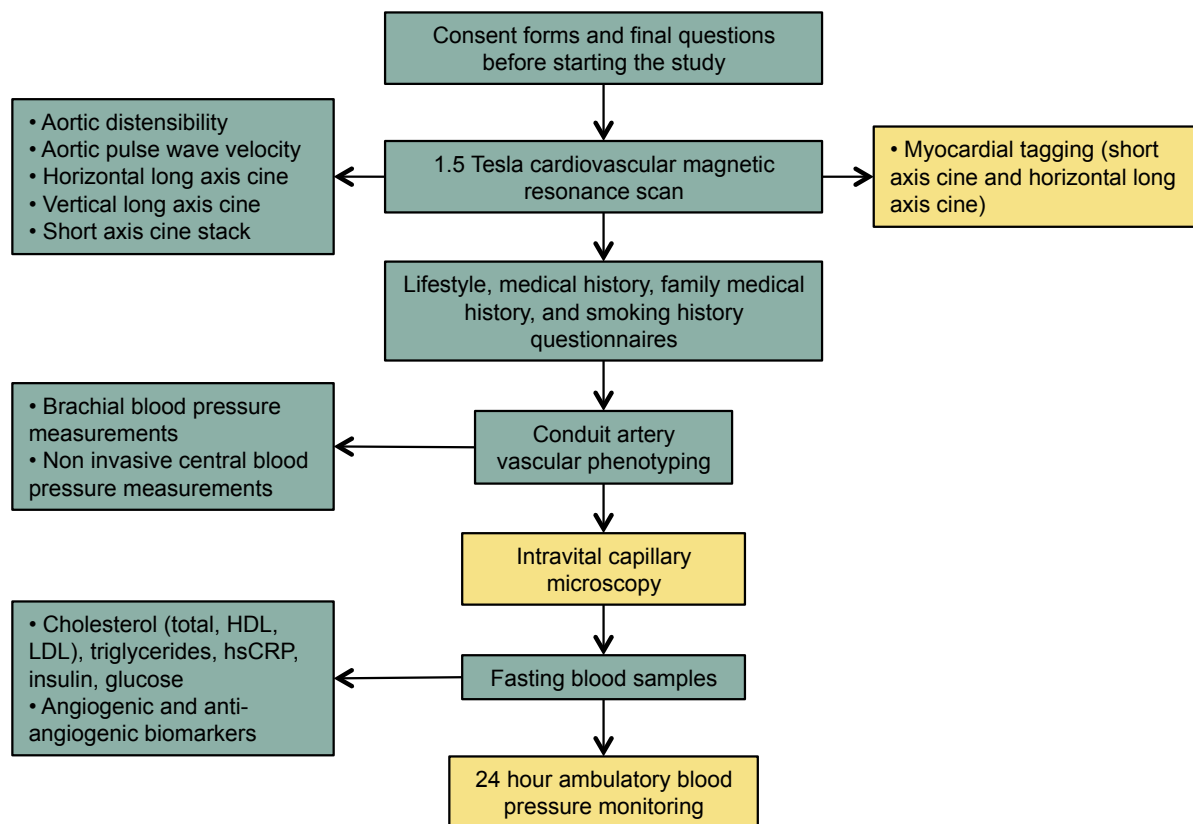


Figure 3.1: Study visit outline. The green boxes indicate procedures that were carried out on all study participants. The yellow boxes indicate procedures that were carried out on a subgroup of study participants (preterm-born young adults ($n = 30$) and term-born young adults ($n = 102$)).

3.2 Cardiovascular Magnetic Resonance

Cardiovascular magnetic resonance imaging was performed on a 1.5 Tesla (Siemens, Sonata) scanner with a 12 channel receiver coil without the use of contrast for all subjects for cardiac and aortic structural and functional imaging. Cardiovascular magnetic resonance was used as it is considered the gold standard for assessing aortic and cardiac structure and function [164; 165; 166]. It provides high resolution, high contrast, multiplanar images that allow for both regional and global analysis of aortic and cardiac structure and function, without geometric assumptions. All cardiovascular imaging was prospectively electrocardiogram (ECG) gated with a precordial three lead ECG.

3.2.1 Aortic Imaging

3.2.1.1 Aortic Pulse Wave Velocity

To measure cardiovascular magnetic resonance based aortic pulse wave velocity, ECG gated, spoiled gradient echo sequences with velocity encoding gradient for phase contrast was applied to measure through plane flow in the ascending and proximal descending aorta at the level of the right pulmonary artery as well as in the descending aorta 11.0 centimetres vertically down from this just below the diaphragm, perpendicular to the vessel (Figure 3.2). Cardiovascular magnetic resonance scan parameters were as follows: effective repetition time 1 RR interval, echo time 2.80 ms, in plane resolution 1.30 mm, slice thickness 5.00 mm, and temporal resolution 10.0 ms. Image analysis was performed using Siemens analytical software (Argus; Siemens Medical Solutions, Erlangen, Germany). Flow images were manually contoured throughout the cardiac cycle and all the frames of the sequence were then integrated over the cardiac cycle (Figure 3.3) [167]. Aortic pulse wave velocity was calculated as the distance between the assessed sites of the aorta (measured on an aortic oblique sagittal image) divided by the time delay between the arrival of the foot of the pulse wave at each site. Table 3.1 shows the intra- and inter-observer coefficients of variation for aortic pulse wave velocity measurements. Table 3.2 shows the raw data for intra-observer aortic arch pulse

wave velocity measurements.

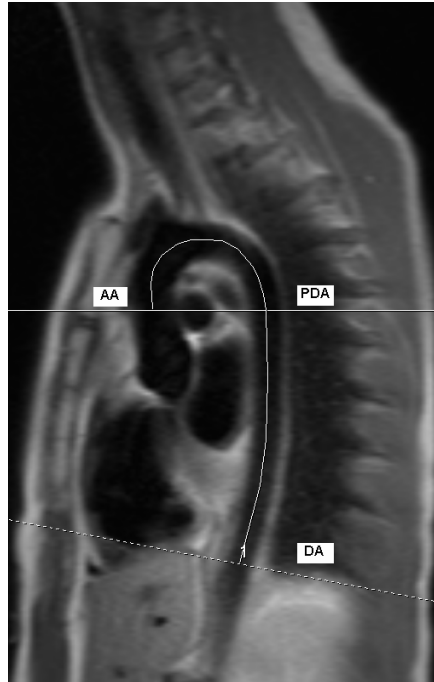


Figure 3.2: Oblique sagittal image of the aorta. Aortic arch pulse wave velocity was measured from the ascending aorta (AA) to the proximal descending aorta (PDA); descending aortic pulse wave velocity was measured from the PDA to the descending aorta (DA); and global aortic pulse wave velocity was measured from the AA to the DA. Aortic distensibility was measured at AA, PDA, and DA.

3.2.1.2 Aortic Distensibility

To gain insight into regional changes in aortic stiffness, aortic distensibility was assessed in three different positions along the aorta. An image obtained at the level of the right pulmonary artery as for aortic pulse wave velocity allows for the cross-sectional assessment of the ascending aorta and proximal descending aorta. A second image planned perpendicular to the vessel 11.0 centimetres vertically down from this just below the diaphragm as for aortic pulse wave velocity allows for the cross-sectional assessment of the distal descending aorta. A third image at the level of the second lumbar vertebrae provides a cross-section of the abdominal aorta. Cardiovascular magnetic resonance parameters: repetition time 2.8 ms, echo time 1.4 ms, in plane resolution 2.0 mm, slice thickness 7.0 mm, temporal resolution 40.0 ms. ECG gated, steady state free precession cine images were acquired during breath hold to determine aortic distensibility. Maximum and minimum aortic cross sectional areas over the

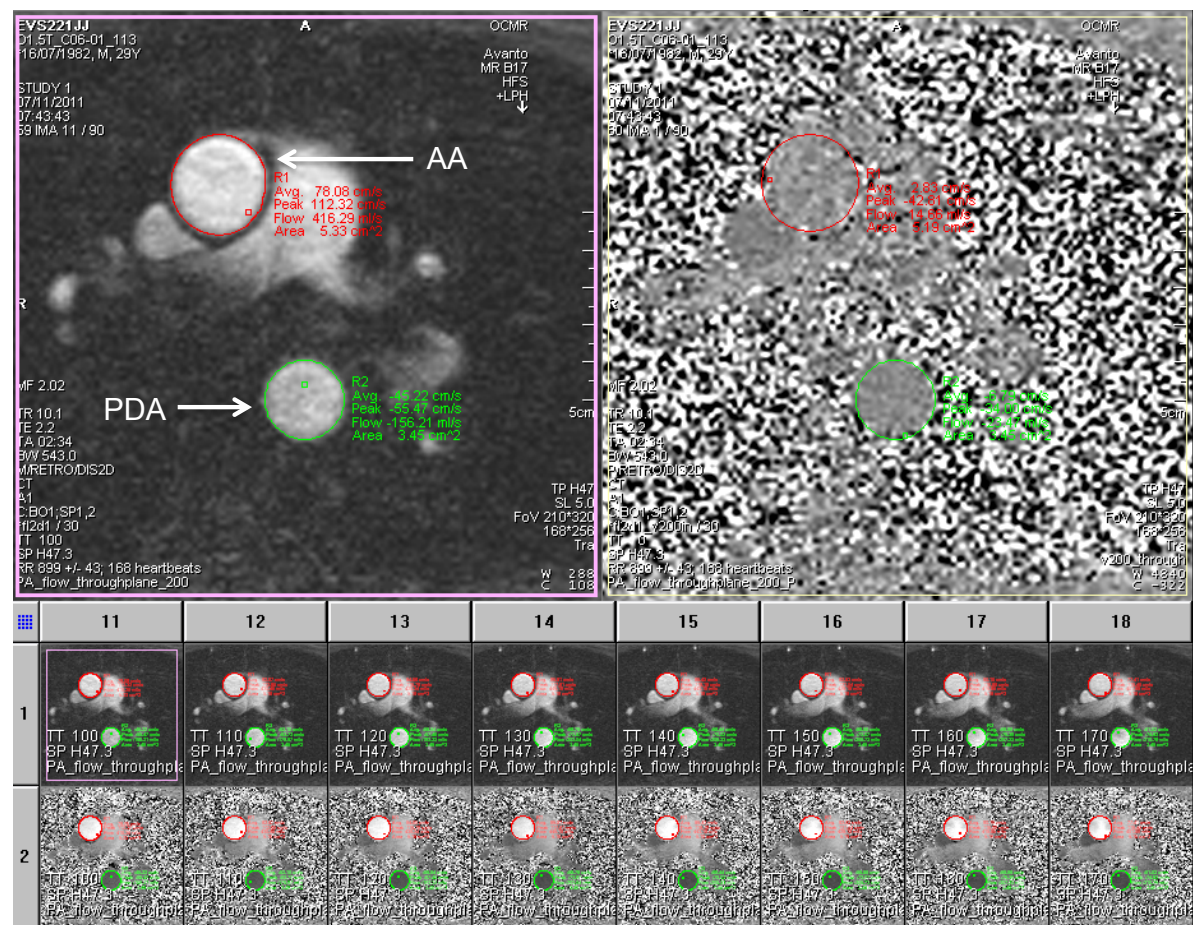


Figure 3.3: Aortic pulse wave velocity analysis. Flow images were manually contoured throughout the cardiac cycle using Argus post-processing software as shown for the ascending aorta (AA) and proximal descending aorta (PDA) in the cross-sectional image above at the level of the right pulmonary artery.

cardiac cycle were determined using semi-automated edge detection algorithms developed using Matlab software (Mathworks Incorporated) and distensibility was calculated as the relative change in area divided by the pulse pressure: (aortic distensibility = $\{ [(\text{maximum area of the aorta} - \text{minimum area of the aorta}) / (\text{minimum area of the aorta})] / \text{aortic pulse pressure} \} \times 10^3$) (Figure 3.4). The blood pressure used for the calculation of aortic distensibility was the surrogate measure of aortic pulse pressure derived using applanation tonometry (see Section 3.7). Table 3.1 shows the intra- and inter-observer coefficients of variation for aortic distensibility measurements.

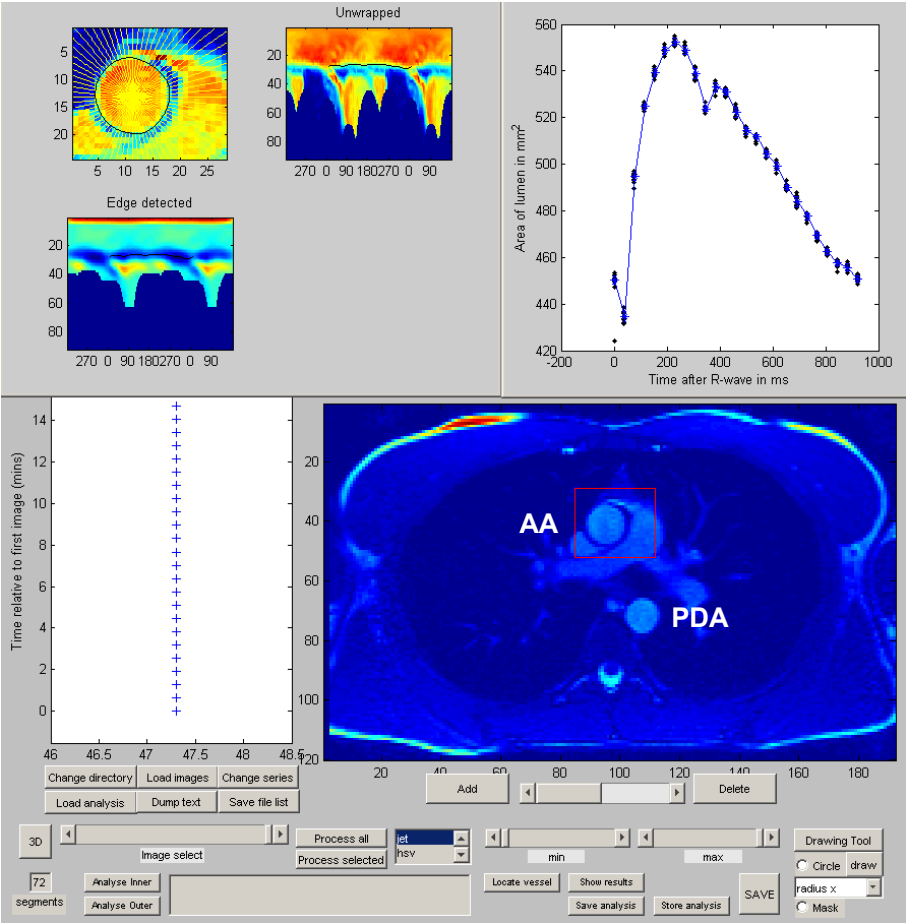


Figure 3.4: Aortic distensibility analysis. In the image above, the bottom panel shows a cross-sectional view of the ascending aorta (AA) and proximal descending aorta (PDA) at the level of the right pulmonary artery, viewed in Matlab. Semi-automated edge detection algorithms developed using Matlab software were applied throughout the cardiac cycle (top left panel) to create the corresponding aortic area curve throughout the cardiac cycle (top right panel). Aortic distensibility = $\{ [(\text{maximum area of the aorta} - \text{minimum area of the aorta}) / (\text{minimum area of the aorta})] / \text{central pulse pressure} \} \times 10^3$.

Table 3.1: Intra- and Inter-Observer Coefficients of Variation for Aortic Pulse Wave Velocity and Aortic Distensibility

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Aortic Pulse Wave Velocity		
Aortic Arch	3.56	4.64
Descending Aorta	8.22	9.43
Global Aorta	3.27	3.39
Aortic Distensibility		
Ascending Aorta	2.62	2.89
Proximal Descending Aorta	2.52	2.47
Distal Descending Aorta	2.30	2.34

CoV indicates coefficient of variation.

Table 3.2: Aortic Arch Pulse Wave Velocity Data for Calculation of Intra-Observer Coefficient of Variation

Measurement 1 (m/s)	Measurement 2 (m/s)
3.56	3.83
5.65	5.34
4.76	4.55
3.78	4.03
4.53	4.22
3.56	3.78
5.65	5.34
4.72	4.65
3.89	4.02
4.51	4.42
3.44	3.65
5.73	5.42

3.2.2 Cardiac Imaging

3.2.2.1 Cine Acquisition

Images were acquired during end expiration breath holding using a steady state free precession sequence with an echo time of 1.5 ms, a repetition time of 3.0 ms, temporal resolution 47.8 ms and a flip angle of 60° . Steady state free precession cine sequences were used to acquire localisation images (horizontal long axis, vertical long axis and short axis views) followed by optimised left ventricular horizontal and vertical long axis cines. These were used to acquire a steady state free precession cine short axis stack aligned to the left ventricle to obtain coverage of the entire left and right ventricles with a slice thickness of 7.0 mm and an inter-slice gap of 3.0 mm. Image analysis for ventricular volumes, mass, and dimensions was performed offline using Siemens analytical software (Argus; Siemens Medical Solutions, Erlangen, Germany).

3.2.2.2 Ventricle Volumes and Mass

Left and right ventricular short axis epicardial and endocardial borders were manually contoured at end-diastole and endocardial borders at end-systole for quantification of mass and volume measurements (Figures 3.5 and 3.6).

Image contrast was adjusted for each slice to delineate epicardial fat from myocardium.

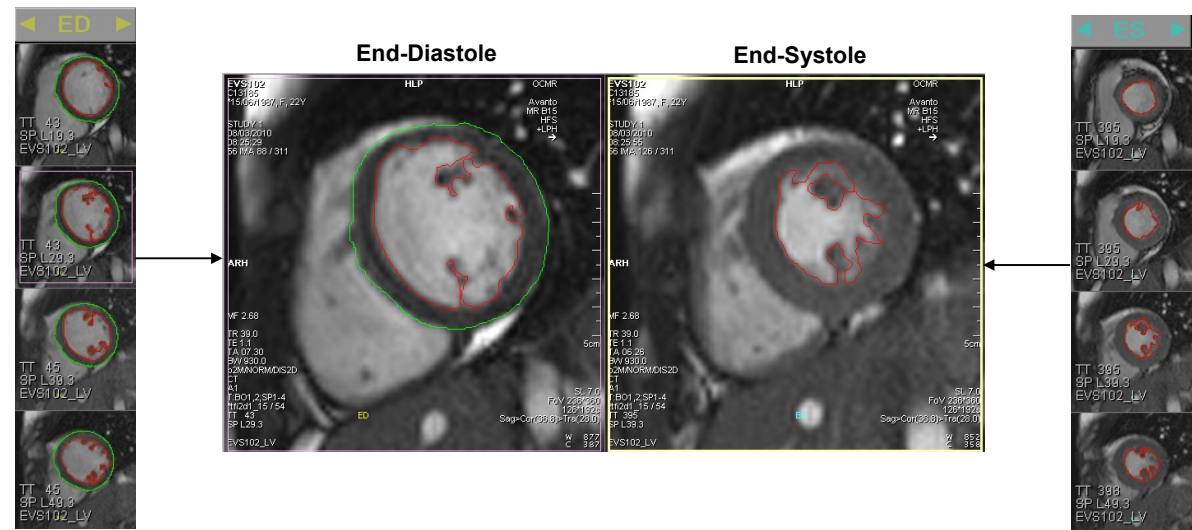


Figure 3.5: Left ventricular volume and mass measurements. The endocardial (red) and epicardial (green) borders were manually contoured at end-diastole and endocardial borders at end-systole for the entire short axis stack.

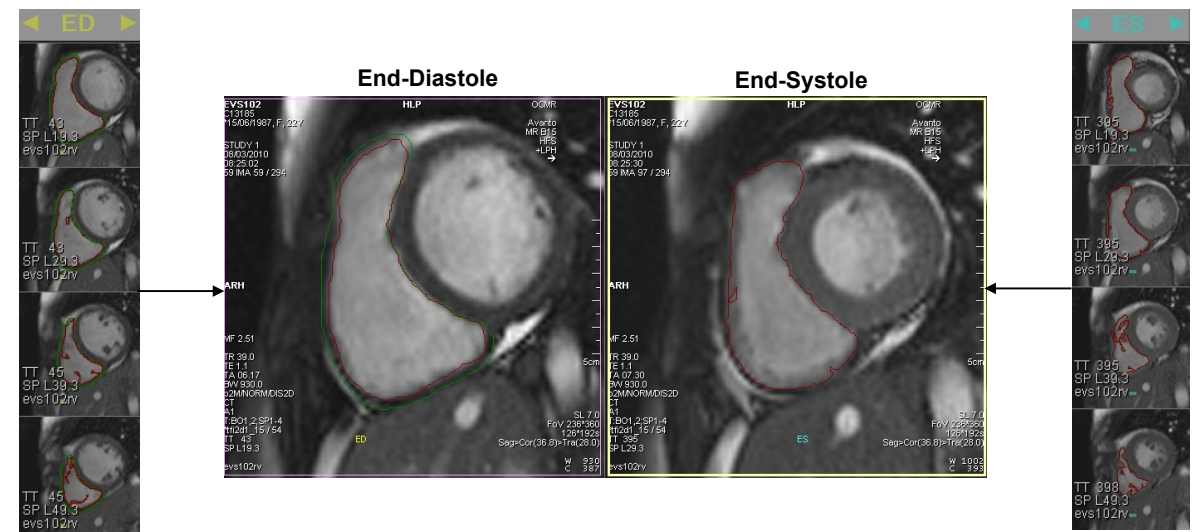


Figure 3.6: Right ventricular volume and mass measurements. The endocardial (red) and epicardial (green) borders were manually contoured at end-diastole and endocardial borders at end-systole for the entire short axis stack.

End-systole was chosen visually as the image phase with the smallest cavity area. For the left ventricle, the basal slice was chosen as the first slice in the short axis stack with at least 50% of the circumference surrounded by ventricular myocardium. Further confirmation of the basal slice was done by comparing the end-diastolic image with subsequent systolic frames and with the slices above and below, as well as by using the cine loops. For the right ventricle, the borders were drawn using a drawing tablet to reduce errors in identification of borders [166]. To ensure accurate identification of the right ventricular basal slice, and structures within this plane, several visual parameters were used and images viewed in cine mode. Right ventricular walls were distinguished from atrial walls as being thicker and trabeculated with inward systolic motion. In addition, for end-systolic frames, identification of the atrioventricular groove by its excursion was used. Due to through-plane motion of the tricuspid valve, the upper posterior part of the right ventricle was typically identified in the basal plane at end-diastole but right atrium at end-systole. Basal slice contours were taken as far as the pulmonary valve. Papillary muscles were included for left and right ventricular mass and excluded for left and right ventricular volume measures. The interventricular septum was considered part of the left ventricle and excluded from right ventricular mass measurements. Left and right ventricular end-systolic volume and end-diastolic volume were used to calculate stroke volume (stroke volume = end-diastolic volume – end-systolic volume). Ejection fraction was calculated as: $\text{ejection fraction} = [(\text{stroke volume}) / (\text{end-diastolic volume})] * 100\%$. Left and right ventricular mass were calculated by subtracting the endocardial volume from the epicardial volume at end-diastole. Left and right ventricular mass were calculated based on prior knowledge of myocardial specific gravity (1.05 g/cm^3). Table 3.3 shows the intra- and inter-observer coefficients of variation for left and right ventricular mass and volume measurements.

3.2.2.3 Left Ventricle Dimensions

Left ventricular wall thickness was measured on the mid-ventricular short axis slice at end-diastole, and internal and external cavity diameters on the mid-ventricular short axis slice at end-diastole between the septum and inferolateral wall (Figure 3.7). Left ventricular length

Table 3.3: Intra- and Inter-Observer Coefficients of Variation for Left and Right Ventricular Mass and Volume Measurements

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Left Ventricle		
Mass	3.52	4.73
End-Diastolic Volume	4.19	4.50
End-Systolic Volume	3.76	4.87
Stroke Volume	4.20	5.03
Ejection Fraction	4.33	5.62
Right Ventricle		
Mass	5.04	6.23
End-Diastolic Volume	4.22	5.35
End-Systolic Volume	5.58	6.61
Stroke Volume	5.31	6.54
Ejection Fraction	5.12	6.67

CoV indicates coefficient of variation.

was measured at end-diastole on the horizontal long axis cine by drawing a line along the mitral valve annulus and measuring the length from the left ventricular apex to the middle of the mitral valve annulus (Figure 3.8). Relative wall thickness was calculated as $(2 \times \text{inferior wall thickness}) / \text{end-diastolic diameter}$. Left ventricle mass-to-volume ratio was calculated as: $\text{left ventricular mass} / \text{end-diastolic volume}$. Table 3.4 shows the intra- and inter-observer coefficients of variation for the left ventricle dimension measurements.

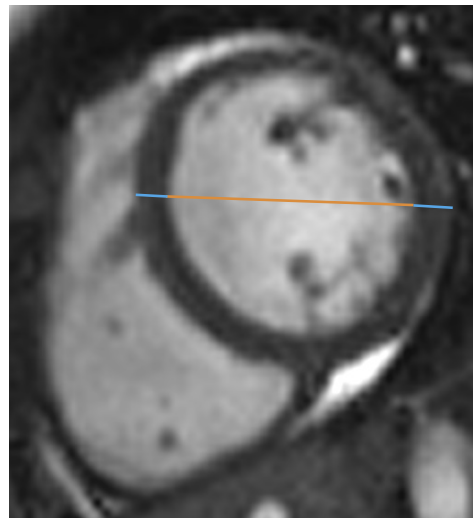


Figure 3.7: Left ventricular internal and external cavity diameter. Left ventricular internal cavity diameter (orange line) and external cavity diameter (orange line plus blue lines) were measured on the mid-ventricular short axis slice at end-diastole between the septum and inferolateral wall.

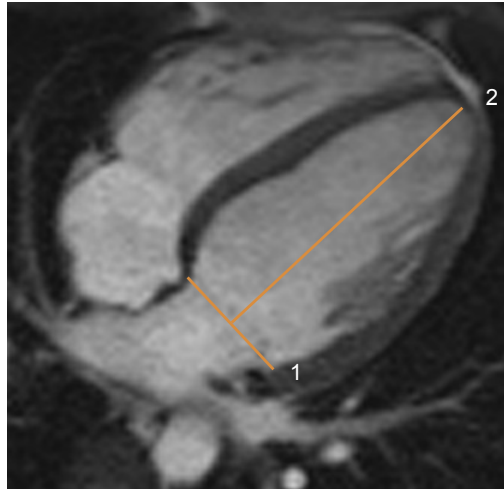


Figure 3.8: Left ventricular length. Left ventricular length was measured at end-diastole on the horizontal long axis cine by drawing a line along the mitral valve annulus (1) and measuring the length (2) from the left ventricular apex to the middle of the mitral valve annulus.

3.2.2.4 Right Ventricle Dimensions

Right ventricular length was measured at end-diastole on the horizontal long axis cine by drawing a line along the tricuspid valve annulus and measuring the length from the right ventricular apex to the middle of the tricuspid valve annulus (Figure 3.9). Right ventricular diameter measurements were made on the horizontal long axis cine at end-diastole within the basal third of the right ventricle below the tricuspid valve and at the level of the right ventricular papillary muscles (Figure 3.9). Table 3.4 shows the intra- and inter-observer coefficients of variation for the right ventricle dimension measurements.

3.2.2.5 Left Ventricle Tagging

A gradient echo based tagging pulse sequence was performed in the horizontal long axis view and in the basal, mid-ventricular and apical short axis slices with a segmented K space, multi-shot sequence (repetition time 25.0 ms, echo time 7.40 ms and flip angle 25.0°). Slice positions were chosen from the images obtained for the steady state free precession short axis cine stack and the horizontal long axis cine. The nearest slice in the short axis cine stack to the base of the left ventricle, in which a complete circle of myocardium was visible throughout the cardiac cycle, was selected as the basal slice. The mid-ventricular and api-



Figure 3.9: Right ventricular dimension measurements. Right ventricular length was measured at end-diastole on the horizontal long axis cine by drawing a line along the tricuspid valve annulus (1) and measuring the length (2) from the right ventricular apex to the middle of the tricuspid valve annulus. Right ventricular diameter measurements were made on the horizontal long axis at end-diastole within the basal third of the right ventricle below the tricuspid valve (3) and at the level of the right ventricular papillary muscles (4).

Table 3.4: Intra- and Inter-Observer Coefficients of Variation for Left and Right Ventricle Dimension Measurements

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Left Ventricle		
Length	3.01	3.43
Wall Thickness	3.08	4.33
Internal Diameter	3.72	3.99
External Diameter	3.41	3.83
Relative Wall Thickness	4.26	4.64
Mass:End-Diastolic Volume	5.21	5.08
Right Ventricle		
Length	3.22	3.74
Internal Diameter 1	4.09	4.23
Internal Diameter 2	2.89	3.00

CoV indicates coefficient of variation. Internal Diameter 1 indicates the internal diameter measurement within the basal third of the right ventricle; Internal Diameter 2 indicates the internal diameter measurement at the level of the right ventricular papillary muscles.

cal slices were then selected with sequential 2.0 cm gaps towards the apex. Semi-automated analysis was done using Cardiac Image Modeller software (CIMTag2D version 7, Auckland MRI Research Group, New Zealand) by aligning a grid to the myocardial tagging planes in end-diastole (Figure 3.10). End-systole was determined visually, and tags were adjusted at each frame throughout the cardiac cycle. Circumferential, longitudinal, and radial myocardial strain and strain rate were calculated by the software from the motion of the intersected tag lines. The segmental peak systolic values were recorded and these were averaged to obtain global values. Regional values were calculated at basal, mid, and apical levels. Table 3.5 shows the intra- and inter-observer coefficients of variation for the left ventricle peak systolic strain values obtained by left ventricle tagging. Due to the poor reproducibility of the longitudinal peak systolic strain and strain rate values, as well as the poor reproducibility of the basal, mid, and apical peak systolic radial strain and strain rate values, these were excluded from any analysis throughout this thesis.

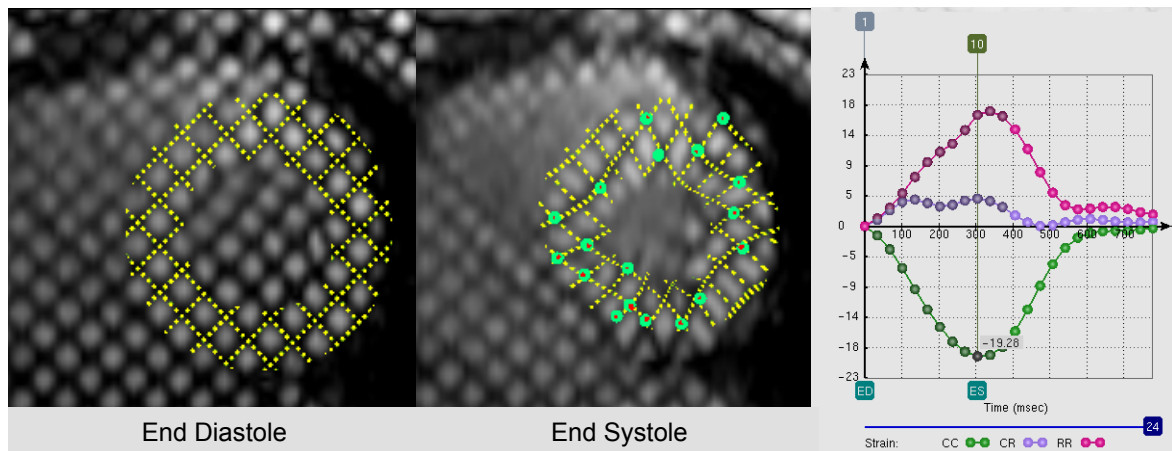


Figure 3.10: Left ventricle tagging. Above is an example of a mid-ventricular short axis cine where the tagging grid was aligned to the myocardial tagging planes in end-diastole (left). End-systole (centre) was determined visually, and tags were adjusted at each frame throughout the cardiac cycle. The corresponding strain curves are shown on the right, with the average circumferential strain curve shown in green.

3.2.2.6 Feature Tracking Analysis

A limitation of the study was that the cardiovascular magnetic resonance tagging sequence was only performed in a subgroup of the cohort. Midway through my doctorate, new soft-

Table 3.5: Intra- and Inter-Observer Coefficients of Variation for Left Ventricle Tagging Measurements

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Longitudinal		
Strain	19.1	21.7
Strain Rate	20.8	23.5
Circumferential		
Basal Strain	6.28	8.74
Basal Strain Rate	11.9	12.3
Mid Strain	5.23	6.42
Mid Strain Rate	10.9	14.6
Apical Strain	5.62	5.99
Apical Strain Rate	12.8	14.3
Radial		
Basal Strain	33.2	43.7
Basal Strain Rate	44.9	49.3
Mid Strain	30.6	33.7
Mid Strain Rate	41.0	44.2
Apical Strain	39.2	43.4
Apical Strain Rate	54.4	56.9

CoV indicates coefficient of variation.

ware, called 2D Cardiac Performance Analysis Software (TomTec, Germany), became available. I assisted Dr Daniel Augustine in a comparison study, demonstrating the good reproducibility of this software, which has recently been published in the *Journal of Cardiovascular Magnetic Resonance* [168]. This software allows for the quantification of myocardial strain, velocity, displacement, and rotation parameters from cardiovascular magnetic resonance steady state free precession short axis and horizontal long axis cine images and takes less than half the time to analyse a dataset than it does for left ventricle tagging [168]. In addition to being able to retrospectively use cine images and providing more myocardial deformation parameters than left ventricle tagging, such as velocity, displacement, and rotation, this software has the added advantage of maintaining tracking throughout the cardiac cycle to allow for diastolic function analysis [168]. Moreover, due to the thinness of the right ventricle myocardial wall, tagging analysis is virtually impossible. However, right ventricle feature tracking analysis also shows good reproducibility as demonstrated in Table 3.7.

Analysis was performed for both the left ventricle and right ventricle. Following uploading of the image, the brightness was optimised to ensure optimal endocardial and blood pool discrimination. For left ventricular longitudinal and right ventricular longitudinal my-

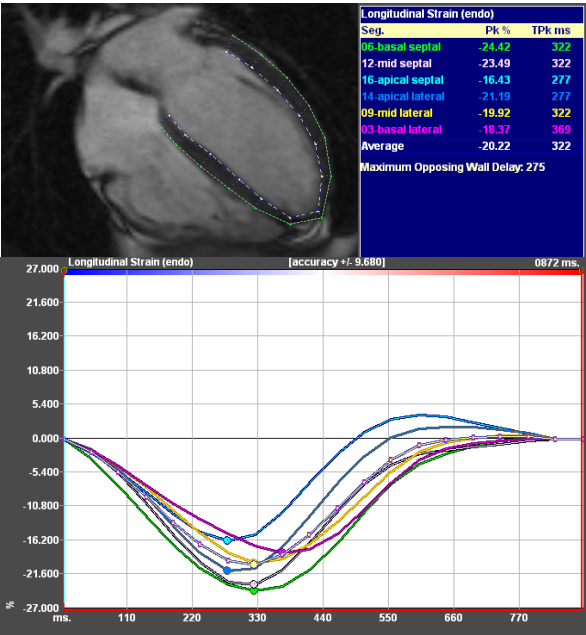


Figure 3.11: Left ventricular longitudinal myocardial deformation analysis. Borders were contoured at end-diastole (top left). The software then tracked the motion of related features adjacent to the endocardial border over the cardiac cycle to allow for analysis of left ventricular longitudinal systolic and diastolic function. The example above shows the longitudinal peak systolic strain values (top right) and curves (bottom panel) for the six left ventricular segments, as well as the average of the six segments (in white).

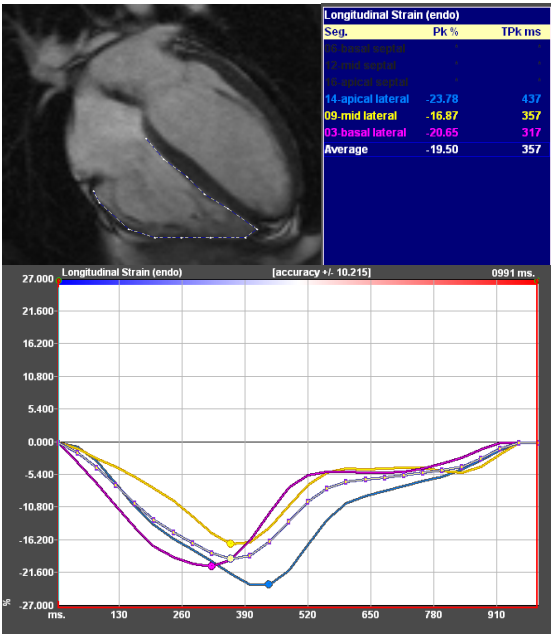


Figure 3.12: Right ventricular longitudinal myocardial deformation analysis. Borders were contoured at end-diastole (top left). The software then tracked the motion of related features adjacent to the endocardial border over the cardiac cycle to allow for analysis of right ventricular longitudinal systolic and diastolic function. The example above shows the longitudinal peak systolic strain values (top right) and curves (bottom panel) for the three right ventricular free wall segments, as well as the average of the three segments (in white).

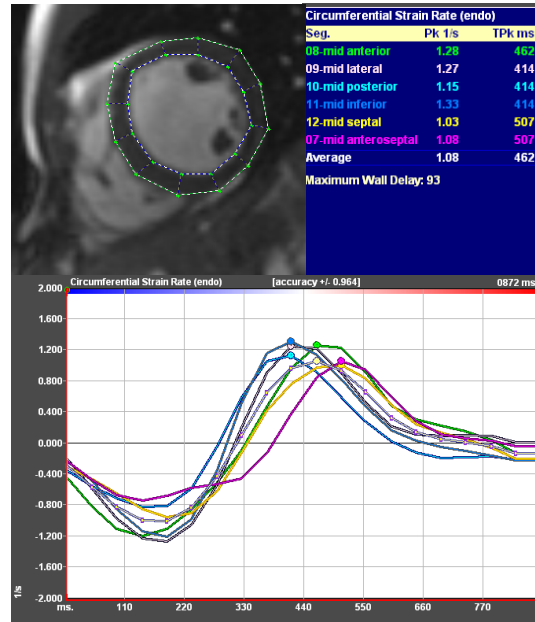


Figure 3.13: Right ventricular circumferential myocardial deformation analysis. Borders were contoured at end-diastole (top left). The software then tracked the motion of related features adjacent to the endocardial border over the cardiac cycle to allow for analysis of left ventricular circumferential systolic and diastolic function. The example above shows the longitudinal peak diastolic strain rate values (top right) and curves (bottom panel) for the six right ventricular segments, as well as the average of the six segments (in white).

ocardial deformation parameters, points were placed along the ventricle endocardial border at end-diastole on the horizontal long axis cines (Figures 3.11 and 3.12). For left ventricular circumferential myocardial deformation parameters, points were placed along the left ventricle endocardial border at end-diastole on the basal, mid, and apical short axis cines (Figure 3.13). The software then tracked the motion of related features adjacent to the endocardial border, such as the cavity tissue boundary or individual patterns along the tissue border, over the cardiac cycle from frame-to-frame. As such, this provided quantification of myocardial deformation during systole and diastole. The averages of segmental strains from the short axis planes were used for endocardial circumferential strain, while averages of segmental strains in the horizontal long axis view were used for global endocardial longitudinal strain. Left ventricular net twist angle was calculated as peak apical endocardial circumferential rotation minus peak basal endocardial circumferential rotation. The septal wall was excluded for right ventricular myocardial deformation analysis. Table 3.6 shows the intra- and inter-observer coefficients of variation for the left ventricle systolic and di-

astolic myocardial deformation values obtained by left ventricle feature tracking analysis. Table 3.7 shows the intra- and inter-observer coefficients of variation for the right ventricle systolic and diastolic myocardial deformation values obtained by right ventricle feature tracking analysis. Due to the poor reproducibility of the right ventricle peak systolic velocity, peak systolic displacement, and peak diastolic velocity measures, these were excluded from any analysis throughout this thesis.

Table 3.6: Intra- and Inter-Observer Coefficients of Variation for Left Ventricle Feature Tracking Analysis

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Systolic		
<u>Longitudinal</u>		
Strain	8.22	11.3
Strain Rate	9.89	13.5
Displacement	8.66	15.7
Velocity	10.3	16.2
<u>Circumferential</u>		
Basal Strain	3.45	5.94
Basal Strain Rate	4.69	5.33
Basal Rotation	5.01	4.99
Basal Rotation Rate	5.32	5.78
Mid Strain	3.32	4.24
Mid Strain Rate	4.69	5.46
Apical Strain	4.26	4.69
Apical Strain Rate	4.82	5.33
Apical Rotation	6.22	6.33
Apical Rotation Rate	6.99	7.43
Net Twist Angle	6.43	6.76
Diastolic		
<u>Longitudinal</u>		
Strain Rate	10.2	13.2
Velocity	10.4	12.8
<u>Circumferential</u>		
Basal Strain Rate	4.55	5.67
Basal Rotation Rate	4.87	5.23
Mid Strain Rate	3.98	5.92
Apical Strain Rate	5.23	5.86
Apical Rotation Rate	5.47	6.77

CoV indicates coefficient of variation.

Table 3.7: Intra- and Inter-Observer Coefficients of Variation for Right Ventricle Longitudinal Feature Tracking Analysis

Measurement	Intra-Observer CoV (%)	Inter-Observer CoV (%)
Systolic		
Strain	9.39	11.4
Strain Rate	10.5	12.1
Displacement	22.2	24.2
Velocity	23.3	26.5
Diastolic		
Strain Rate	11.8	13.4
Velocity	21.0	23.2

CoV indicates coefficient of variation.

3.3 Intravital Capillary Video Microscopy

Studying capillaries in humans is difficult without causing changes that will affect parameters of interest. Intravital capillary video microscopy is a non-invasive measure that has allowed the dynamic study of dermal capillaries, which are easily accessible for imaging and unaffected by outside interference [169]. The examination of functional capillary density (baseline number) and capillary recruitment (capillaries perfused with erythrocytes following vessel occlusion, referred to as structural capillary density) allows a non-invasive assessment of tissue perfusion at the level of the microvasculature.

The participant rested in the supine position on the bed for 10 minutes prior to starting, with their left hand lying flat on a micrometer stage. A small blood pressure cuff was placed around the proximal phalanx of the middle finger of the left hand for venous occlusion measures, and a thin layer of nail varnish was applied to the skin of the dorsal surface of the middle phalanx of the middle finger of the left hand to reduce light rarefaction. This area of the finger was examined using a Leica Stereo Microscope at 200 times magnification and Bosch Dinlon CCD Analogue Camera with the images displayed on a Norban Vista black and white high resolution monitor. A Schott LED light with a wavelength spectrum (450 to 610 nm) that captures the absorption spectrum of haemoglobin was directed at the finger to image the capillaries. Calibration was done using a 1.0 mm glass stage graticule (Knight Optical UK). For the baseline recordings, an area of skin was found that contained a promi-

nent landmark such as a bubble in the nail varnish or a blemish in the skin. This area of the skin was then recorded using a Sony DVO-1000MD DVD recorder for one minute, during which time the focus was constantly but gradually altered in order to focus to different depths within the tissue and for all capillaries to come into focus at various stages during the one minute recording. After one minute, the finger was gently moved using a micrometer positioning stage so that an adjacent field of capillaries was visible, and the same procedure undertaken. This was repeated until six adjacent fields were recorded as shown in Figure 3.14. After completion of the baseline recordings, the small blood pressure cuff around the proximal phalanx was inflated to 50 mmHg for five minutes to cause venous occlusion. The same six fields of view as for the baseline recordings were then imaged for one minute each. This allowed for the determination of baseline capillary perfusion (functional capillary density) as well as the structural density of capillaries (venous occlusion). Intravital capillary microscopy analysis is described in Chapter 4.

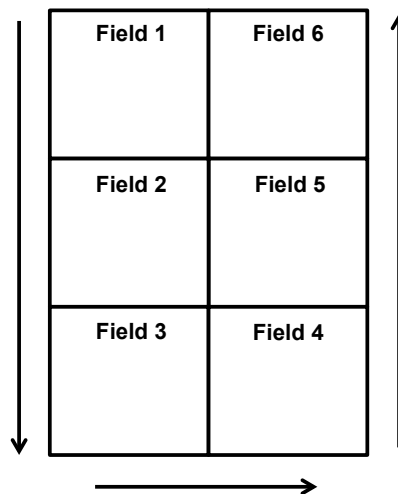


Figure 3.14: Imaging fields for intravital capillary video microscopy.

3.4 Anthropometry and Lifestyle Questionnaire

Height was measured to the nearest centimetre and weight to the nearest 0.2 kilogram, with subjects wearing light clothing, using a combined digital height and weight measurement

station (Seca, UK). Body surface area (BSA) was calculated using the Du Bois formula ($BSA (m^2) = 0.20247 \times \text{Height (m)}^{0.725} \times \text{Weight (kg)}^{0.425}$). Data on medical history, smoking history, parental medical history, and lifestyle were obtained using a validated questionnaire (see Appendix A) [163]. For each participant, additional information was also collected on maternal and paternal smoking prior to, during, and after the pregnancy of interest (see Appendix B).

3.5 Blood Sample Collection

Blood samples were drawn, centrifuged at $3,000 \times g$ for 15 minutes at 4°C and separated within 30 minutes into cryovials. They were then stored for later analysis at -80°C . Fasting blood biochemistry was measured at the Oxford John Radcliffe Hospital Biochemistry Laboratory using routine validated assays, with clinical level quality controls. Low density lipoprotein cholesterol was calculated using the Friedewald formula (low density lipoprotein cholesterol = total cholesterol – high density lipoprotein cholesterol – (triglycerides / 2.17)) [170]. Homeostatic model assessments for insulin sensitivity (HOMA-S), β cell function (HOMA-B), and insulin resistance (HOMA-IR) were calculated based on fasting insulin and glucose levels using a HOMA calculator (www.dtu.ox.ac.uk/homacalculator/index.php).

3.6 Enzyme-Linked Immunosorbent Assays

Quantikine enzyme-linked immunosorbent assays (ELISAs) were done to measure free vascular endothelial growth factor A (VEGF-A), soluble vascular endothelial growth factor receptor 1 (sVEGF R1 or sFlt-1), and soluble endoglin (sENG) concentrations. While there exist other members of the VEGF family, such as VEGF-B, VEGF-C, VEGF-D and placental growth factor, VEGF-A, which is the product of three major isoforms as a result of alternative splicing, was measured because of its key role in angiogenesis and vascular physiology [171], as well as the high affinity binding between sFlt-1 and VEGF-A [172]. All three ELISAs use the quantitative sandwich enzyme immunoassay technique (Quantikine, R & D Systems Europe, Abingdon, UK).

3.6.1 Sample Preparation

Stored serum samples (frozen at -80°C) were used for all ELISAs. Samples were thawed on ice, with only a single freeze-thaw cycle. Samples were spun for five minutes at $1000 \times g$ to separate any remaining debris from the supernatant. The sample supernatant was then transferred to new eppendorf tubes. The samples were not diluted.

3.6.2 Standard Preparation

Standards were prepared by reconstitution with 1.0 mL of deionised water to produce a stock solution (concentration dependent on analyte kit). The standard was mixed to ensure complete reconstitution and left to sit. After 15 minutes, the standard mix was gently agitated prior to making dilutions. For sENG and sFlt-1, a dilution series was used such that the first dilution was 1:10, and thereafter dilutions were 1:2. 900 μL of calibrator diluent was pipetted into the 1:10 eppendorf tube. 500 μL of calibrator diluent was pipetted into the remaining eppendorf tubes. 100 μL of the stock solution was pipetted into the first tube (1:10 dilution), and then 500 μL from this tube into the next (1:20 dilution). Eppendorf tubes were mixed thoroughly before the next transfer. This was continued up until a 1:640 dilution, leaving seven standard dilutions. For VEGF-A, a dilution series was used such that all dilutions were 1:2. 500 μL of calibrator diluent was pipetted into all eppendorf tubes. 500 μL of the stock solution was pipetted into the first tube (1:2 dilution), and then 500 μL from this tube into the next (1:4 dilution). Eppendorf tubes were mixed thoroughly before the next transfer. This was continued up until a 1:64 dilution, leaving seven standard dilutions.

3.6.3 Assay Procedure

After preparing the samples and standards, 100 μL of assay diluent was added to each well of a 96 well plate. For VEGF-A and sFlt-1 100 μL of sample, standard or control was pipetted into each well. For sENG, 50 μL of sample, standard or control was pipetted into each well. Assay diluent was used as the control for all ELISAs. All samples, standards, and controls

were plated in duplicate. The plates were then incubated at room temperature.

Following incubation, each well of the 96 well plates was aspirated and washed using 400 μL of washing buffer on an automatic plate washer. The process was repeated three times for a total of four washes. 200 μL of conjugate was then added to each well. The plates were then incubated for two hours at room temperature. Following incubation, the wash process was repeated as before. 200 μL of substrate solution was then added to each well. The plates were then incubated for 30 minutes at room temperature in darkness to protect them from light. 50 μL of stop solution was then added to each well, and the plates were tapped and placed on the plate shaker at low speed for five minutes to ensure thorough mixing of the substrate solution and stop solution. The optical density of each well was measured at 450nm using a FLUOstar Omega microplate reader (BMG Labtech, KBioscience, USA) within 30 minutes. Omega Data Analysis software was used to analyse the data. Duplicate readings for each standard, control, and sample were averaged, and the average zero standard optical density was subtracted. Standard curves were created by generating a four parameter logistic curve-fit. A coefficient of variation between duplicates of less than 15% was considered acceptable.

3.7 Brachial and Aortic Blood Pressure Measurements

The participant rested in the supine position on the bed for 10 minutes prior to blood pressure recordings. Three brachial blood pressure measurements were recorded on the left arm with an automatic digital monitor (HEM 705CP, OMRON, Japan) and the second and third measurements were averaged for analysis [159; 160; 161]. Aortic blood pressure was assessed non-invasively by applanation tonometry to derive ascending aortic pressure waveforms (SphygmoCor Analysis System, Australia) [173]. The concept of deriving a non-invasive measure of aortic blood pressure stemmed from the knowledge that arterial pressure changes greatly from the aorta to peripheral sites. As such, peripheral blood pressure measures cannot be used as direct surrogate measures of aortic blood pressure [174]. Thus, applanation tonometry has been used to develop a non-invasive method to derive aortic blood pressure measurements. The radial artery is considered the best site for achieving acceptable

arterial waveforms as it is well supported by bony tissue and is easily accessible [175]. This has led to a number of comparison studies of radial artery applanation tonometry measurements with aortic catheterisation measurements, which has allowed for the development of transfer functions to derive aortic pressure waveforms from radial artery pressure waveforms [176; 177; 178]. As a result of the nature of this type of validation work, the population groups used, and the overall limited amount of proper validation studies to date, concerns have been raised as to the accuracy and applicability of this method for deriving non-invasive aortic blood pressure measurements in different population groups given the likely variability in vascular and haemodynamic properties [179]. Nevertheless, radial applanation tonometry remains the most widely accepted surrogate measure of aortic blood pressure in the literature, and was thus incorporated as part of the investigations presented in this thesis.

In order to measure aortic blood pressure, pressure waveforms were obtained from the left radial artery, with the wrist slightly extended and supported. The radial waveform was displayed in real time and assessed visually to ensure that artefact from movement and respiration was avoided. The mean of 15 to 20 sequential radial artery waveforms were used to calculate an average radial artery waveform and to generate the corresponding ascending aortic pressure waveform using a transfer function. Aortic blood pressure was then calculated by calibration against left brachial blood pressure with the assumption that mean arterial pressure is constant over large arteries.

3.8 Ambulatory Blood Pressure Monitoring

24 hour blood pressure monitoring was done on the left arm using an automatic digital monitor (A & D Instruments Ltd., Japan). A blood pressure cuff was fitted to each participant's arm at the end of the study visit. Three blood pressure measurements were done using a stethoscope to listen for Korotkoff sounds at the same time as the automatic recordings in order to check accuracy of the device. Manual measures within ± 5 mmHg of the automatic recording were considered acceptable. The blood pressure monitor was then set to make an automatic recording every 30 minutes on the half hour from 07:00 until 23:00, and then

every hour on the hour between 23:00 and 07:00. Participants were asked to keep their arm still during the recordings but to continue their day as normal. All participants completed a diary, recording when they went to bed and when they woke up as well as activities throughout the day. Results of the 24 hour blood pressure monitoring were separated into daytime and nighttime recordings.

3.9 Statistical Analysis

Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) Version 19. Normality of variables was assessed by visual assessment of normality curves and the Shapiro-Wilk test. Comparison between groups for continuous variables was performed using a two-sided, independent samples Student's t-test or one-way ANOVA for normally distributed data and Mann Whitney and Kruskal-Wallis tests for skewed data. For categorical variables, comparison was done using a Chi-Square test. Linear regression models were performed using a forced entry method. Pearson correlations (r) were used for bivariable associations. For bivariable and multivariable regression models, unstandardised regression coefficients (B) were used, where a unit increase in the independent variable yields an increase in the dependent variable equal to B . Results are presented as mean $\pm$ standard deviation. P values <0.05 were considered statistically significant.

4

METHODS DEVELOPMENT

This chapter describes the development of a new analysis method for intravital capillary video microscopy, using semi-automated Image Pro Plus analysis software rather than commonly used acetate counting. I show that this new analysis method reduces the analysis time and has better intra- and inter-observer reproducibility.

4.1 Introduction

As mentioned in Chapter 3, intravital capillary video microscopy is a non-invasive measure that has allowed the dynamic study of dermal capillaries, which are easily accessible for imaging and unaffected by outside interference [169]. The current method to analyse capillary density is done offline by overlaying a clear acetate sheet with a circle of known area on a video monitor and playing back the recorded video loops, manually marking the capillaries. This method is laborious and limited by difficulty to track movement of capillaries due to inherent displacement of the field of view with subject movement. Furthermore, storing analysed images for future reference using the manual acetate measurement is impractical. Due to these limitations, I have developed a modification of this analysis method using Image Pro Plus software, which allows for computer-based analysis and the possibil-

ity to store analysed images for future reference. This new method aims to provide easier frame-by-frame analysis and less opportunity to miscount capillaries, which will decrease analysis time and improve reproducibility.

4.2 Methods

4.2.1 Study Population

12 young adults attended the John Radcliffe Hospital in Oxford in the morning following a 12 hour overnight fast.

4.2.2 Image Acquisition

Images were acquired as described in Section 3.3 of Chapter 3. The baseline recordings were used for comparing the two analysis methods.

4.2.3 Acetate Analysis Method

The standard method of intravital capillary video microscopy analysis using a clear acetate to count capillaries was used. Images were played back using the Sony DVO-1000MD DVD recorder on the Norban Vista black and white high resolution monitor. A clear acetate sheet with a permanent circle (diameter of actual circle = 119.2 mm; corrected area for magnification = 0.40 mm^2) was used to count the number of capillaries that fell within the circle by holding it up against the monitor (Figure 4.1). Capillaries were then marked on the acetate using a marker. This was repeated for all six fields of view and capillary density was calculated as a number per mm^2 . These recordings were then reanalysed at a later date by me to determine intra-observer reproducibility. The analysis was then repeated by a second observer to determine inter-observer reproducibility.

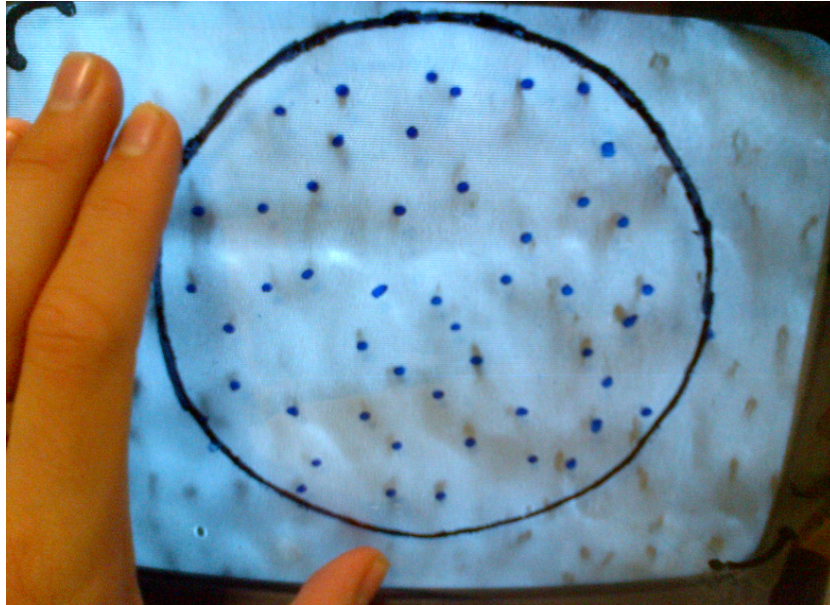


Figure 4.1: Acetate sheet capillary density analysis. A clear acetate sheet with a permanent circle (diameter of actual circle = 119.2 mm; corrected area for magnification = 0.40 mm^2) was used to count the number of capillaries that fell within the circle by holding it up against a black and white high resolution monitor while playing back the recorded clips. Capillaries are manually marked, rewinding and playing back through the video clips to check for missed vessels. This was repeated for all six fields of view at baseline and the six fields of view post venous occlusion.

4.2.4 Image Pro Plus Analysis Method

I have developed a new method to analyse capillary density utilising Image Pro Plus software (Media Cybernetics Inc., USA). Images were loaded into Image Pro Plus using a digital converter to convert analogue recordings to digital files. A circle of known area (0.40 mm^2) was drawn on the centre of the field of view at the beginning of each one minute recording (Figure 4.2). Using the count tool, capillaries can be counted as per the acetate method and the file saved for future reference. This was repeated for all six fields of view and capillary density was calculated as a number per mm^2 . These recordings were then reanalysed at a later date by me to determine intra-observer reproducibility. The analysis was then repeated by a second observer to determine inter-observer reproducibility.

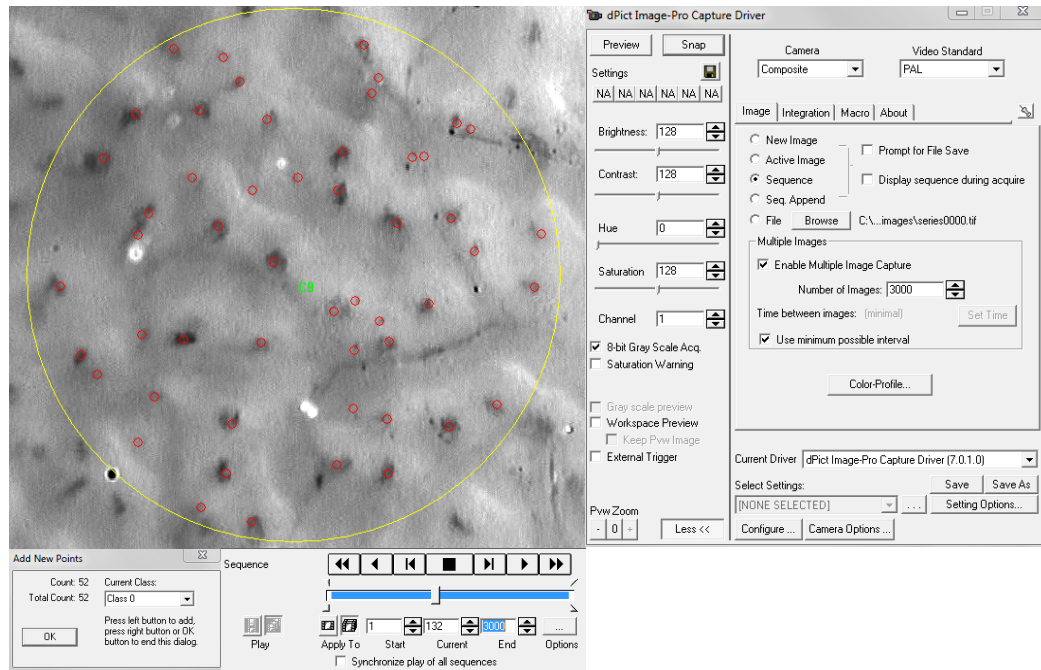


Figure 4.2: Image Pro Plus capillary density analysis. A circle of known area (0.40 mm^2) was drawn on the centre of the field of view of the uploaded sequence of images (frame rate = 25 frames/second). Capillaries are manually tagged, scrolling back and forth between frames to check for missed vessels. This was repeated for all six fields of view at baseline and the six fields of view post venous occlusion.

4.2.5 Statistical Analysis

The first and second measurements done by me (observer 1) for both methods were used to determine intra-observer reproducibility. Inter-observer reproducibility was done by comparing the first measurement done by observer 1 with the measurement done by observer 2. Inter- and intra-observer reproducibility for each method was assessed using the coefficient of variation. Agreement between the acetate and Image Pro Plus analysis methods was assessed using Bland-Altman analysis [180] to identify the bias and the limits of agreement with 95% confidence intervals using the first measurement for each method done by observer 1.

4.3 Results

4.3.1 Acetate Analysis Method

The intra- and inter-observer coefficients of variation for the average of the six fields using the acetate method were 6.02% and 7.24%. Table 4.1 demonstrates the intra- and inter-observer reproducibility across the six fields. The average analysis time for the six fields of view for the acetate method was 23 minutes 10 seconds.

4.3.2 Image Pro Plus Analysis Method

The intra- and inter-observer coefficients of variation for the average of the six fields using the Image Pro Plus method were 3.00% and 5.06%. Table 4.1 demonstrates the intra- and inter-observer reproducibility across the six fields. The average analysis time for the six fields of view for the acetate method was 10 minutes 35 seconds with an additional six minutes to load the images.

Table 4.1: Intra- and Inter-Observer Coefficients of Variation for the Six Fields of View for Capillary Density Analysis using the Acetate Method and Image Pro Plus Method

Field	Acetate Method		Image Pro Plus Method	
	Intra-observer CoV (%)	Inter-observer CoV (%)	Intra-observer CoV (%)	Inter-observer CoV (%)
1	4.74	6.37	4.16	4.11
2	7.40	9.59	2.63	4.73
3	9.56	7.67	3.03	5.67
4	9.49	9.54	3.20	6.12
5	6.60	11.04	4.10	6.15
6	4.94	9.35	3.63	7.39
Average of 6 fields	6.02	7.24	3.00	5.06

CoV indicates coefficient of variation.

4.3.3 Agreement Between Methods

Figure 4.3 is the Bland-Altman plot comparing the acetate analysis method with the Image Pro Plus analysis method. The bias was 3.16 capillaries/mm² and the standard devia-

tion of the bias was 3.09 capillaries/mm². The 95% limits of agreement were -3.02 to 9.33 capillaries/mm².

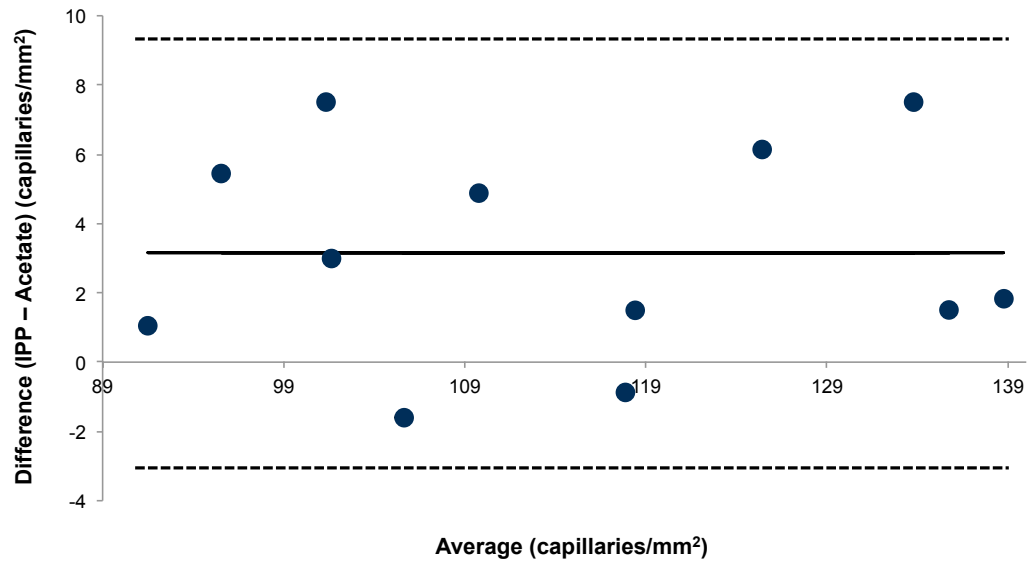


Figure 4.3: Bland-Altman Plot comparing the Image Pro Plus and acetate methods of capillary density analysis. The solid line indicates the mean difference between the two measurements, while the dotted lines indicate $\pm$ two standard deviations of the mean difference between the two measurements. IPP indicates Image Pro Plus.

4.4 Discussion

This work demonstrates that Image Pro Plus is a suitable method to replace the standard method of capillary density analysis using a transparent acetate. The Image Pro Plus method shows better intra- and inter-observer reproducibility compared to the acetate method, with good agreement between the methods.

The acetate method of analysis is laborious. The Image Pro Plus method has the advantage of not having to hold the acetate and trying to move it with the moving field of view while scrolling through video loops. This also means that the risk of dropping the acetate and having to restart the analysis is no longer an issue. Furthermore, it is easy to scroll back and forth through the video clips using Image Pro Plus to check the marked capillaries, which also makes it easier to determine what is and what is not a capillary as the frame rate can be

easily altered. After converting the images to digital files, you can also adjust the contrast much more easily to improve viewing of the capillaries without affecting the magnification of the field of view. Finally, the final count and image with the marked capillaries can be saved for each video loop for future reference using Image Pro Plus. While the capillary densities were slightly higher using the Image Pro Plus analysis method, the bias was systematic. This is thus likely the result of the aforementioned advantages.

I have shown that using Image Pro Plus is not only more practical, but also reduces the analysis time of six fields of view by 12 minutes and 35 seconds. This amount of time is significant, as the intravital capillary video microscopy protocol I used also involves venous occlusion measures, adding six more fields of view and six minutes of additional recordings. Thus, using Image Pro Plus saves about 25 minutes of analysis time per individual. Furthermore, I showed that both the intra- and inter-observer coefficients of variation were better for the Image Pro Plus analysis method. Though this system is much more expensive, the time saved and improved reproducibility are major advantages, for both small-scale and large-scale studies.

An inherent limitation of capillary microscopy is that a stable image is required for analysis, and while there are several methods to hold the finger in place without obstructing flow, it is usually very difficult to ensure that there is no movement of any kind during the recording, such as physiological tremor and cardiac rhythm. While Image Pro Plus analysis makes dealing with such movements easier, there is still room for improvement. Using the current algorithm for Image Pro Plus automatic vessel detection does not accurately detect capillaries, but this may be a possibility in the future.

4.5 Conclusions

In summary, the Image Pro Plus analysis method significantly reduces the analysis time, is more practical, and shows improved reproducibility. As such, Image Pro Plus was considered a suitable replacement of the acetate method for capillary density analysis by intravital capillary video microscopy.

5

POSTNATAL INTRAVENOUS LIPIDS AND CARDIOVASCULAR DEVELOPMENT

This chapter sets out to demonstrate the impact of postnatal intravenous lipid exposure in infants born preterm on aortic and left ventricular function in young adulthood. In order to do this, I have created a nested case-control study within the preterm-born cohort of young adults (*cohort 1*) to ensure similar perinatal and adult characteristics between cases and controls. In addition, 40 term-born young adult controls (*cohort 2*) of similar age and sex were included in this investigation as a comparison of normal values for the outcome measures. Cardiovascular magnetic resonance imaging was used to assess aortic and left ventricular function. It was found that intravenous lipid infusions during the first nine weeks of life correlated strongly with a rise in total cholesterol levels at this time. Interestingly, this rise in total cholesterol in the first nine weeks of life relates strongly to increased aortic stiffness in young adulthood, most notably in the abdominal aorta. Furthermore, the postnatal rise in total cholesterol also relates to reduced left ventricular systolic function in young adulthood. I have published this work in *Arteriosclerosis, Thrombosis, and Vascular Biology* as the first author [159].

5.1 Introduction

Intravenous lipids are used in parenteral nutrition for infants born preterm and are proven to be beneficial for neonatal brain and central nervous system development [112]. They consist of triglycerides in the form of soybean oil emulsified by egg phospholipid and glycerol [113]. Hypercholesterolaemia secondary to intravenous lipid use is well described, likely driven by endogenous cholesterol synthesis as a result of delayed lipid clearance [113; 114; 115].

Hyperlipidaemia is a key biological driver for atherogenesis [116; 117; 118]. Lipid rich fatty streaks first emerge in the aortic wall during fetal life and peak in prevalence during the first year following birth [119; 120]. Napoli *et al.* [121] demonstrated that newborn offspring of hypercholesterolaemic mothers who have been exposed to abnormal lipid profiles have significantly more aortic fatty streaks which persist into adolescence. Whether changes in the aorta are seen in preterm-born individuals without a maternal history of hypercholesterolaemia who were exposed to hyperlipidaemia in early postnatal life rather than *in utero* is unknown. However, this is of potential importance because of its clinical relevance to a broader population and postnatal nutrition.

Therefore, I studied preterm-born young adults, whose mothers were normocholesterolaemic, and as part of their routine clinical care, had received a brief, artificial, nutritional elevation in lipid levels postnatally in the form of an intravenous lipid infusion. As fatty streaks first appear in the aorta [119], and since animal models have demonstrated that arterial stiffness is the first pathophysiological change that occurs during this process [181], I studied global aortic stiffness in early adulthood, measured as pulse wave velocity; a measure known to be relevant to later cardiovascular outcome in large scale studies [182]. Cardiovascular magnetic resonance imaging was used as it allows for direct imaging of the aorta and the opportunity to study regional variation in aortic stiffness [164]. This information was used to determine whether the abdominal aorta, known to be most affected by fatty streaks in early life, had the greatest changes in aortic stiffness in the preterm-born young adults exposed to intravenous lipids.

Furthermore, because animal models have also shown a direct relationship between diet-induced hyperlipidaemia and left ventricular dysfunction [122; 123], I also investigated whether exogenous lipid exposure had effects beyond the vasculature and used cardiovascular magnetic resonance to study long-term variation in left ventricular structure and function.

5.2 Methods

5.2.1 Study Population

This chapter includes a subgroup of the preterm-born young adults (*cohort 1*). In addition to being randomised to banked breast milk or feeding formula, intravenous lipids were given simultaneously to newborns in the cohort of individuals born preterm based on variable local guidelines. Preterm-born individuals received intravenous lipids in the form of commercially available 10% or 20% Intralipid. They were given up to 2 to 3 g/kg/day of Intralipid, conforming with Intralipid administration practice at that time [115; 183; 184]. Eighteen participants in the study cohort of individuals born preterm were identified as having received postnatal intravenous lipids and who were not exposed to maternal hypercholesterolaemia (Figure 5.1). To limit the confounding effect of other potential variables at birth related to the clinical decision to give intravenous lipids, for each case, two controls who had not received intravenous lipids were identified who had similar gestational age, birth weight *Z* score, sex, key maternal risk factors, pregnancy complications and postnatal complications (Table 5.1).

In addition, 40 individuals from the young adult term-born control group with equivalent age and sex (*cohort 2*), that were not exposed to postnatal intravenous lipids, were included to provide normal reference ranges for the left ventricular and aortic measures, performed using the same techniques.

Table 5.1: Perinatal Characteristics and Maternal Conditions

	Preterm-Born Young Adults			Term-Born Young Adults	
	IV Lipids n = 18	No IV Lipids n = 36	P Value Ψ	No IV Lipids n = 40	P Value Υ
Birth Characteristics					
Males, n (%)	6 (33.3)	12 (33.3)	>0.99	14 (35.0)	0.99
Gestational Age (Weeks)	28.9 ± 2.1	29.3 ± 1.9	0.49	39.9 ± 0.7	<0.001
Birth Weight Z score	-1.19 ± 1.00	-1.14 ± 1.07	0.55	-0.14 ± 0.28	<0.001
Small for Gestational Age, n (%)	4 (22.2)	8 (22.2)	>0.99	0 (0)	0.006
Maternal Conditions					
Diabetes Mellitus, n (%)	1 (5.56)	3 (8.33)	0.71	2 (5.0)	0.83
Hypercholesterolaemia, n (%)	0 (0%)	0 (0)		0 (0)	
Hypertension, n (%)	3 (16.7)	6 (16.7)	>0.99	4 (10.0)	0.66
Ischaemic Heart Disease, n (%)	0 (0)	0 (0)		0 (0)	
Cerebrovascular Accident, n (%)	0 (0)	0 (0)		0 (0)	
Maternal Pregnancy Conditions/Complications					
Preeclampsia During Pregnancy, n (%)	6 (33.3)	10 (27.8)	0.67	0 (0)	<0.001
Smoking First Trimester, n (%)	6 (33.3)	10 (27.8)	0.67	9 (22.5)	0.81
Smoking Second and Third Trimester, n (%)	4 (22.2)	7 (19.4)	0.77	8 (20.0)	0.94
Steroids Given, n (%)	5 (27.8)	9 (25.0)	0.83		
Spontaneous Delivery, n (%)	11 (61.1)	23 (63.9)	0.84		
Neonatal Condition/Well-being					
Put on Ventilation, n (%)	13 (72.2)	24 (66.7)	0.68		
Days on Ventilation, Mean ± SD	5.33 ± 5.36	4.39 ± 7.59	0.64		
Infections Based on Positive Blood Culture in First Nine Weeks, n (%)	3 (16.7)	5 (13.9)	0.79		
Apgar Score 1 minute, n (%)					
0 - 3	5 (27.8)	9 (25.0)	0.84		
4 - 6	8 (44.4)	19 (52.8)			
7 - 10	5 (27.8)	9 (25.0)			
Apgar Score 5 minutes, n (%)					
0 - 3	2 (11.1)	3 (8.33)	0.94		
4 - 6	6 (33.3)	13 (36.1)			
7 - 10	10 (55.6)	20 (55.6)			

IV indicates intravenous. Values as Mean ± Standard Deviation unless stated otherwise.

Ψ = Preterm-born young adults given intravenous lipids vs preterm-born young adults not given intravenous lipids.

Υ = Preterm-born young adults given intravenous lipids vs preterm-born young adults not given intravenous lipids vs term-born young adults.

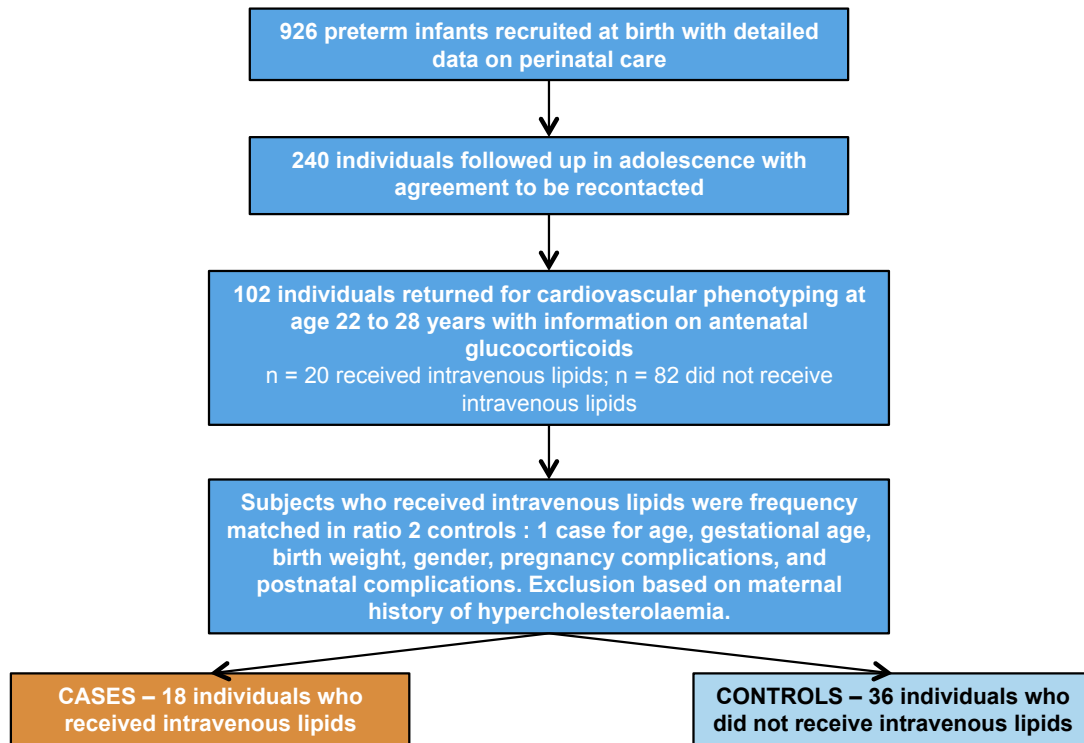


Figure 5.1: Preterm study cohort. Two hundred and forty individuals with agreement to be recontacted from a cohort of 926 preterm-born individuals (birth weight less than 1,850 g) were followed up in adolescence. One hundred and two of these individuals were seen in young adulthood to undergo cardiovascular phenotyping. Individuals that received intravenous lipids and those that did not were matched in a 1 to 2 ratio based on early life characteristics, with 18 that received intravenous lipids and 36 that did not.

5.2.2 Study Visit

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity and blinded analysis. The following methods were used for this chapter as described in detail in Chapter 3:

- 3.2: Cardiovascular Magnetic Resonance

- 3.2.1: Aortic Imaging

- 3.2.1.1: Aortic Pulse Wave Velocity

- 3.2.1.2: Aortic Distensibility

- 3.2.2: Cardiac Imaging

3.2.2.1: Cardiac Cine Acquisition

3.2.2.2: Left Ventricular Mass and Volumes

- 3.4: Anthropometry and Lifestyle Questionnaire
- 3.5: Blood Sampling and Biochemistry
- 3.7: Blood Pressure Measurements

5.2.3 Statistical analysis

Statistical analysis was carried out using SPSS Version 19 as described in Section 3.9 of Chapter 3. It was determined that 36 preterm-born controls would provide 80% power at $P = 0.05$ to identify a 0.66 standard deviation difference in aortic pulse wave velocity between preterm-born young adults that were and were not given intravenous lipids in early postnatal life. This difference is equivalent to 0.80 m/s, which is similar to the differences previously observed between subclinical risk factor groups [185].

5.3 Results

5.3.1 Population Characteristics in Early Postnatal Life

Clinical infant neonatal characteristics were confirmed to be similar in each recruitment centre, but there was significant variation in whether a centre used intravenous lipids (Table 5.2). As a result, it was possible to frequency match those who received intravenous lipids with individuals who had similar perinatal characteristics but who did not receive intravenous lipids. Table 5.1 demonstrates that the preterm-born individuals included in the analysis who had or had not received intravenous lipids were similar for all maternal, labour, and postnatal characteristics. Furthermore, as the preterm-born individuals had been randomised to milk feeding regimes at birth, it was possible to ensure that other dietary intakes that may have affected the results were equivalent between the preterm-born young adults that did and did not receive intravenous lipids (Table 6.3). There were no differences in the

number of individuals in the preterm-born groups in terms of antenatal glucocorticoid exposure (Table 5.1) or postnatal minimum, mean or maximum cortisol levels (Table 5.4). Thyroid function as assessed by postnatal minimum, mean, and maximum triiodothyronine levels also did not differ between the groups (Table 5.4). The comparison group of young adult term-born individuals also showed no difference in sex, maternal characteristics, and smoking during pregnancy as compared to the groups of preterm-born individuals. However, as expected, gestational age, birth weight *Z* score, percentage born small for gestational age, and number of individuals whose mothers had gestational hypertension or preeclampsia differed between the young adults born term and both groups of preterm-born young adults (Table 5.1).

Table 5.2: Percentage of Individuals at the Five UK Centres from the Original Birth Cohort that Received Intravenous Lipids

Centre	Original cohort (n = 926)
1	36.0%
2	2.27%
3	46.9%
4	17.9%
5	17.2%

Table 5.3: Number of Individuals Randomised to Banked Breast Milk or Formula in the Preterm-Born Young Adult Groups

	IV Lipids (n = 18)	No IV Lipids (n = 36)	<i>P</i> Value
Fed Banked Breast Milk, n (%)	4 (22.2%)	10 (27.8%)	0.66
Fed Formula Milk, n (%)	14 (77.8%)	26 (72.2%)	

IV indicates intravenous.

5.3.2 Population Characteristics in Young Adult Life

In early adult life, there were no significant differences between the preterm-born individuals that received postnatal intravenous lipids and those that did not in terms of age, blood pressure levels, anthropometry, smoking history, lipid profile, measures of glucose homeostasis or other biochemistry measures (Table 5.5). The term-born young adults were aged

Table 5.4: Thyroid Function and Cortisol Levels (Age 0 to 9 Weeks) in the Preterm-Born Young Adult Groups

	IV Lipids (n = 18)	No IV Lipids (n = 36)	P Value
Triiodothyronine Levels			
Minimum (ng/dL)	0.41 ± 0.31	0.44 ± 0.29	0.73
Mean (ng/dL)	0.56 ± 0.15	0.56 ± 0.26	0.99
Maximum (ng/dL)	0.72 ± 0.17	0.70 ± 0.29	0.75
Cortisol Levels			
Minimum (nmol/L)	999.6 ± 670.1	1063.9 ± 798.2	0.76
Mean (nmol/L)	1217.1 ± 556.4	1350.9 ± 701.8	0.45
Maximum (nmol/L)	1863.3 ± 548.8	1734.7 ± 695.6	0.46

IV indicates intravenous; T3, triiodothyronine. Values as Mean ± Standard Deviation.

25.4 ± 1.8 years with an average body mass index of 23.5 ± 2.3 g/m², thus neither of these two parameters differed across the three groups (ANOVA $P = 0.86$ and 0.21, respectively). There were no differences between the groups in terms of parental or grandparent family medical history (Table 5.1 and Table 5.6) and no differences between the groups in terms of diet, medical history, alcohol intake, exercise or sociodemographics ($P > 0.05$).

Table 5.5: Characteristics in Young Adult Life of Preterm-Born Individuals

	IV Lipids n = 18	No IV Lipids n = 36	P Value
Age (years)	25.1 ± 1.5	25.3 ± 1.5	0.61
Body Mass Index (kg/m ²)	23.0 ± 3.2	24.8 ± 7.0	0.32
Waist to Hip Ratio	0.76 ± 0.08	0.78 ± 0.09	0.32
Smokers, n (%)	5 (27.8%)	8 (22.2%)	0.65
Total Cholesterol (mmol/L)	4.71 ± 0.65	4.76 ± 0.94	0.25
HDL-C (mmol/L)	1.52 ± 0.31	1.56 ± 0.47	0.78
LDL-C (mmol/L)	2.57 ± 0.59	2.85 ± 0.80	0.24
Triglycerides (mmol/L)	1.09 ± 0.70	0.95 ± 0.47	0.40
CRP (mmol/L)	1.26 ± 1.53	2.74 ± 4.36	0.82
Glucose (mmol/L)	5.09 ± 0.51	4.89 ± 0.41	0.17
Insulin (pmol/L)	61.5 ± 30.2	58.0 ± 29.9	0.70
HOMA-IR (mmol*pmol/L ²)	1.15 ± 0.56	1.08 ± 0.57	0.61
HOMA-S (%)	106.4 ± 48.7	115.5 ± 54.4	0.56
HOMA-β (%)	100.1 ± 28.5	104.7 ± 40.3	0.68
Systolic Blood Pressure, mmHg	121.0 ± 12.9	118.5 ± 11.6	0.49
Diastolic Blood Pressure, mmHg	72.7 ± 6.1	71.6 ± 9.8	0.67
Mean Arterial Pressure, mmHg	88.9 ± 7.2	87.4 ± 9.7	0.60
Pulse Pressure, mmHg	48.2 ± 11.5	46.9 ± 9.4	0.66

IV indicates intravenous; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol;

CRP, C-reactive protein; HOMA, homeostasis model assessment; IR, insulin resistance; S, insulin sensitivity; β, beta cell function. Values as Mean ± Standard Deviation.

Table 5.6: Paternal and Grandparent Family History of Preterm-Born Individuals

	IV Lipids n = 18	No IV Lipids n = 36	P Value
Paternal Conditions			
Diabetes Mellitus, n (%)	0 (0%)	3 (8.33%)	0.21
Hypercholesterolaemia, n (%)	1 (5.56%)	1 (2.78%)	0.61
Hypertension, n (%)	4 (22.2%)	6 (16.7%)	0.62
Ischaemic Heart Disease, n (%)	2 (11.1%)	3 (8.33%)	0.74
Cerebrovascular Accident, n (%)	0 (0%)	2 (5.56%)	0.31
Grandmother Conditions (Maternal and Paternal)			
Ischaemic Heart Disease, n (%)	2 (11.1%)	2 (5.56%)	0.46
Cerebrovascular Accident, n (%)	1 (5.6%)	0 (0%)	0.15
Grandfather Conditions (Maternal and Paternal)			
Ischaemic Heart Disease, n (%)	2 (11.1%)	4 (11.1%)	>0.99
Cerebrovascular Accident, n (%)	2 (11.1%)	5 (13.9%)	0.78

IV indicates intravenous.

5.3.3 Aortic Function

5.3.3.1 Intravenous Lipid Administration

Figure 5.2 demonstrates that preterm-born individuals who received intravenous lipids postnatally had a 29% greater aortic pulse wave velocity in early adulthood (5.89 ± 1.23 vs 4.57 ± 1.17 m/s, $P < 0.001$). Aortic pulse wave velocity in the term-born controls was 4.48 ± 0.66 m/s, which was similar to the preterm-born individuals not exposed to intravenous lipids ($P = 0.68$), but significantly lower than the preterm-born individuals who had received intravenous lipids ($P < 0.001$). Data on regional aortic distensibility was then used to determine which areas of the aorta had the greatest change in aortic stiffness. There was a significant reduction in distensibility in the abdominal aorta (9.74 ± 4.27 vs 12.91 ± 4.11 mmHg⁻¹ x 10³, $P = 0.01$), but not in the ascending or proximal descending aorta (Figure 5.3). Abdominal aortic distensibility in the term-born controls was 13.6 ± 4.5 mmHg⁻¹ x 10³, which was similar to the preterm-born individuals who did not receive intravenous lipids postnatally ($P = 0.47$), but significantly higher than the preterm-born individuals who received intravenous lipids postnatally ($P = 0.003$).

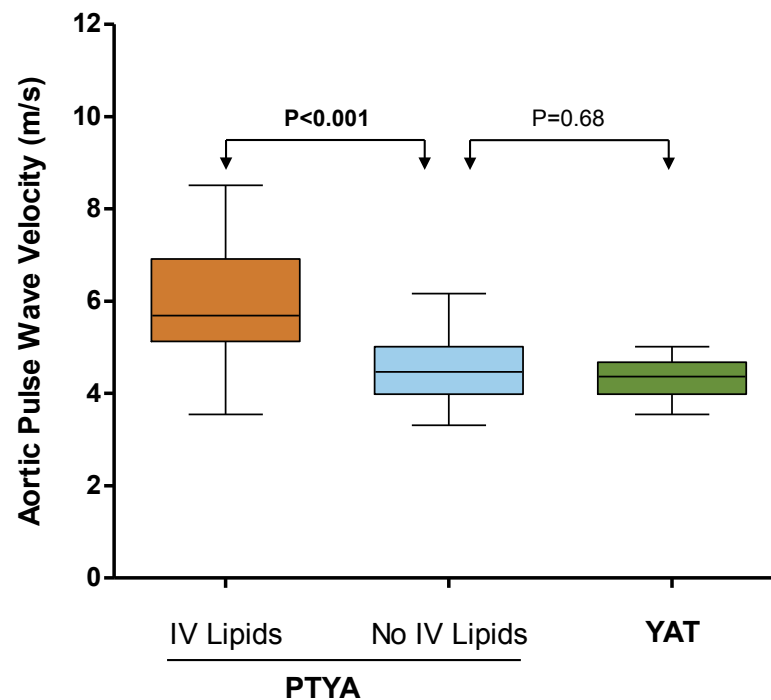


Figure 5.2: Aortic pulse wave velocity for preterm-born young adults (PTYA) that received intravenous lipids (orange) and those that did not receive intravenous lipids (blue), as well as young adult controls born term (YAT, green). Aortic pulse wave velocity was significantly higher in young adulthood in the preterm-born group of individuals that received intravenous lipids compared to the preterm-born group of individuals that did not receive intravenous lipids (5.89 ± 1.23 vs 4.57 ± 1.17 m/s, $P < 0.001$) at age 0 to 9 weeks. Aortic pulse wave velocity in the term-born young adult controls was 4.48 ± 0.66 m/s, which was similar to the preterm-born individuals not exposed to intravenous lipids ($P = 0.68$), but significantly lower than the preterm-born individuals who had received intravenous lipids ($P < 0.001$). Data shown as box and whisker plot: minimum to maximum.

5.3.3.2 Association between Intravenous Lipids, Neonatal Biochemistry, and Aortic Stiffness

As intravenous lipid administration is known to alter a range of circulating metabolic parameters, I explored relations between three different intravenous lipid parameters and maximum cholesterol, triglyceride, glucose, and phospholipid levels during the first nine weeks of life. Figure 5.4 demonstrates that, as expected, maximum cholesterol, triglyceride, glucose and phospholipid levels were all significantly associated ($P < 0.05$) with at least one of the intravenous lipid parameters. However, only postnatal maximum cholesterol level was related to both postnatal intravenous lipid administration and the primary endpoint of aortic pulse wave velocity in young adulthood ($r = 0.62$, $P < 0.001$). Figure 5.5, Panel A shows

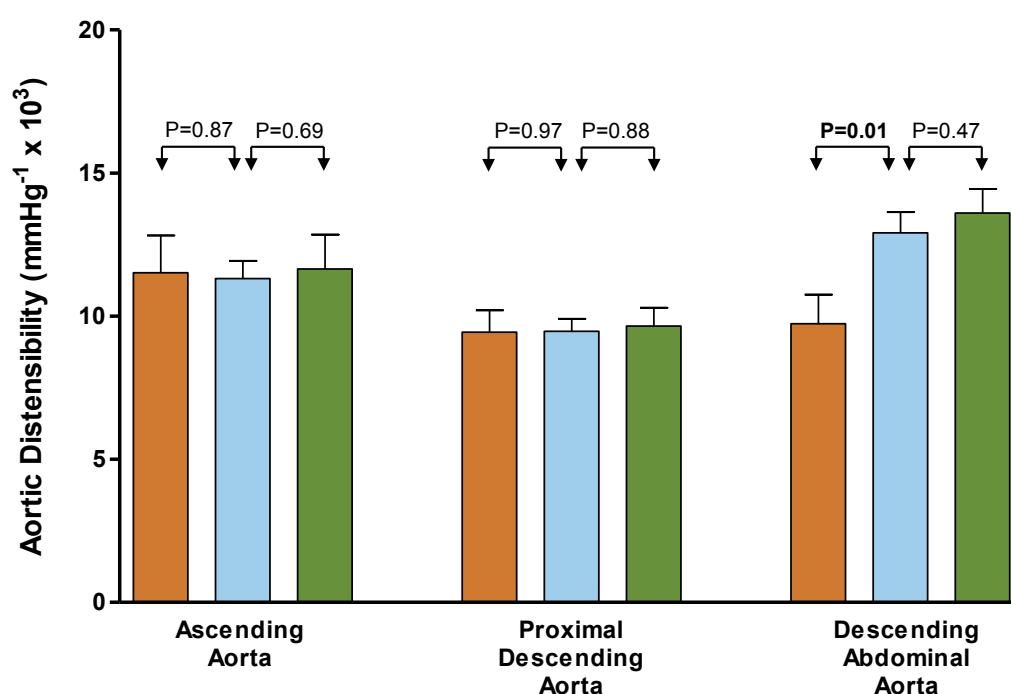


Figure 5.3: Aortic distensibility for preterm-born young adults (PTYA) that received intravenous lipids (orange) and those that did not receive intravenous lipids (blue), as well as young adult controls born term (YAT, green). There were no differences between preterm-born individuals that did and did not receive intravenous lipids during early postnatal life in ascending aortic or proximal descending aortic distensibility in young adulthood, though descending abdominal aortic distensibility was greatly reduced in the group that received intravenous lipids (9.74 ± 4.27 vs 12.91 ± 4.11 $\text{mmHg}^{-1} \times 10^3$, $P = 0.01$). Abdominal aortic distensibility in the term-born controls was 13.6 ± 4.5 $\text{mmHg}^{-1} \times 10^3$, which was similar to the preterm-born individuals who did not receive intravenous lipids postnatally ($P = 0.47$), but significantly higher than the preterm-born individuals who received intravenous lipids postnatally ($P = 0.003$). Data shown as mean $\pm$ standard error of the mean.

the graded relation between postnatal maximum cholesterol and aortic pulse wave velocity in young adulthood. The preterm-born individuals that did and did not receive postnatal intravenous lipids were then separated to determine the association between postnatal maximum cholesterol and aortic pulse wave velocity in each group. There was a graded association between the level of cholesterol obtained in the first nine weeks of life and aortic pulse wave velocity in young adulthood in the preterm-born young adults that received intravenous lipids postnatally ($r = 0.73$, 95% confidence interval 0.020 to 0.059, $P = 0.001$) (Figure 5.5, Panel B) with a similar positive, but non-significant, graded association in the preterm-born young adult group that did not receive postnatal intravenous lipids ($r = 0.35$, $P = 0.20$). Similarly, Figure 5.6, Panel A shows the inverse association between postnatal maxi-

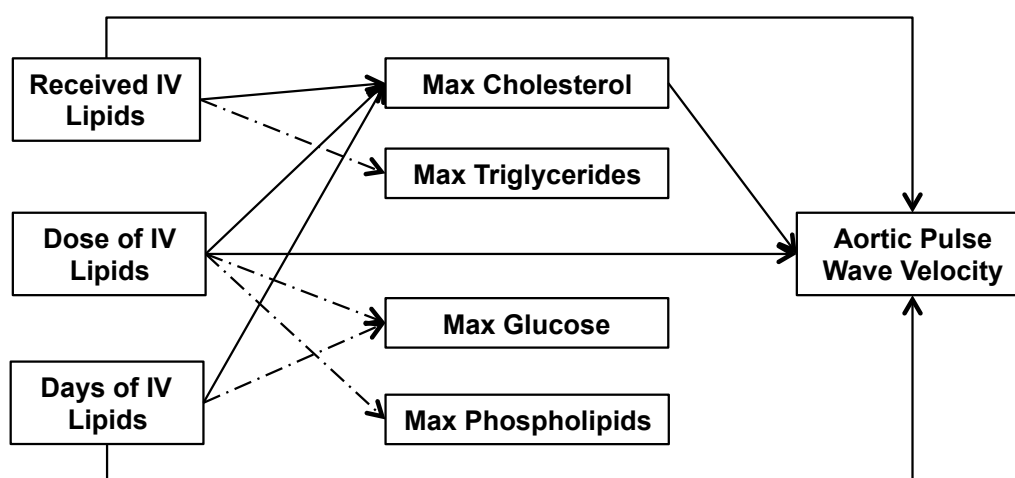


Figure 5.4: Associations between intravenous lipid parameters and biochemistry parameters in the first nine weeks of life with aortic pulse wave velocity at age 25 years. Dotted lines = $P < 0.05$; solid lines = $P < 0.001$. Maximum cholesterol level was the only biochemistry parameter in the first nine weeks of life that was significantly associated with whether or not intravenous lipids were given ($r = 0.47$, $P < 0.001$), dose of intravenous lipids ($r = 0.56$, $P < 0.001$) and number of days given intravenous lipids ($r = 0.57$, $P < 0.0001$) in early postnatal life, as well as with aortic pulse wave velocity in young adult life ($r = 0.62$, $P < 0.001$). Intravenous lipid parameters were closely interrelated ($P < 0.001$) and there were no associations between intravenous lipid parameters and minimum or mean cholesterol, triglyceride, glucose, and phospholipid levels.

imum cholesterol and abdominal aortic distensibility in young adulthood. The preterm-born individuals that did and did not receive postnatal intravenous lipids were then separated, and it was shown there was a graded, inverse association between the level of cholesterol obtained in the first nine weeks of life and abdominal aortic distensibility in young adulthood in the preterm-born young adults that received intravenous lipids postnatally ($r = -0.53$, 95% confidence interval -0.066 to -0.0061 , $P = 0.02$) (Figure 5.6, Panel B).

5.3.3.3 Intravenous Lipid and Cholesterol Levels in Neonatal and Young Adult Life

Figure 5.7 demonstrates that preterm-born individuals given intravenous lipids had on average a 1.26 mmol/L higher maximum cholesterol level during neonatal life ($P = 0.006$) than the preterm-born individuals not given intravenous lipids, whereas minimum cholesterol levels were similar between groups at this age. The data indicated that the maximum cholesterol level in the preterm-born individuals given intravenous lipids was likely to be representative of mean circulating cholesterol levels during intravenous lipid infusion, independent of

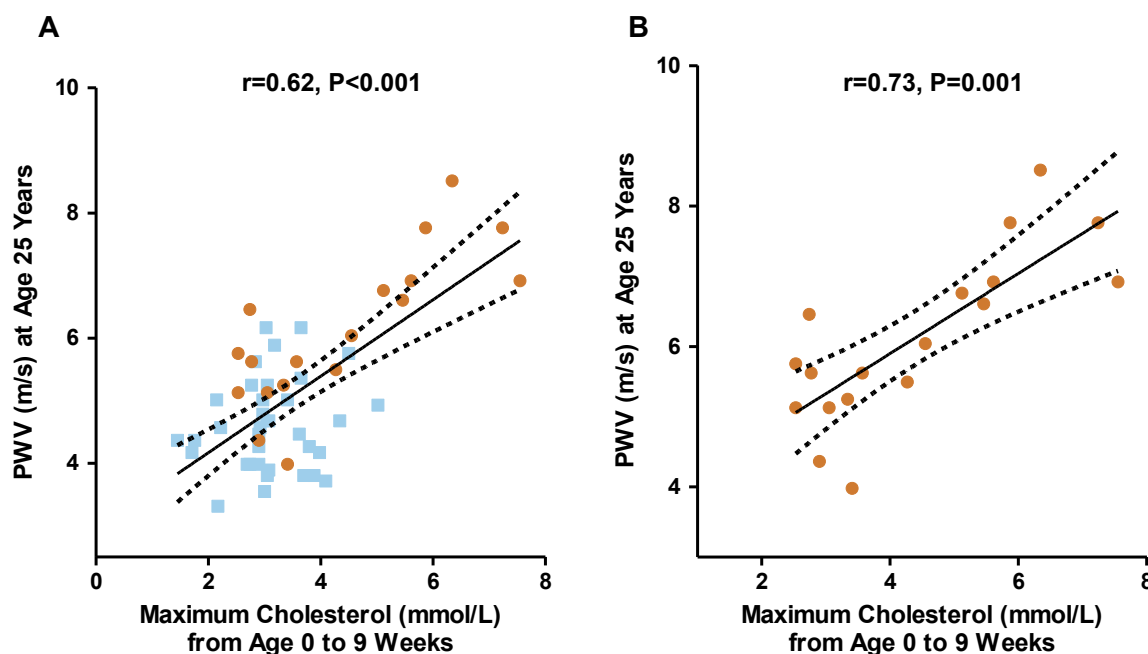


Figure 5.5: Postnatal maximum cholesterol and aortic pulse wave velocity in young adulthood. **A**, The association between maximum cholesterol in early postnatal life and aortic pulse wave velocity in young adult life in preterm-born individuals ($r = 0.62$, $P < 0.001$). Blue squares = preterm-born individuals that did not receive intravenous lipids postnatally; orange circles = preterm-born individuals that received intravenous lipids postnatally. **B**, The association between maximum cholesterol in early postnatal life and aortic pulse wave velocity in young adult life in all those who received intravenous lipids ($r = 0.73$, $P = 0.001$). PWV indicates aortic pulse wave velocity.

native cholesterol levels (Figure 5.7). Intravenous lipids were given postnatally for an average of 11.2 ± 10.0 days (range = 2 to 36 days), and the area under the curve of cholesterol measurements during the nine week period was closely related to the number of days given intravenous lipids and the dose of intravenous lipids given ($r = 0.79$, $P < 0.001$ and $r = 0.77$, $P < 0.001$, respectively). Figure 5.8 is an example of the cholesterol curve for one preterm-born individual who received intravenous lipids for 33 days and had a corresponding sustained increase in cholesterol for a similar period of time before returning to a 'baseline' cholesterol level.

The cholesterol values in neonatal life were compared to those reported by Sporik *et al.* [186], who showed mean cholesterol values at birth of 1.8 ± 0.5 mmol/L in healthy term-born infants. This level was similar to the mean values for both the preterm-born group that received intravenous lipids (1.73 ± 0.7 mmol/L) and the preterm-born group that did not receive intravenous lipids (1.82 ± 0.6 mmol/L). Furthermore, cholesterol levels in young

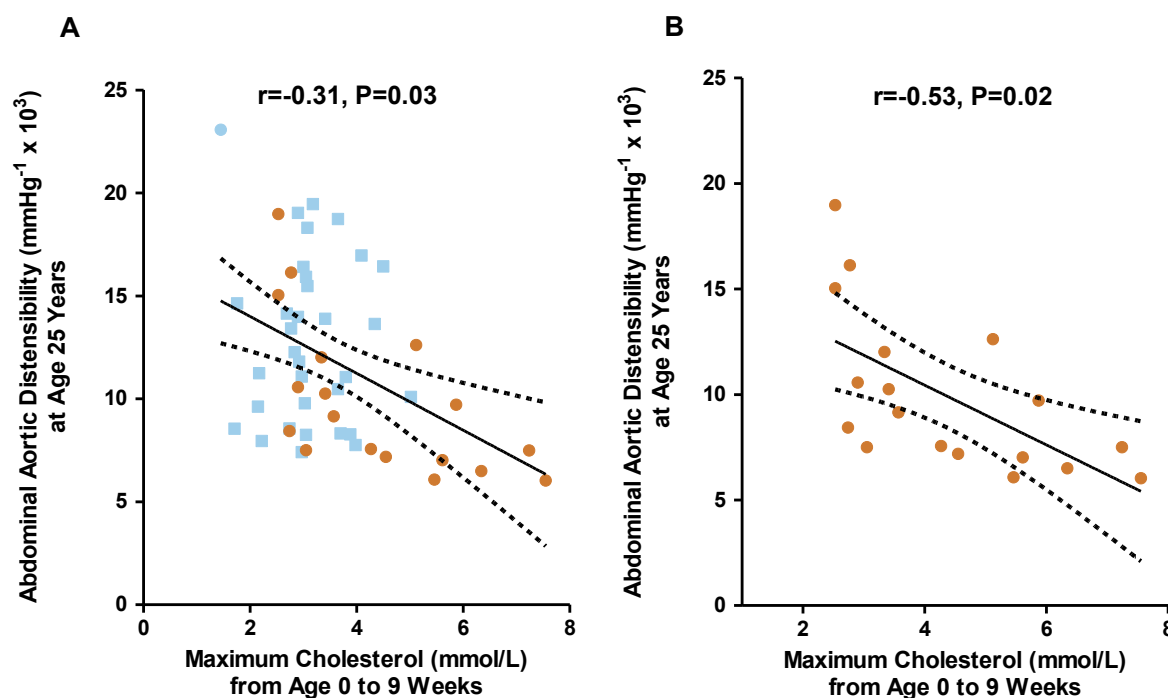


Figure 5.6: Postnatal maximum cholesterol and abdominal aortic distensibility in young adulthood. **A**, The association between maximum cholesterol in early postnatal life and abdominal aortic distensibility in young adult life in preterm-born individuals ($r = -0.31$, $P = 0.03$). Blue squares = preterm-born individuals that did not receive intravenous lipids postnatally; orange circles = preterm-born individuals that received intravenous lipids postnatally. **B**, The association between maximum cholesterol in early postnatal life and abdominal aortic distensibility in young adult life in all those who received intravenous lipids ($r = -0.53$, $P = 0.02$).

adult life (Figure 5.7) also did not differ between the groups of preterm-born individuals and were independent of maximum cholesterol levels during the first nine weeks of life. These findings suggest that underlying fasting lipid metabolism was similar between groups.

5.3.3.4 Early Cholesterol Levels and Other Predictors of Pulse Wave Velocity in Young Adulthood

The relative importance of early cholesterol levels compared to other predictors of aortic pulse wave velocity in young adulthood was then explored. Parameters with $P < 0.50$ for the entire group of preterm-born individuals were included in a forced entry linear regression model (Table 5.7). In the case of blood pressure measurements, the parameter that most strongly associated to aortic pulse wave velocity (mean arterial pressure) was included in the regression model as all blood pressure measures were strongly interrelated. Aortic pulse

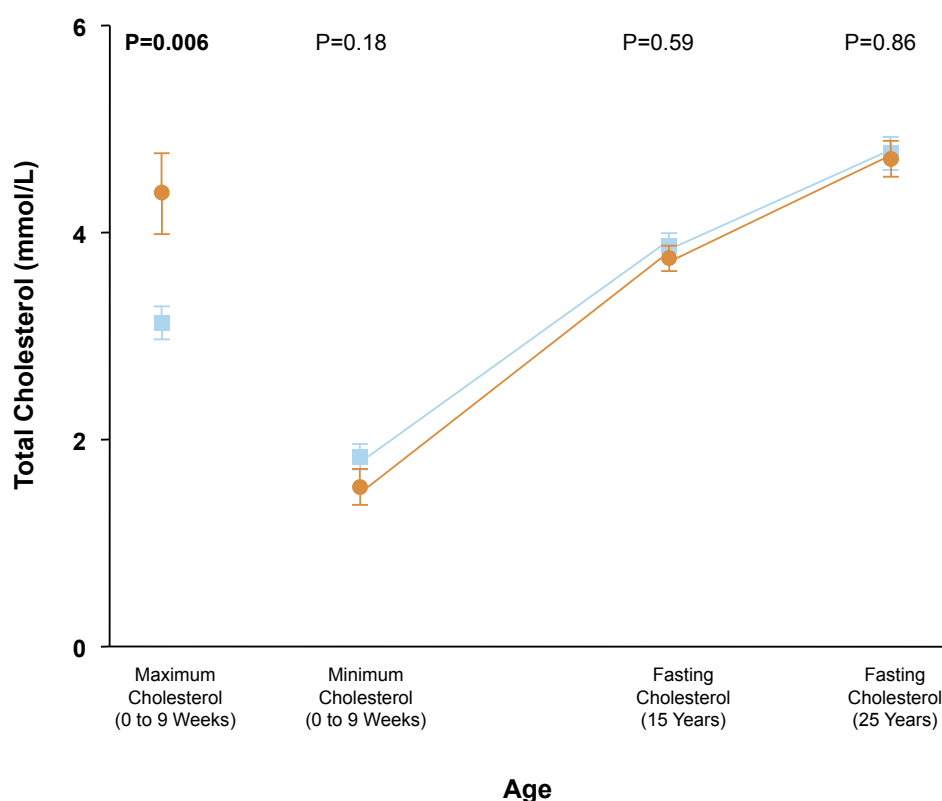


Figure 5.7: Difference in cholesterol levels between preterm-born individuals that received intravenous lipids (orange) and those that did not receive intravenous lipids (blue) in early postnatal life (0 to 9 weeks), adolescence (age 15 years) and young adulthood (age 25 years). Individuals that received intravenous lipids achieved significantly higher maximum cholesterol levels during the first nine weeks of life (4.38 ± 1.65 vs. 3.12 ± 0.78 mmol/L, $P = 0.006$). There were no differences between the two groups in terms of minimum cholesterol levels in the first nine weeks of life, fasting total cholesterol levels at age 15 years or at age 25 years. Data shown as mean $\pm$ standard error of the mean.

wave velocity was included as the dependent variable with maximum cholesterol at age 0 to 9 weeks, mean arterial pressure, low density lipoprotein cholesterol, body mass index, and smoking status in young adult life as independent variables. Maximum cholesterol from age 0 to 9 weeks was an independent predictor of aortic pulse wave velocity in adult life ($B = 0.045 \log(\text{m/s}) / (\text{mmol/L})$, 95% confidence interval: 0.027 to 0.062, $P < 0.001$). This means that for every one unit (in mmol/L) increase in postnatal maximum cholesterol levels, there was a $10^{0.045}$ fold (or 10.9%) increase in aortic pulse wave velocity in young adulthood. I also ensured there was no association between recruitment centre and cardiovascular end points in those who did not receive intravenous lipids, and repeated analysis excluding the recruitment centre with the highest use of intravenous lipids. This did not change the association

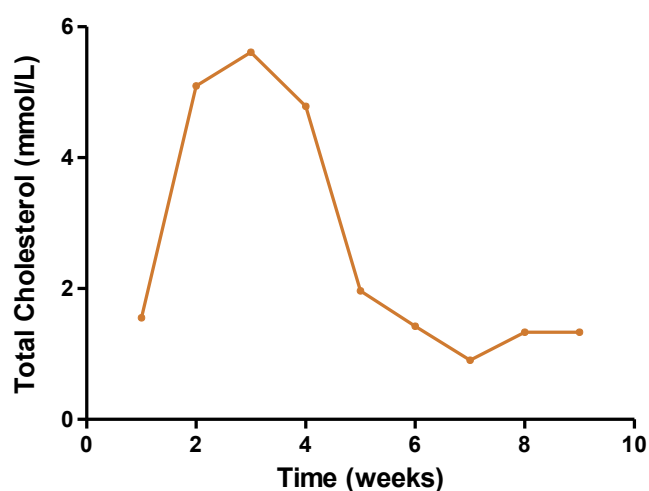


Figure 5.8: An example of a cholesterol curve for an individual who received intravenous lipids for 33 days. As can be seen, there was a two and a half fold increase in total cholesterol levels over a 30 day period. The total cholesterol levels then returned to baseline.

between intravenous lipid use and cardiovascular endpoints.

5.3.4 Left Ventricular Function

5.3.4.1 Intravenous Lipid Administration

The hypothesis that intravenous lipids were also associated with long-term changes in left ventricular structure and function was then explored. There were no differences in left ventricular mass indexed for body surface area or ejection fraction in young adult life between preterm-born individuals that received intravenous lipids and those that did not (67.7 ± 12.5 g/m² vs 65.7 ± 10.9 g/m², $P = 0.53$ and $64.1 \pm 5.6\%$ vs $64.0 \pm 6.8\%$, $P = 0.98$). Data collection on left ventricular strain, a marker of subclinical variation in left ventricular function, was included as part of the protocol part way through the study and was available for a subgroup of participants (seven who received intravenous lipids and 12 who did not receive intravenous lipids postnatally). Figure 5.9 demonstrates that individuals born preterm who had received intravenous lipids postnatally had a significant reduction in myocardial left ventricular circumferential peak systolic apical strain in young adult life (-17.9 ± -3.3 vs $-21.6 \pm -1.8\%$, $P = 0.006$). Left ventricular circumferential peak systolic apical strain in the term-born young

Table 5.7: Bivariable Regression Associations with Log Transformed Aortic Pulse Wave Velocity for the Full Group of Young Adults Born Preterm

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Sex	-0.010	-0.064	0.045	0.73
Gestational Age	0.00097	-0.014	0.013	0.88
Birth Weight Z score	1.75×10^{-6}	-0.029	0.021	0.77
Newborn Days on Ventilation	0.0010	-0.0015	0.0033	0.53
Maximum Cholesterol Level Achieved (Age 0 to 9 Weeks)	0.045	0.029	0.061	<0.001
Age	0.0070	-0.01	0.025	0.42
Smoking	0.021	-0.040	0.079	0.50
Body Mass Index	-0.0011	-0.0057	0.0030	0.49
Waist:Hip Ratio	0.025	-0.27	0.32	0.86
Systolic Blood Pressure	0.0020	-0.0011	0.0040	0.17
Diastolic Blood Pressure	0.0030	-0.0012	0.0053	0.19
Pulse Pressure	0.0010	-0.0016	0.0034	0.58
Mean Arterial Pressure	0.0031	0.0010	0.0056	0.11
Total Cholesterol	0.0030	-0.031	0.037	0.86
HDL Cholesterol	0.013	-0.055	0.081	0.70
LDL Cholesterol	-0.020	-0.058	0.018	0.30
Triglycerides	0.0082	-0.043	0.059	0.75

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL indicates low density lipoprotein cholesterol.

adult controls was $-21.1 \pm -2.5\%$, which differed significantly from the preterm-born young adults who received intravenous lipids postnatally ($P = 0.005$), but did not differ significantly from the preterm-born young adults who did not receive intravenous lipids postnatally ($P = 0.55$). Figure 5.10 is an example of left ventricular deformation in young adulthood from end-diastole (Panel A, left image) to end-systole (Panel A, right image) in a preterm-born individual that received intravenous lipids in the first nine weeks of life (Figure 5.10 Panel A, top) and a preterm-born individual that did not receive intravenous lipids in the first nine weeks of life (Figure 5.10 Panel A, bottom). The associated changes in left ventricular circumferential peak systolic apical strain throughout the cardiac cycle are shown in Figure 5.10, Panel B.

5.3.4.2 Association with Total Cholesterol Level

The nature of the association between total cholesterol level, intravenous lipid administration and left ventricular strain in the preterm-born individuals was then explored. Left ven-

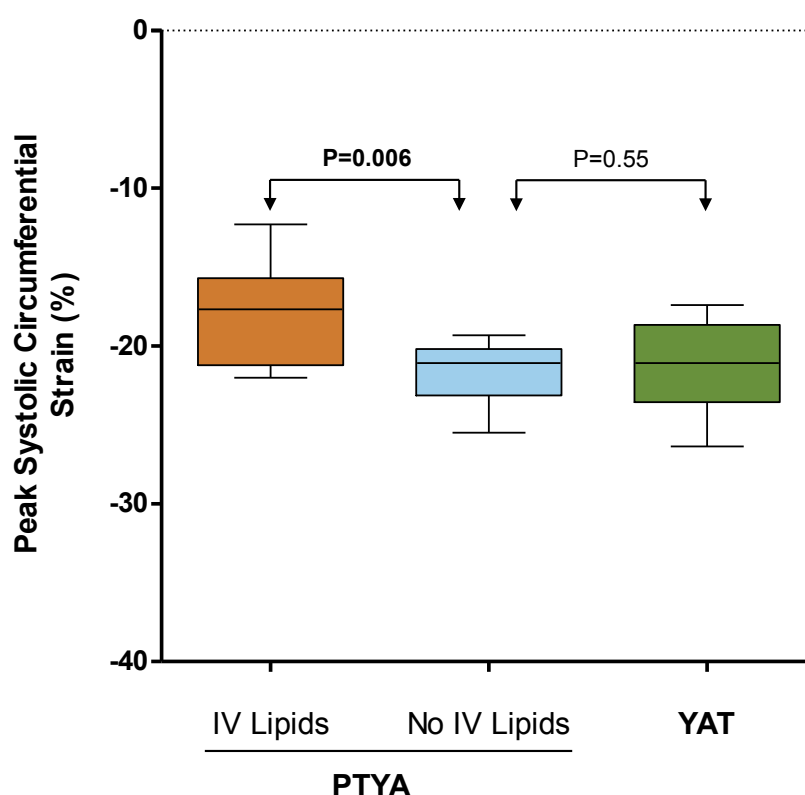


Figure 5.9: Mean apical peak systolic circumferential strain in the left ventricle for preterm-born young adults (PTYA) that received intravenous lipids (orange) and did not receive intravenous lipids (blue) in the first nine weeks of life, as well as young adult controls born term (YAT). Preterm-born individuals that received intravenous lipids postnatally had significantly lower apical peak systolic circumferential strain in young adulthood than preterm-born individuals that did not receive intravenous lipids (-17.9 ± 3.3 vs -21.6 ± 1.8 %, $P = 0.006$). Left ventricular apical peak systolic circumferential strain in the term-born young adult controls was -21.1 ± 2.5 %, which differed significantly from the preterm-born young adults who received intravenous lipids postnatally ($P = 0.005$), but did not differ significantly from the preterm-born young adults who did not receive intravenous lipids postnatally ($P = 0.55$). Data shown as box and whisker plot: minimum to maximum.

tricular circumferential peak systolic apical strain was significantly associated with the dose of intravenous lipids received ($r = 0.61$, $P = 0.006$) and the number of days given intravenous lipids ($r = 0.51$, $P = 0.03$) in early postnatal life. Similar to the association between maximum cholesterol level in the first nine weeks of life and aortic function in young adulthood, there was a graded association between maximum cholesterol level in the first nine weeks of life and left ventricular circumferential peak systolic apical strain ($r = 0.57$, $P = 0.01$). This association was driven by the relationship within the preterm-born individuals that received intravenous lipids, but there were no associations with other biochemical parameters during

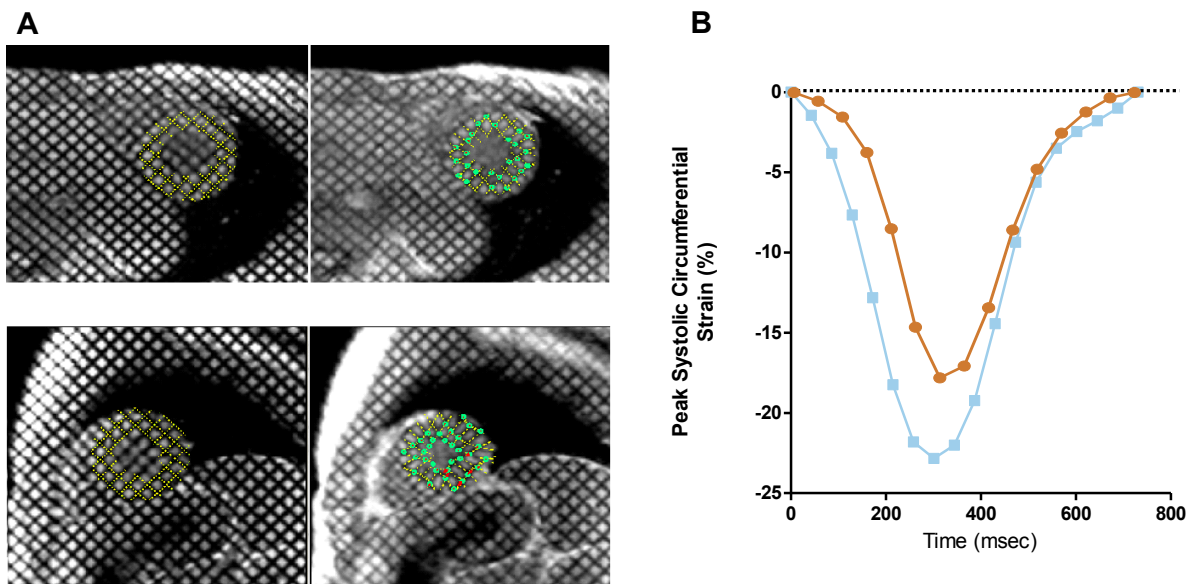


Figure 5.10: **A**, Example of a tagged apical short axis slice of the left ventricle during end-diastole (on the left) and end-systole (on the right) in an individual that received intravenous lipids (top) and an individual that did not receive intravenous lipids (bottom). Tags are set at end-diastole and adjusted at each frame through the cardiac cycle until end-systole to define the degree of myocardial deformation. **B**, Examples of the change in apical circumferential strain over the cardiac cycle in the left ventricle for the two individuals demonstrated in 'A' (Intravenous lipids = orange; no intravenous lipids = blue). The reduction in circumferential strain at end systole implies reduced contraction of the myocardium.

neonatal life and left ventricular function.

5.3.4.3 Myocardial and Aortic Function

Left ventricular circumferential peak systolic apical strain was also significantly associated with aortic pulse wave velocity ($r = -0.56$, $P = 0.01$) in early adulthood. I therefore explored whether the changes in left ventricular strain I had identified in the preterm-born young adults who received intravenous lipids postnatally could be explained by the association between intravenous lipids and aortic stiffness, or whether these were independent phenomena. In a forced entry regression model with left ventricular circumferential peak systolic apical strain as the dependent variable and maximum neonatal cholesterol level and aortic pulse wave velocity in young adult life as independent variables, both maximum cholesterol level ($P = 0.01$) and aortic pulse wave velocity ($P = 0.05$) remained as independent predictors of left ventricular circumferential peak systolic apical strain.

5.4 Discussion

This study demonstrates that intravenous lipid exposure during neonatal life in infants born preterm is related to changes in aortic and left ventricular function in young adult life. Furthermore, the impact on aortic stiffness and left ventricular strain in preterm-born young adults is graded depending on the maximum cholesterol level attained and amount of time elevated during this early period of cardiovascular development. These associations are independent of native cholesterol levels during the first decades of life, suggesting that nutritional elevation of cholesterol in critical periods of early development in preterm-born individuals may have a direct impact on the cardiovascular system.

Preterm-born infants are at risk of significant morbidity and intravenous lipids are proven to be beneficial for neonatal brain and central nervous system development [112]. Therefore, the findings are not necessarily of clinical relevance to the use of intravenous lipids in this setting. Rather, the interest related to the fact that, because intravenous lipids consist of triglycerides emulsified by egg phospholipid and glycerol, they are known to lead to various hyperlipidaemias including hypercholesterolaemia [113; 115; 183]. Thereby I was able to make use of this unique clinical situation to explore experimentally the impact in preterm-born individuals of artificial modification of lipid levels early in life on aortic and cardiac development. A particular strength of this cohort of preterm-born individuals is that they had taken part in a randomised trial of milk feeding regimes at birth and therefore other nutritional influences were controlled for between groups. Furthermore, the cohort had the advantage of prospective follow-up over a 25 year period with detailed biochemical data during the first nine weeks of life, adolescence, and early adulthood, making this investigation possible to conduct.

A potential limitation might have been that other clinical factors related to the decision to give intravenous lipids also related to long-term differences in aortic and myocardial function. Therefore, rather than a comparison of preterm-born individuals who received intravenous lipids to those who did not, I designed a nested case-control study that used the

detailed perinatal data that was available to me in the follow-up group to identify a preterm-born control group matched for potential confounding characteristics and complications including maternal history, pregnancy complications, and neonatal care [157]. This was possible because there was significant variation in clinical use of intravenous lipids between centres at the time of the study, and therefore, an infant with the same clinical characteristics may or may not have received intravenous lipids. The detail of data collection also allowed for the assessment of pregnancy history and postnatal cortisol levels to determine whether the findings may relate to differences in prenatal or postnatal glucocorticoid exposure [187]. Furthermore, I studied postnatal triiodothyronine levels to ensure that this did not account for variation in total cholesterol levels in early postnatal life or cardiovascular endpoints in young adulthood. It is possible that some individuals with selective variation in thyroxin levels or subclinical hypothyroidism, characterised by increased thyroid stimulating hormone, were not identified. Thus, it is not possible based on the data collected at birth to exclude the possibility that some of the variation in lipid levels relates to subclinical hypothyroidism in certain individuals.

The findings are consistent with the rise in endogenous cholesterol induced by the intravenous lipids being the key factor in the association rather than other parameters elevated by intravenous nutrition, such as circulating triglyceride, glucose, or phospholipid levels. Total cholesterol level was the only parameter associated with cardiovascular function and the association was heavily graded, supporting a dose effect. The analysis of the association between postnatal intravenous lipid use and the cholesterol curve over the first nine weeks of life suggests the maximum cholesterol level reflects circulating total cholesterol levels during exposure to intravenous lipids. Furthermore, this analysis suggests that this elevation in total cholesterol is transitory, with a return to baseline levels after the initial intravenous lipid exposure. Despite these close associations, it is possible that other metabolic changes induced by intravenous lipid use that were not measured, that are also exclusively reflected in the change in cholesterol level, may account for these findings. In particular, intravenous lipid administration results in an elevation of free cholesterol as a result of an exceeded capacity to clear phospholipid, likely in part due to saturation of the lecithin cholesterol acyl-

transferase pathway. This leads to a rise in cholesteryl esters but greater proportional rise in phospholipid and free cholesterol in the form of lipoprotein X in the low density lipoprotein fraction [114]. Further work is needed to determine how this alteration in lipid profile and subsequent clearance by the neonate results in long-term changes in cardiac and vascular pathophysiology.

Aortic pulse wave velocity was used as the primary endpoint as a ‘gold standard’ measurement of global aortic stiffness [182]. Aortic pulse wave velocity is known to be relevant to later cardiovascular outcome and improve cardiovascular risk prediction when combined with other cardiovascular risk factors [182; 188; 189]. Cardiovascular magnetic resonance provides an accurate and highly reproducible method for pulse wave velocity measurement [164; 185; 190]. Due to direct visualisation of the aorta, the measure accounts for aortic geometry and is unaffected by body size, in contrast to other non-invasive methods that rely on surface measures of distance between measurement sites [191]. Values of aortic pulse wave velocity depend on methodology used and vary with age. The control groups of young adults born preterm and term, not exposed to postnatal intravenous lipids, have comparable aortic pulse wave velocity measures to other similarly aged cohorts measured using cardiovascular magnetic resonance [192; 193]. In older cohorts using measures based on carotid-femoral tonometry mean values as high as 9.3 m/s are reported [182]. Preterm-born young adults who received intravenous lipids were identified as having a 1.32 m/s (1.47 standard deviations) greater aortic pulse wave velocity than preterm-born young adults who did not receive postnatal intravenous lipids or the term-born young adult controls. Outcome data in relation to aortic pulse wave velocity is not yet available based on measures performed in young adulthood. Nonetheless, if this magnitude of difference is maintained into later life, we know from studies based on other methods for assessment of pulse wave velocity that a 1 m/s increase in aortic pulse wave velocity relates to a 14% age and sex adjusted increase in total cardiovascular events in middle-aged and elderly individuals [194]. The difference in aortic pulse wave velocity in young adulthood identified between preterm-born individuals that did and did not receive intravenous lipids in early postnatal life is greater than that associated with other risk factors such as obesity [195] and smoking [185].

Aortic stiffness is biologically the most plausible intermediate cardiovascular phenotype to alter long-term following exposure to lipids during this critical period of development [188]. The most significant changes in aortic stiffness were found to be in the abdominal aorta. Human post mortem studies have demonstrated that the abdominal aorta is one of the sites with the greatest amount of lipid deposition [120; 121]. Although fatty streaks tend to regress after the first year of life, the data in this study would be consistent with a hypothesis that the increased cholesterol evident within the abdominal aortic wall during this critical period of development has a long-term selective impact on this region of the aorta. As previously mentioned, the postnatal period in these preterm-born individuals represents a particularly sensitive period of aortic development that normally occurs during the last trimester. During this time proportions of elastin and collagen, relevant to arterial stiffness, are established within the aortic wall [82]. Lipid exposure, and particularly cholesterol exposure, has been shown to downregulate elastin and collagen cross-linking *in vitro* in animal cell models [196]. Furthermore, Massaeli *et al.* [197] showed that cholesterol negatively impacts the organisation of actin and myosin in rabbit aortic vascular smooth muscle cells, relevant to contractility. Thus, it is reasonable to speculate that postnatal exposure to intravenous lipids and the resultant endogenous elevation in cholesterol levels in humans could have a similar impact on the development of the aorta. Alternatively, the lipid exposure during this critical period may have a direct programming effect on lipid metabolism that leads to chronic changes in ability to handle a lipid load and persistently elevated postprandial cholesterol levels. Unfortunately, data on postprandial lipids in later life was not collected, and thus I am not able to exclude this possibility. As such, a valid alternative hypothesis would be that the changes in aortic and left ventricular function are the result of this chronic, rather than acute, damage. Aortic stiffness is of particular relevance to blood pressure [198; 199], and the trend towards higher blood pressure in the group of preterm-born young adults who received intravenous lipids in early postnatal life may be an early indicator of changes that will amplify over time.

The finding of a relation between abnormally elevated lipid levels in early postnatal life and left ventricular function in young adulthood in humans is intriguing. Acute direct effects

of a high lipid diet on left ventricular function have been reported in several animal models [122; 123; 200]. Whether such insults have long-term effects, and are particularly influential during early stages of cardiac development, has not been studied. I was able to include myocardial tagging as a sensitive marker of early changes in myocardial function in a proportion of the young adults. A graded association was demonstrated between intravenous lipid dose and length of exposure postnatally with a reduction in left ventricular circumferential peak systolic apical strain. As with the aortic measures, the associated elevation in cholesterol secondary to intravenous lipid infusion appeared to be the main mediator of the association and was related in a graded manner to apical systolic strain. This association was independent of that between aortic stiffness and left ventricular strain. The perinatal stage is important for the increase in expression of cardiac specific sarcoplasmic reticulum calcium ATPase and α and β myosin heavy chain contractile proteins (fast and slow contractile ATPase activity, respectively). Huang *et al.* [122] showed that rabbits fed a high lipid diet had a significant reduction in myocyte systolic contractile function along with a downregulation of cardiac specific sarcoplasmic reticulum calcium ATPase expression and a large increase in the ratio of myosin heavy chain β to α myosin heavy chain expression in the rabbit myocyte. They concluded that altered calcium handling and myocyte heavy chain expression explained the reduced myocyte contractility in the group fed the lipid rich diet. Ónody *et al.* [200] have presented similar findings and it will be of interest to determine whether, in humans, intravenous lipid exposure during critical periods of early postnatal life have a similar negative impact on cardiac myocytes.

Cholesterol levels as early as the first few months of life are known to reflect cholesterol levels in later life [186; 201], and this study demonstrates that a measure of minimum cholesterol within days of birth can predict fasting cholesterol levels in young adults. However, intravenous lipids led to a significantly higher level of total cholesterol than in the preterm-born young adults who did not receive intravenous lipids, and the change in cholesterol was independent of native cholesterol levels in adolescence and young adulthood. Further work is also required to understand the relevance of these findings to the narrower nutritional variation in cholesterol in normal neonatal life. The data showed a graded association be-

tween neonatal maximum cholesterol level and later cardiovascular function that extended throughout the range of cholesterol levels. However, although there was a positive graded association in the group of preterm-born young adults who did not receive postnatal intravenous lipids, this relationship did not reach significance. The variation in aortic stiffness in the group of preterm-born young adults who did not receive postnatal intravenous lipids was smaller, and similar to the levels in the term-born young adult control group. A larger study will be needed to explore the impact of normal ranges of cholesterol in neonatal life on aortic function. The nutritional status of the pregnant mother may also be relevant for the wider population of term-born individuals as the period during which cholesterol was modified in the preterm-born individuals relates to a stage of aortic growth and development that usually occurs *in utero* during the last trimester [82; 121].

5.5 Conclusion

In conclusion, the work in this chapter demonstrates that intravenous lipid administration leads to an artificial elevation of total cholesterol levels in immediate postnatal life in infants born preterm, which is associated with long-term changes in aortic and left ventricular function proportional to the degree of cholesterol elevation. The preterm neonatal period is particularly sensitive and there is significant public health interest in the best management of these infants. The public health relevance of the findings in the wider population also need to be understood as, for the first time, it has been demonstrated that nutrition high in lipids during sensitive periods of early human development has a long-term negative impact on the cardiovascular system.

6

ANTENATAL STEROIDS, AORTIC STIFFNESS, AND GLUCOSE METABOLISM

This chapter was designed to demonstrate the impact of antenatal glucocorticoid exposure on aortic stiffness in young adults born preterm. To do this, I created a nested case-control study within the cohort of young adults born preterm (*cohort 1*), based on a similar concept as I used in Chapter 5 to investigate the long-term cardiovascular impact of intravenous lipids given in early postnatal life. This nested case-control design was used to ensure similar perinatal and adult characteristics between cases and controls. In addition, 95 term-born adults (from *cohort 2* and *cohort 3*) were included to create a normal ageing profile for the aortic measures. Cardiovascular magnetic resonance imaging was used to assess aortic and left ventricular function. Antenatal glucocorticoid exposure is associated with a localised increase in aortic arch stiffness, similar in magnitude to term-born individuals a decade older. The change in aortic arch stiffness does not relate to changes in glucose metabolism that are also evident in these individuals. I have published this work in *Pediatrics* as a joint first author [160].

6.1 Introduction

Glucocorticoids are potent regulators of growth and development. In animal studies, transient glucocorticoid exposure, during critical windows of fetal life, results in long-term alterations in elastin synthesis [202], vascular function [203; 204; 205; 206], baroreceptor function [207], sympathetic outflow, hypothalamic pituitary adrenal axis, nephrogenesis, the renin angiotensin system, and glucose homeostasis [208]. Whether *in utero* exposure to glucocorticoids has similar long-term effects in humans has, traditionally, been difficult to study experimentally. However, preterm birth is a unique clinical scenario in which a range of interventions, including antenatal glucocorticoids, are administered during a critical developmental period [209; 210; 211; 212; 126]. To date, brachial arterial pressure is the only vascular measure that has been assessed in young adults exposed to antenatal glucocorticoids, with no evidence of blood pressure elevation [131]. However, it is likely that cuff sphygmomanometry does not provide a sensitive or specific enough measurement of vascular function for the expected changes within this population group. Animal models have demonstrated that the aorta is the site most affected, whereby elastin deposition within the aortic wall is critically altered by glucocorticoid exposure in late gestation [92]. Therefore, I have used the preterm-born cohort to test the specific hypothesis that *in utero* glucocorticoid exposure in humans leads to long-term alterations in aortic function. Furthermore, I have determined the relevance of alterations in glucose metabolism, previously observed in preterm-born young adults exposed to antenatal glucocorticoids [131], to this association. The degree of aortic stiffening was then related to that observed during ageing in the normal population to gauge the relative impact of antenatal glucocorticoid exposure on long-term cardiovascular risk for those born preterm.

In addition, because animal models have also shown an early switch from a myocardial hyperplastic to hypertrophic growth phase following intravenous administration of cortisol [133], I also investigated whether antenatal glucocorticoids had effects beyond the vasculature and used cardiovascular magnetic resonance to study long-term alterations in left ven-

tricular structure and function.

6.2 Methods

6.2.1 Study population

This chapter includes a subgroup of the young adults born preterm (*cohort 1*). The mothers of 16 preterm-born young adults received antenatal glucocorticoids. Since antenatal glucocorticoid use varied significantly between recruitment centres (Table 6.1), consistent with local practice in the 1980s, it was possible to frequency match these individuals to 32 preterm-born young adults whose mothers had not received antenatal glucocorticoids, in a nested case-control design (Figure 6.1). Frequency matching was performed based on the variables listed in Table 6.2.

Table 6.1: Percentage of Individuals at the Five UK Centres from the Original Birth Cohort Exposed to Antenatal Glucocorticoids

Centre	Original cohort (n = 926)
1	6.57%
2	21.8%
3	7.58%
4	31.8%
5	11.0%

In addition, 95 term-born individuals aged 20 to 39 years (*cohort 2* and *cohort 3*) were included to provide age stratified, normal ranges for the aortic outcome measures.

6.2.2 Study Visit

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity and blinded analysis. The following methods were used for this chapter as described in detail in Chapter 3:

- 3.2: Cardiovascular Magnetic Resonance

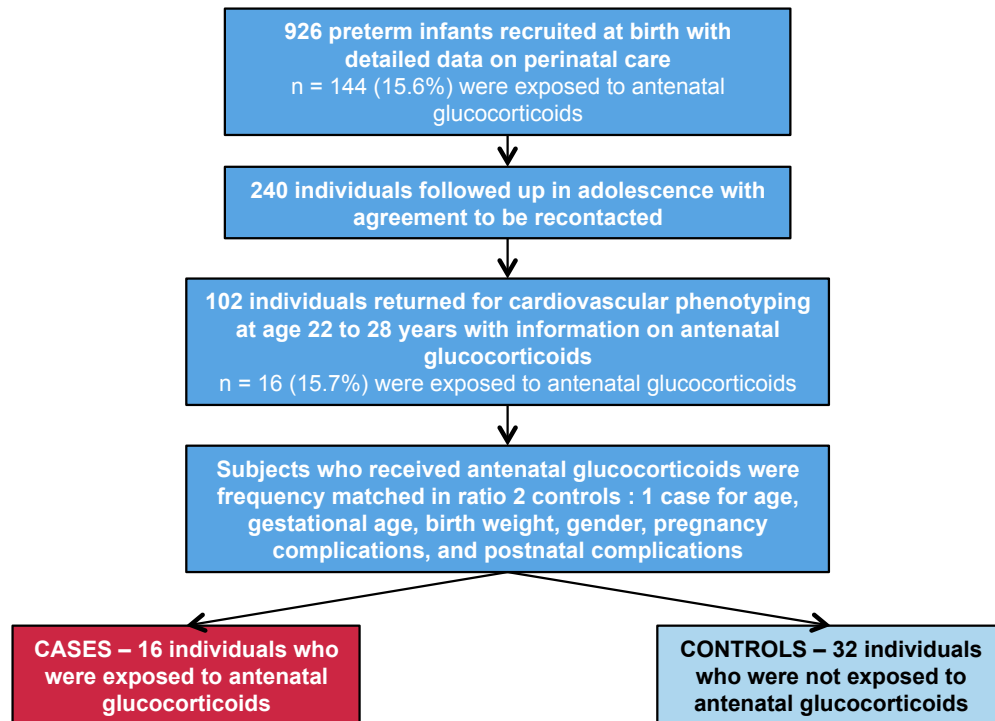


Figure 6.1: Preterm study cohort. Two hundred and forty individuals with agreement to be recontacted from a cohort of 926 individuals (birth weight less than 1,850 g) were invited for cardiovascular follow-up in young adulthood. Of these, 102 agreed to undergo detailed cardiovascular phenotyping. Of these, 16 individuals had been exposed to antenatal glucocorticoids and were matched in a 1:2 ratio based on early life characteristics with 32 who were not.

3.2.1: Aortic Imaging

3.2.1.1: Aortic Pulse Wave Velocity

3.2.1.2: Aortic Distensibility

3.2.2: Cardiac Imaging

3.2.2.1: Cardiac Cine Acquisition

3.2.2.2: Left Ventricular Mass and Volumes

- 3.4: Anthropometry and Lifestyle Questionnaire
- 3.5: Blood Sampling and Biochemistry
- 3.7: Blood Pressure Measurements

Table 6.2: Birth, Parental and Neonatal Characteristics of Preterm-Born Young Adults Exposed and Not Exposed to Antenatal Glucocorticoids

	No Glucocorticoids (n = 32)	Glucocorticoids (n = 16)	P Value
Birth Characteristics			
Males, n (%)	16 (50.0)	8 (50.0)	>0.99
Birth weight, grams	1241.9 ± 250.5	1178.0 ± 283.8	0.43
Birth weight Z score	-0.48 ± 1.08	-0.58 ± 0.85	0.74
Gestational age, weeks	29.3 ± 1.9	28.9 ± 1.6	0.46
Apgar Score at 5 min	7.25 ± 2.65	6.63 ± 2.19	0.42
Spontaneous Delivery, n (%)	23 (71.9)	12 (75.0)	0.82
Pregnancy Characteristics			
Gestational Hypertension	1 (3.13)	0 (0)	0.48
Preeclampsia	7 (21.9)	3 (18.8)	0.8
Maternal Smoking, n (%)	6 (18.8)	3 (18.8)	>0.99
Paternal Smoking, n (%)	11 (34.4)	2 (12.5)	0.11
Postnatal Complications			
Oxygen Supplementation ≥30%, n (%)	23 (71.9)	11 (68.8)	0.88
Length of Oxygen therapy, days	8.34 ± 12.3	9.38 ± 11.15	0.78
Incidence of Ventilation, n (%)	20 (62.5)	11 (68.8)	0.67
Duration of ventilation, days	6.15 ± 3.8	6.18 ± 5.27	0.81
Pneumonia, n (%)	7 (21.9)	3 (18.8)	0.80
Culture positive septic episodes, n (%)	5 (15.6)	4 (25.0)	0.43

Values as Mean ± Standard Deviation unless stated otherwise.

6.2.3 Statistical analysis

Statistical analysis was carried out using SPSS Version 19 as described in Section 3.9 of Chapter 3. As there were 16 preterm-born young adults exposed to antenatal glucocorticoids in the cohort, it was determined that frequency matching one case to two preterm-born young adult controls not exposed to antenatal glucocorticoids would provide 80% power, at $P = 0.05$, to identify a 0.74 standard deviation difference between groups, equivalent to a 0.80 m/s change in aortic pulse wave velocity and $2.60 \text{ mmHg}^{-1} \times 10^{-3}$ change in aortic arch distensibility.

6.3 Results

6.3.1 Study population

Table 6.2 demonstrates that there were no significant differences in early life characteristics between the young adults born preterm that were and were not exposed to antenatal glucocorticoids. Furthermore, as the preterm-born individuals had been randomised to milk feeding regimes at birth, it was possible to ensure that other dietary intakes that may have affected the results were equivalent between the preterm-born young adults that did and did not receive intravenous lipids (Table 6.3). There were also no differences in weight gain in the first year of life between the preterm-born groups ($P = 0.60$). In early adult life, there were no significant differences between groups in age (Table 6.4), family cardiovascular medical history, personal medical history, or lifestyle ($P > 0.05$). The control group of 95 term-born individuals consisted of 39 (41%) males, had an average age of 28 years (range: 20 to 39 years), mean birth weight of 3365.2 ± 494.5 g, and mean gestational age of 39.6 ± 0.9 weeks.

Table 6.3: Number of Individuals Randomised to Banked Breast Milk or Formula in the Preterm-Born Young Adult Groups

	No Glucocorticoids (n = 32)	Glucocorticoids (n = 16)	P Value
Fed Banked Breast Milk, n (%)	6 (18.8%)	4 (25.0%)	0.62
Fed Formula Milk, n (%)	26 (81.3%)	12 (75.0%)	

6.3.2 Antenatal Glucocorticoids and Cardiovascular Risk Factors

There were no significant differences in anthropometry, blood pressure, left ventricular or lipid profile measures between preterm-born young adults exposed and not exposed to antenatal glucocorticoids (Table 6.4).

There was a significant reduction in β cell function (HOMA-B) in preterm-born young adults exposed to antenatal glucocorticoids ($P = 0.02$) (Figure 6.2, Panel C), with lower insulin levels ($P = 0.04$) and higher glucose levels ($P = 0.08$) (Figure 6.2, Panels A and B). As information on fasting glucose and insulin was available for a proportion of subjects (seven

Table 6.4: Baseline Characteristics of Preterm-Born Individuals in Young Adulthood

	No Glucocorticoids (n = 32)	Glucocorticoids (n = 16)	P Value
Demographics			
Age (years)	24.9 ± 1.3	25.3 ± 0.9	0.31
Males, n (%)	16 (50)	8 (50)	>0.99
Smokers, n (%)	5 (15.6)	3 (18.8)	0.75
Body Mass Index (kg/m ²)	23.9 ± 3.9	23.3 ± 2.32	0.51
Lipid Profile (mmol/L)			
Total Cholesterol	4.76 ± 0.70	5.23 ± 1.06	0.09
HDL-C	1.54 ± 0.35	1.66 ± 0.47	0.36
LDL-C	2.70 ± 0.75	3.00 ± 0.99	0.27
Triglycerides	0.93 ± 0.45	0.93 ± 0.28	>0.99
Peripheral Blood Pressure (mmHg)			
Systolic	116.5 ± 11.4	120.8 ± 10.2	0.21
Diastolic	70.6 ± 9.0	71.6 ± 6.3	0.68
Mean Arterial	85.8 ± 8.8	88.0 ± 6.5	0.39
Pulse Pressure	45.7 ± 9.6	49.1 ± 9.4	0.25
Central Blood Pressure (mmHg)			
Systolic	106.5 ± 10.5	108.4 ± 8.4	0.54
Diastolic	74.9 ± 7.8	73.7 ± 6.4	0.58
Mean Arterial	85.5 ± 8.2	85.3 ± 6.7	0.93
Pulse Pressure	31.6 ± 6.5	34.7 ± 4.9	0.10
Left Ventricular Measurements			
LV Mass (g/m ²)	61.1 ± 9.0	62.9 ± 11.2	0.56
Ejection Fraction (%)	61.7 ± 6.2	64.6 ± 4.7	0.13
Cross-sectional Aortic Areas (cm²)			
Ascending Aorta	3.91 ± 0.81	3.92 ± 0.89	0.98
Proximal Descending Aorta	2.18 ± 0.50	2.16 ± 0.45	0.94
Distal Descending Aorta	1.68 ± 0.35	1.69 ± 0.40	0.93
Aortic Lengths (cm)			
Aortic Arch	8.20 ± 1.07	8.26 ± 0.82	0.84
Total	19.0 ± 2.4	19.3 ± 1.0	0.67

HDL-C indicates high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LV, left ventricular. Values as Mean ± Standard Deviation unless stated otherwise.

cases and 15 controls) from the follow-up at age 15 years, I also investigated whether similar trends were evident in adolescence. The difference in glucose levels was significant in adolescence ($P = 0.03$) with a difference in HOMA-B of the same magnitude; although this did not reach significance ($P = 0.20$). At this age, levels of insulin were similar between preterm-born individuals that were and were not exposed to antenatal glucocorticoids. There were no differences in insulin sensitivity at either age point (HOMA-S) (Figure 6.2, Panel D).

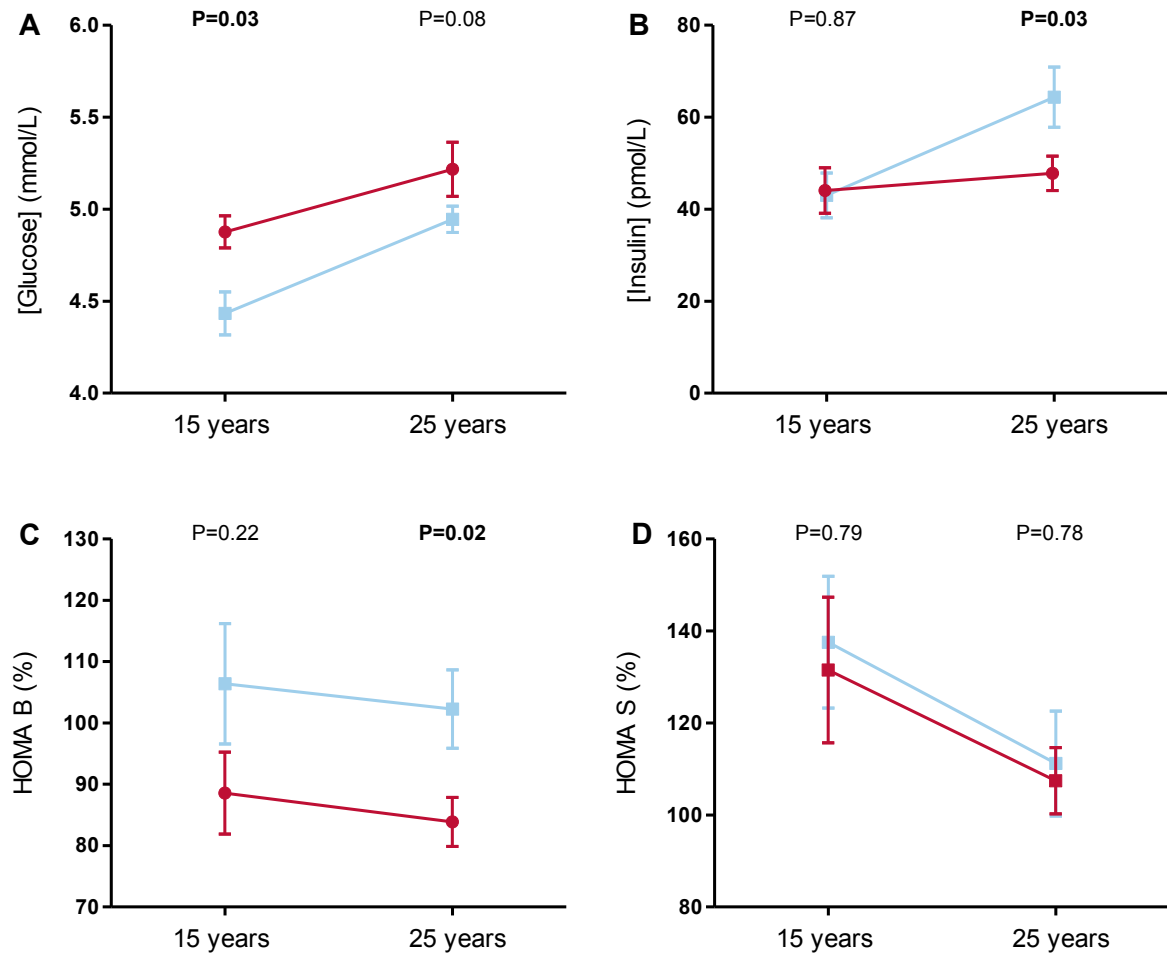


Figure 6.2: Glucose homeostasis in individuals born preterm at age 15 years and age 25 years. **A**, Glucose. **B**, Insulin. **C**, HOMA-B. **D**, HOMA-S. Values shown for preterm-born individuals who were exposed to antenatal glucocorticoids (red) or were not exposed to antenatal glucocorticoids (blue). Data shown as mean $\pm$ standard error of the mean. Preterm-born young adults exposed to antenatal glucocorticoids and not exposed to antenatal glucocorticoids compared at age 25 years ($n = 16$ and $n = 32$, Glucocorticoids and No Glucocorticoids) and 15 years ($n = 7$ and $n = 15$, Glucocorticoids and No Glucocorticoids) with an independent samples Student's t -test ($P < 0.05$, bold).

6.3.3 Antenatal Glucocorticoids and Aortic Stiffness

I then studied whether antenatal glucocorticoid exposure was associated with variation in aortic stiffness. There was no significant difference in global aortic pulse wave velocity between the preterm-born individuals that were and were not exposed to antenatal glucocorticoids in young adulthood ($P = 0.25$). However, aortic pulse wave velocity was 1.02 m/s faster in the arch of the aorta in young adulthood in those exposed to antenatal glucocorticoids ($P = 0.006$) (Figure 6.3, Panel A). This regional increase in aortic stiffness was confirmed by aortic distensibility measures. There was a significant reduction in aortic arch distensibility (9.88 ± 3.21 vs 13.6 ± 3.9 mmHg⁻¹ x 10³, $P = 0.002$) and preserved levels more distally along the aorta (Figure 6.3, Panel B). These results could not be explained by variation in aortic geometry, including regional aortic cross-sectional areas and lengths (Table 6.4). I also looked at the flow profiles at the different sections of the aorta by averaging the individual data for each group separately to ensure there were no differences in the upslope gradient that may have modified the ability to accurately identify the intercept. The gradient and shape of the curves were the same in both groups of preterm-born individuals, with the only difference being the earlier arrival of the pulse wave at the proximal descending aorta in preterm-born young adults exposed to antenatal glucocorticoids. This finding was consistent with the calculated aortic pulse wave velocity measures (Figure 6.4).

6.3.4 Antenatal Glucocorticoids and Aortic Ageing

The levels of aortic stiffness in the preterm-born young adults were then compared to those observed in individuals born full term. In the normal population, as age increases, there is a proportional increase in aortic stiffness in the ascending aorta and aortic arch [213]. Therefore, age was plotted against ascending aortic distensibility for the 95 subjects born term to uncomplicated pregnancies aged 20 to 39 years (Figure 6.5). It was demonstrated that there was a significant, inverse, linear relationship between age and aortic arch distensibility ($r = -0.48$, $P < 0.001$). Based on this standard association, I used the mean and standard error of the mean of aortic arch distensibility observed in the two preterm-born groups to interpo-

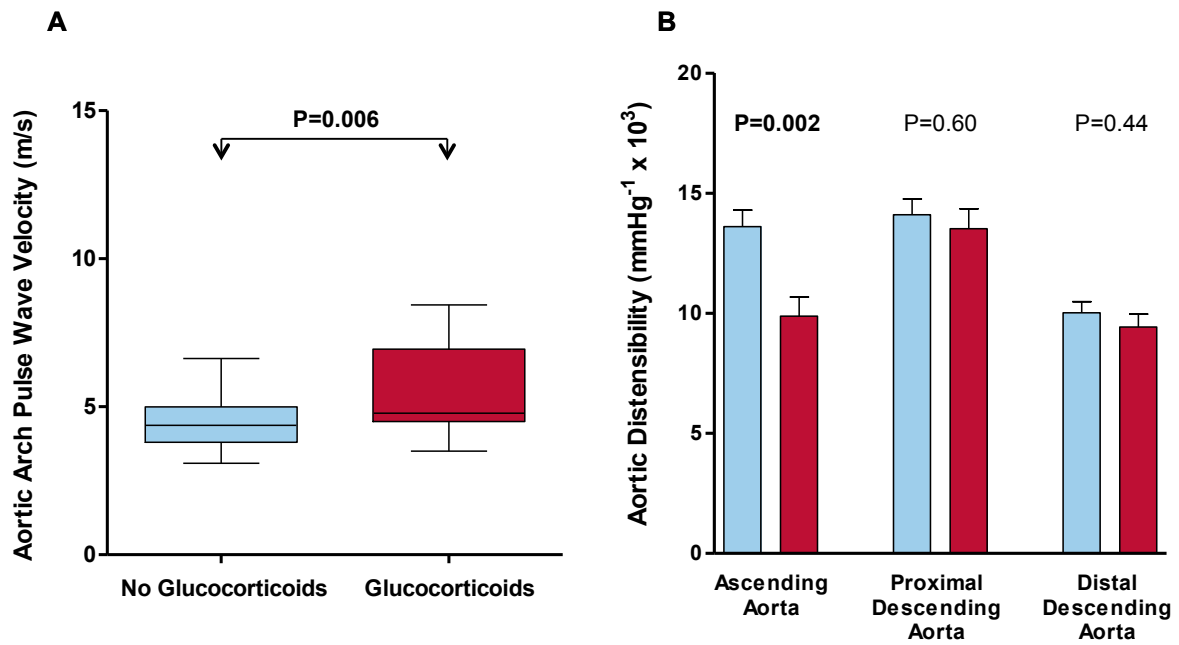


Figure 6.3: Increased aortic stiffness in preterm-born young adults exposed to antenatal glucocorticoids. **A**, Aortic arch pulse wave velocity for preterm-born young adults who were not exposed to antenatal glucocorticoids ($n = 32$) and those that were exposed to antenatal glucocorticoids ($n = 16$). Aortic arch pulse wave velocity was log transformed for normal distribution. Aortic arch pulse wave velocity was significantly higher in the group exposed to antenatal glucocorticoids (5.45 ± 1.41 vs 4.47 ± 0.91 m/s, $P = 0.006$). Data shown as box and whisker: minimum to maximum. **B**, Regional differences in aortic distensibility. Ascending aortic distensibility was greatly reduced in the group exposed to antenatal steroids (9.88 ± 3.21 vs 13.6 ± 3.9 $\text{mmHg}^{-1} \times 10^3$, $P = 0.002$), though there were no differences in stiffness in the proximal descending aorta or distal descending aorta. Data shown as mean $\pm$ standard error of the mean.

late ‘aortic ages’. For those not exposed to antenatal glucocorticoids, the interpolated aortic age was 22.2 ± 1.9 years, similar to the actual mean age of the preterm cohort. For those exposed to antenatal glucocorticoids, the interpolated aortic age was nearly ten years greater than their chronological age (32.6 ± 2.2 years) (Figure 6.5).

6.3.5 Glucose Homeostasis, Aortic Stiffness, and Antenatal Glucocorticoids

Glucose homeostasis variables were correlated with both ascending aorta distensibility and aortic arch pulse wave velocity (HOMA-B and aortic arch pulse wave velocity, $r = -0.34$, $P = 0.03$; aortic arch distensibility, $r = 0.31$, $P = 0.05$) within the preterm-born individuals. Regression analyses were therefore performed to determine the independence of the association

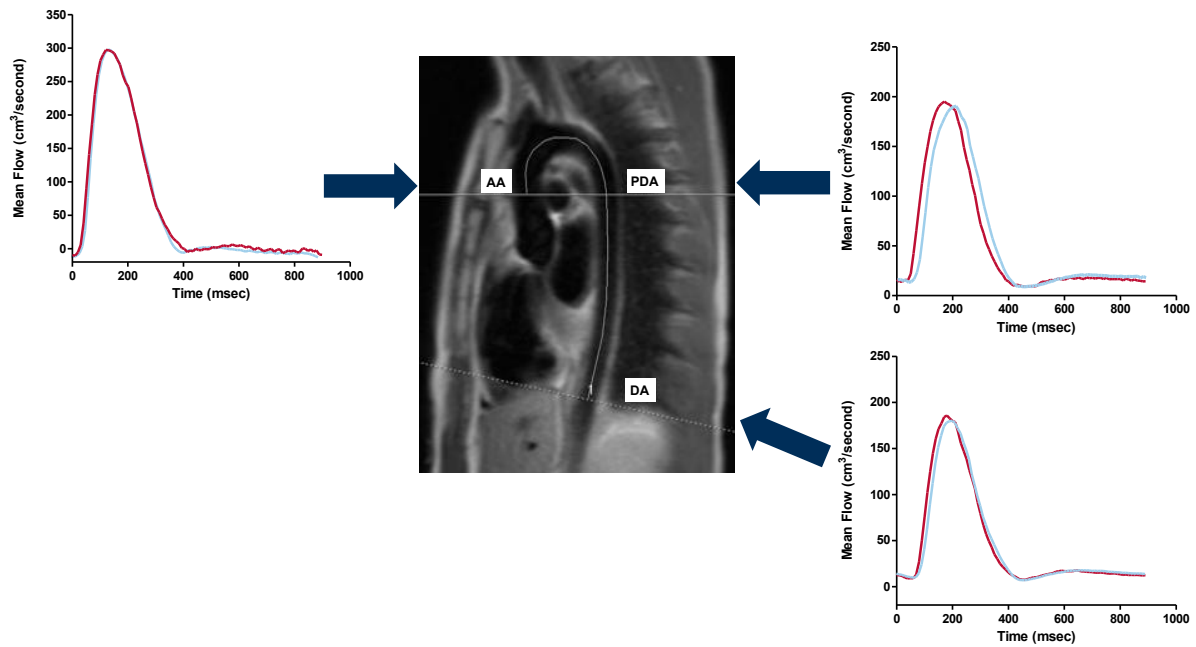


Figure 6.4: Oblique sagittal image of the aorta with regional flow profiles. Aortic pulse wave velocity was measured from the ascending aorta (AA) to the proximal descending aorta (PDA) and distal descending aorta (DA). Aortic distensibility was measured at the AA, PDA and DA. Aortic distensibility = $\{ [(\text{maximum area of the aorta} - \text{minimum area of the aorta}) / (\text{minimum area of the aorta})] / \text{central pulse pressure} \} \times 10^3$. Flow profiles are shown at the level of the AA, PDA, and DA represent the average levels of flow during the cardiac cycle for preterm-born young adults exposed to antenatal glucocorticoids (red) and preterm-born young adults that were not exposed to antenatal glucocorticoids (blue).

between antenatal glucocorticoids and aortic stiffness from other factors.

Five variables achieved a correlation with ascending aorta distensibility with a P value <0.05 and were included in the regression model (Table 6.5). Using forced entry regression with sex, gestational age, high density lipoprotein cholesterol, antenatal glucocorticoid exposure, and systolic blood pressure as the independent variables, antenatal glucocorticoid exposure remained as an independent predictor of ascending aorta distensibility ($B = -3.39 \text{ mmHg}^{-1} \times 10^3/\text{group}$, 95% confidence interval: -5.36 to -1.42 , $P = 0.001$). Antenatal glucocorticoid exposure was a dichotomous categorical variable with preterm-born young adults not exposed to antenatal glucocorticoids as '1' and those exposed to antenatal glucocorticoids as '2'.

Using the same method for aortic arch pulse wave velocity, six variables achieved a correlation with aortic arch pulse wave velocity with a P value <0.05 and were included in the

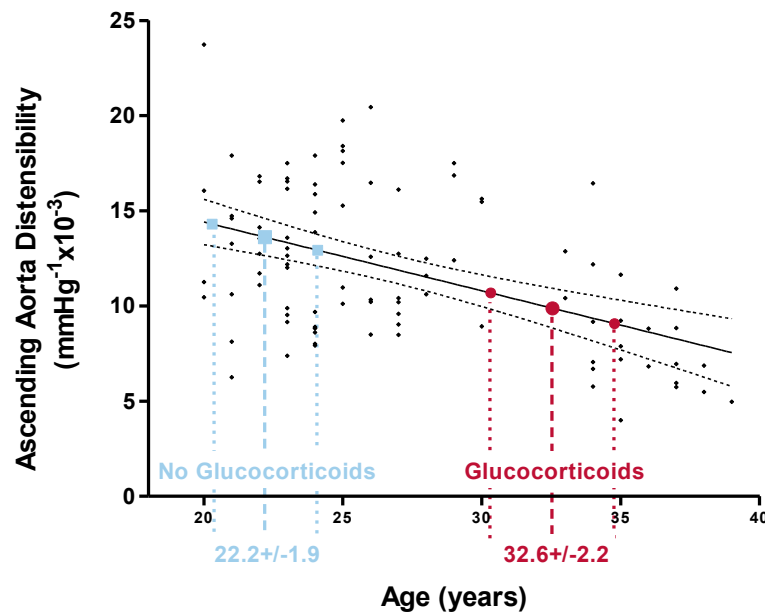


Figure 6.5: Interpolated aortic ‘age’ of preterm-born young adults who were exposed to antenatal glucocorticoids and those who were not. Linear regression was used to plot Age vs Aortic Distensibility (black line, with 95% confidence intervals) for a cohort of 95 term-born controls recruited at age 20 to 39 years who underwent the same experimental protocol as preterm-born individuals. Data points for term-born individuals are shown as small black diamonds. Fit line with 95% confidence intervals as shown ($r = -0.48$, $P < 0.001$). The group mean ascending aorta distensibility $\pm$ standard error of the mean was used to interpolate age from the standard curve for preterm-born young adults who were exposed to antenatal glucocorticoids (red) or were not exposed to antenatal glucocorticoids (blue). Based on their ascending aortic distensibility, this model estimated the age of the preterm-born young adults not exposed to antenatal glucocorticoids as 22.2 ± 1.9 years. For the preterm-born young adults exposed to antenatal glucocorticoids, the aortic ‘age’ was estimated to be 32.6 ± 2.2 years.

regression model (Table 6.6). However, as three of these were blood pressure parameters, which were closely interrelated, only the most strongly related blood pressure parameter (systolic blood pressure) to aortic arch pulse wave velocity was included in the regression model. Using forced entry regression with birth weight Z score, antenatal glucocorticoid exposure, systolic blood pressure, and HOMA-B as the independent variables, antenatal glucocorticoid exposure remained as an independent predictor of aortic arch pulse wave velocity ($B = 0.062 \log(\text{m/s})/\text{group}$, 95% confidence interval: 0.0053×10^{-3} to 0.12 , $P = 0.03$). This means that there was a $10^{0.062}$ fold (or 15.3%) increase in aortic arch pulse wave velocity in young adulthood if exposed to antenatal glucocorticoids.

Table 6.5: Bivariable Regression Associations with Ascending Aorta Distensibility

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Sex	3.65	1.54	5.77	0.001
Gestational Age	0.69	0.047	1.33	0.04
Birth Weight Z score	-0.24	-1.44	0.97	0.69
Newborn Days on Ventilation	-0.098	-0.36	0.17	0.46
Antenatal Glucocorticoid Exposure	-3.74	-6.00	-1.48	0.002
Age	-0.10	-1.10	0.90	0.84
Smoking	-0.71	-3.90	2.47	0.65
Body Mass Index	-0.027	-0.38	0.32	0.88
Waist:Hip Ratio	-9.97	-22.0	2.07	0.10
Systolic Blood Pressure	-0.11	-0.21	-0.007	0.04
Diastolic Blood Pressure	-0.076	-0.20	0.048	0.23
Pulse Pressure	-0.055	-0.16	0.053	0.06
Mean Arterial Pressure	-0.12	-0.26	0.012	0.07
Total Cholesterol	0.31	-0.91	1.52	0.61
HDL Cholesterol	4.02	1.06	6.98	0.009
LDL Cholesterol	0.21	-1.04	1.46	0.74
Triglycerides	-1.11	-2.52	0.31	0.12
Insulin	0.034	-0.0036	0.071	0.08
Glucose	0.59	-2.31	3.50	0.68
HOMA-B	0.022	-0.014	0.057	0.22
HOMA-S	-0.020	-0.043	0.0031	0.09

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; HOMA-B, Homeostasis Model Assessment β cell function; HOMA-S, Homeostasis Model Assessment insulin sensitivity.

6.4 Discussion

This study demonstrates for the first time that young people born preterm, whose mothers received antenatal glucocorticoids, have a regional increase in aortic stiffness in the ascending aorta and aortic arch. Furthermore, they have changes in glucose metabolism, which are independent of the impact of antenatal glucocorticoids on aortic function.

Aortic stiffness predicts future development of hypertension and is an independent predictor of coronary artery disease, stroke, and mortality [214]. In older adults, a 1 m/s increase in pulse wave velocity relates to a 14% increase in total cardiovascular events [194]. This magnitude of increase in pulse wave velocity is similar to that identified in this study in aortic arch pulse wave velocity between young adults born preterm that were and were not exposed to antenatal glucocorticoids. While there are no studies of aortic stiffness in young populations with long enough follow-up to provide outcome data, increased availability of

Table 6.6: Bivariable Regression Associations with Aortic Pulse Wave Velocity

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Sex	-0.027	-0.085	0.031	0.36
Gestational Age	-0.0047	-0.021	0.012	0.57
Birth Weight Z score	-0.028	-0.056	0.0011	0.05
Newborn Days on Ventilation	-0.0049	-0.011	0.0015	0.13
Antenatal Glucocorticoid Exposure	0.082	0.024	0.14	0.006
Age	0.0092	-0.015	0.034	0.46
Smoking	0.043	-0.035	0.12	0.27
Body Mass Index	0.0022	-0.0065	0.011	0.62
Waist to Hip Ratio	0.26	-0.037	0.56	0.08
Systolic Blood Pressure	0.0037	0.0012	0.0062	0.004
Diastolic Blood Pressure	0.0031	4.20×10^{-5}	0.0061	0.05
Pulse Pressure	0.0015	-0.0012	0.0041	0.27
Mean Arterial Pressure	0.0045	0.0013	0.0077	0.007
Total Cholesterol	-0.0025	-0.033	0.028	0.87
HDL Cholesterol	-0.046	-0.12	0.032	0.24
LDL Cholesterol	0.0026	-0.028	0.034	0.87
Triglycerides	-7.76	-0.044	0.028	0.67
Insulin	-0.00068	-0.0016	0.00026	0.15
Glucose	0.059	-0.011	0.13	0.10
HOMA-B	-0.00098	-0.0018	-0.0014	0.02
HOMA-S	0.00017	-0.00043	0.0076	0.57

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; HOMA-B, Homeostasis Model Assessment β cell function; HOMA-S, Homeostasis Model Assessment insulin sensitivity.

imaging techniques such as cardiovascular magnetic resonance has demonstrated that ageing during the first decades of life is characterised by increases in ascending aortic stiffness [214; 215]. This phenomenon was also demonstrated in the group of term-born individuals. As such, this data was used to determine that preterm-born individuals whose mothers received antenatal glucocorticoids had levels of aortic stiffness equivalent to adults in their thirties.

Changes in vascular structural matrix proteins are a key determinant of vascular ageing [216], and the third trimester, when antenatal glucocorticoids are given in human pregnancy, is the period of most rapid aortic remodeling during life [82]. At this time, there is accelerated arterial elastin accumulation and cross-linking [92; 217], resulting in an acute increase in aortic wall stiffness and aortic pulse wave velocity [82; 218]. This is thought to be a preadaptation to the dramatic increase in newborn blood flow after cessation of placental blood flow and initiation of pulmonary gas exchange. In animal models, antenatal glucocor-

ticoids modified this maturation process and increased the elastin precursor, tropoelastin, in the fetal aorta [92]. Furthermore, antenatal glucocorticoids also enhanced the expression of lysyl oxidase; an enzyme critical in crosslinking elastin and collagen [219]. However, Doppler studies showed profound increases in fetal blood flow sustained for up to 96 hours following glucocorticoid exposure [220]. Differential flow exposure during development could lead to disparity in aortic geometry or upper body vessel formation, which would complicate comparisons of aortic stiffness [213]. However, there were no variations in aortic flow patterns or geometry between the preterm-born groups in this study. As increased flow also drives elastin accumulation, the observations would be consistent with specific effects of glucocorticoids on elastin maturation. The histological structure of the aorta varies along its length, allowing it to function as a reservoir and conductive vessel to turn pulsatile into laminar flow [86], and the ascending aorta is richest in elastin [221; 222]. In Chapter 5, I demonstrated that postnatal intravenous lipid exposure in preterm-born individuals had a selective impact on the distal aorta. This region is preferentially associated with lipid accumulation in the arterial wall in neonatal life [160] and, together, these observations highlight the value of detailed cardiovascular phenotyping to disentangle complex interactions between interventions and aortic development.

Infants whose mothers received antenatal glucocorticoids have a lower incidence of hypotension and decreased need for pressor support postnatally [223; 224], consistent with a glucocorticoid-induced accelerated maturation of aortic structure. A key advantage of this maturation is that it optimises central haemodynamic function and preserves myocardial and cerebral perfusion pressure in neonates born preterm. In the current study, there were no differences in left ventricular measures related to whether or not subjects were exposed to antenatal glucocorticoids. However, once these individuals reach later adult life, the degree of increase in aortic stiffness observed is likely to have an adverse cardiovascular effect. Longer term cardiovascular follow-up in this cohort will therefore be of interest.

Those exposed to antenatal glucocorticoids had a specific reduction in β cell function. A follow-up study of adults aged 30 years whose mothers took part in a randomised controlled trial of antenatal betamethasone [131] also demonstrated differences in glucose metabolism

in response to an oral glucose tolerance test, with higher plasma insulin levels in those exposed to glucocorticoids. Oral glucose tolerance tests were not performed in this study, but the findings in young adult life were replicated based on samples collected ten years earlier. The mean gestational age in the preterm-born cohort was 29 weeks, whereas in the previous study around 30% of individuals were born term. The impact of glucocorticoid exposure on glucose metabolism may vary with gestational period as glucocorticoid signalling is most relevant to pancreatic islet formation earlier in fetal development [225]. Further work will be able to define whether effects vary dependant on dose and time of glucocorticoid administration.

A significant strength of this study is the prospective collection of maternal and perinatal data, which allowed for the identification of a precisely defined cohort of preterm-born individuals exposed to antenatal glucocorticoids. Although the study is the largest prospective follow-up study of preterm-born individuals with this level of detail on cardiovascular phenotyping in young adulthood, the proportion exposed to antenatal glucocorticoids is relatively small and they were not randomised. However, at the time of recruitment, use varied significantly between centres. I was therefore able to frequency match cases to controls with equivalent perinatal characteristics, which would not be possible in younger cohorts due to widespread use of antenatal glucocorticoids. In the late 1980s and throughout the 1990s, postnatal glucocorticoids were also used in the United Kingdom to treat preterm-born infants with bronchopulmonary dysplasia [226]. It is possible that postnatal glucocorticoid exposure may also have detrimental effects on aortic development. However, participants in this study were recruited prior to this period and thus it is not possible to assess the impact of this intervention in this cohort. It would have also been of interest to determine whether those exposed to multiple doses of antenatal glucocorticoids had greater changes in aortic arch stiffness and β cell function, especially given the conflicting views in the literature as to the pros and cons of this treatment approach [127; 128; 129]. However, this information was not available for this cohort but will be of interest and importance for future investigations.

It is possible that antenatal glucocorticoid use has led to survival of infants who would not have survived otherwise, resulting in a cohort with more severe aortic dysfunction. How-

ever, the findings remain of clinical relevance to the long-term cardiovascular health management of those exposed to antenatal glucocorticoids. Interestingly, 30 to 50% of antenatal glucocorticoid doses are currently given to women who go on to deliver after 34 weeks gestation [131; 212] and, therefore, whose infant may not gain any specific benefit from the therapy. For instance, Porto *et al.* [227] demonstrated in a randomised clinical trial of antenatal glucocorticoid treatment at 34 to 36 weeks of pregnancies at risk of imminent preterm delivery that infants whose mothers were randomised to the treatment group showed no reduction in the incidence of neonatal respiratory disorders. While other studies have made similar conclusions, there are also suggestions that infants born after 34 weeks may still benefit from treatment with antenatal glucocorticoids [127; 228]. It will be of interest to understand whether these individuals born near term or at term also have differences in aortic function in later life.

6.5 Conclusion

Antenatal glucocorticoids for women at risk of preterm delivery are the standard of obstetric care and reduce postnatal premature infant morbidity. Between 1975 and 2000, the percentage of preterm-born infants exposed to antenatal glucocorticoids rose from 8% to 75% [211]. This study demonstrates that, 25 years later, *in utero* glucocorticoid exposure is associated with changes in glucose metabolism and localised changes in aortic function in young people. Although antenatal glucocorticoids irrefutably improve the survival of preterm-born infants, the impact on the cardiovascular system may need to be considered in the longer term care of this population when they reach adulthood.

7

PRETERM BIRTH AND THE ADULT LEFT VENTRICLE

This chapter sets out to determine if young adults born preterm have altered left ventricular structure and function. Cardiovascular magnetic resonance and a computational atlas were used to compare the geometry and function of the left ventricle in the cohort of young adults born preterm (*cohort 1*); compared to the cohort of young adults born term (*cohort 2*); and the cohort of adults a decade older born term (*cohort 3*). It was determined that preterm birth is associated with an increase in left ventricular mass, which is inversely related to gestational age and independent of variation in blood pressure. Preterm-born young adults also have shorter left ventricles, smaller internal left ventricular cavity diameters, and a displaced left ventricular apex compared to term-born controls. Furthermore, preterm-born young adults show distinct differences in left ventricular function related to preterm birth, which is significantly worse in those whose mothers had preeclampsia. I have published this work in *Circulation* as the first author [161].

7.1 Introduction

Birth is associated with a switch in cardiomyocyte phenotype from a fetal hyperplastic pattern to neonatal hypertrophic response [98]. Animal models demonstrate this switch in cardiomyocyte growth pattern also occurs at the time of preterm delivery [111]. Moreover, these cardiomyocytes, which are still relatively immature during the last trimester, are then exposed to significant flow changes as the low resistance placental circulation transforms into a high resistance arterial system [109]. Experimental studies demonstrate that, in this setting, cardiomyocytes undergo accelerated hypertrophy with an increase in interstitial myocardial collagen deposition and that the induced changes are sufficient to remodel the left ventricle [98; 110; 111].

While cardiovascular magnetic resonance allows for accurate non-invasive assessment of left ventricular structure and function in humans [165], computational atlas formation has also introduced the possibility to capture three-dimensional geometric variation within populations to explore risk factor influences [229; 230]. These techniques were used to reveal for the first time the impact of preterm birth on long-term changes in left ventricular structure and function in humans. Furthermore, I went on to investigate whether key perinatal factors associated with preterm birth, such as maternal preeclampsia, *in utero* growth restriction, days of mechanical ventilation, and variation in postnatal weight gain, had additional impacts on the left ventricle relevant to adult cardiovascular health.

7.2 Methods

7.2.1 Study Population

This chapter includes all 102 young adults born preterm (*cohort 1*), 102 young adults born term frequency matched for age and sex (*cohort 2*), and 30 adults a decade older frequency matched for sex (*cohort 3*). Details of these three cohorts are outlined in Chapter 2.

7.2.2 Study Visit

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity and blinded analysis. The following methods were used for this chapter as described in detail in Chapter 3:

- 3.2: Cardiovascular Magnetic Resonance
 - 3.2.2: Cardiac Imaging
 - 3.2.2.1: Cardiac Cine Acquisition
 - 3.2.2.2: Left Ventricular Mass and Volumes
 - 3.2.2.3: Left Ventricular Dimensions
 - 3.2.2.6: Left Ventricular Myocardial Deformation
- 3.4: Anthropometry and Lifestyle Questionnaire
- 3.5: Blood Sampling and Biochemistry
- 3.7: Brachial and Aortic Blood Pressure Measurements

7.2.3 Cardiac Atlas for Assessment of Left Ventricular Geometry

Creation of a cardiac statistical atlas of all cardiovascular magnetic resonance images was undertaken in collaboration with the Department of Computer Science, University of Oxford and Department of Biomedical Engineering, King's College London based on recently published methods [230]. The end-diastolic frame from the DICOM file for each slice of the left ventricular short axis cine stack that included the manually contoured endocardial and epicardial contours drawn using Argus were retrieved and rebuilt into a single DICOM file with MatLab R2011b (The Mathworks, Natick, USA) (Figure 7.1 and Figure 7.2). The file was converted into a binary segmentation image representing the left ventricle, and a mesh was fitted to this myocardial anatomy, achieving subvoxel accuracy (average fitting error of 1.24

mm) [230]. The left ventricular anatomy of each subject was then described with a mesh defined by a set of 3456 nodal variables (or degrees of freedom). Principal component analysis was undertaken to identify the key modes of variation of the shape, reducing the parametric space for comparisons from 3456 to six dimensions. The left ventricular meshes for the population have been made available to the scientific community at: amdb.isd.kcl.ac.uk [231].

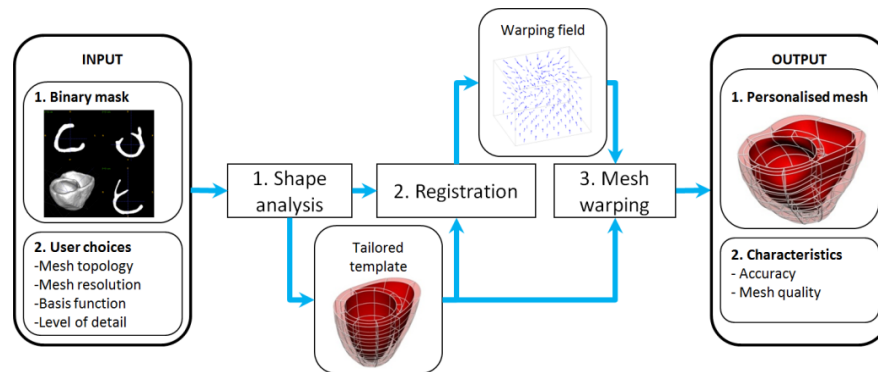


Figure 7.1: General overview of the meshing process.

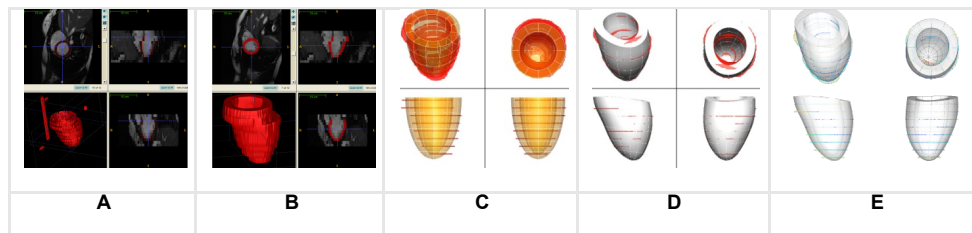


Figure 7.2: Image and meshing process applied in one of the cardiovascular magnetic resonance cases. A: Results of the manual segmentation done with Argus post-processing software, retrieved and visualised in ITK Snap. B: Results of the extraction of the binary mask of the myocardium. C: Mesh initialisation, comparing the iso surface of the binary mask (red) with the tailored mesh template (gold semi transparent). D: Customised mesh (white) overlaid to the isosurface of the binary mask (red). E: Measurement of the fitting error (colour coded from 0 mm (blue) to 5 mm (red)) as the distance between the contours defined in 'A' to the mesh.

7.2.4 Statistical Analysis

Statistical analysis was carried out using SPSS Version 19 as described in Section 3.9 of Chapter 3. 102 subjects per group provided 80% power at $P = 0.05$ to identify a 0.38 standard deviation difference between young adult groups, equivalent to a 10 gram difference in left ventricular mass, and a 0.58 standard deviation difference between each young adult group and the older adults. Where multiple comparisons were performed between groups, P values were adjusted using the Bonferroni method. Comparisons between preterm-born young adults with term-born young adults were adjusted for age and sex, while comparisons between preterm-born young adults and term-born young adults with the older term-born older adults were adjusted for sex.

7.3 Results

7.3.1 Study Population Characteristics

In adult life, there were no significant differences in number of smokers, personal and family medical history, or lifestyle factors such as socioeconomic status, physical activity or diet ($P > 0.05$). Adults born preterm were shorter and weighed more than the young adult term-born individuals (Table 7.1) and had a distinct metabolic profile (Table 7.2). Total and low density lipoprotein cholesterol, triglyceride, glucose, and insulin levels were also significantly increased in the young adults born preterm compared to the young adults born term. Brachial and aortic blood pressure parameters were also elevated compared to the term-born individuals of similar age, with levels similar to term-born adults a decade older (Table 7.2).

7.3.2 Elevated Left Ventricular Mass in Preterm-Born Young Adults

Young adults born preterm had a 19.0 gram higher left ventricular mass and significantly increased left ventricular mass indexed to body surface area compared to term-born young adults (left ventricular mass: 121.1 ± 27.3 vs 102.1 ± 26.8 grams, $P < 0.001$ and left ventricular

Table 7.1: Characteristics of Cohorts

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	P Value Ψ	Term-born adults (n = 30)	P Value Υ	P Value Δ
Demographics						
Gestational Age (wks)	30.3 $\pm$ 2.5	39.6 $\pm$ 0.9	<0.001	39.8 $\pm$ 0.8	<0.001	>0.99
Age (years)	25.1 $\pm$ 1.4	25.0 $\pm$ 2.6		35.5 $\pm$ 1.8		
Males, n (%)	47 (46.1)	47 (46.1)		14 (46.7)		
Smokers, n (%)	20 (19.6)	20 (19.6)	>0.99	3 (10.0)	0.63	0.63
Anthropometrics						
Birth weight (g)	1297.0 $\pm$ 286.8	3460.0 $\pm$ 417.0	<0.001	3301.9 $\pm$ 423.9	<0.001	0.33
Height (cm)	169.1 $\pm$ 10.0	173.5 $\pm$ 9.0	<0.001	169.3 $\pm$ 9.6	>0.99	0.01
Weight (kg)	73.0 $\pm$ 20.5	69.3 $\pm$ 12.5	0.33	79.8 $\pm$ 28.9	0.46	0.01
BMI (kg/m ²)	24.9 $\pm$ 5.4	22.9 $\pm$ 3.1	0.003	27.1 $\pm$ 9.6	0.33	<0.001
BSA (/m ²)	1.81 $\pm$ 0.21	1.83 $\pm$ 0.20	>0.99	1.84 $\pm$ 0.21	>0.99	>0.99
Waist:Hip	0.78 $\pm$ 0.10	0.81 $\pm$ 0.06	0.91	0.81 $\pm$ 0.08	>0.99	>0.99

BMI indicates body mass index; BSA, body surface area.

Values as Mean $\pm$ Standard Deviation unless stated otherwise. *P* values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

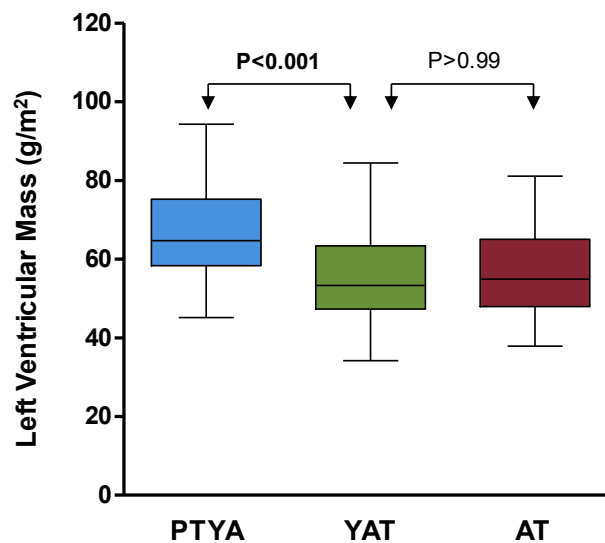


Figure 7.3: Left ventricular mass indexed to body surface area. Left ventricular mass indexed to body surface area was higher in preterm-born young adults (PTYA, blue; 66.5 ± 10.9 g/m²) than young adults born at term (YAT, green; 55.4 ± 11.4 g/m², $P < 0.001$) and adults a decade older born at term (AT, red; 57.0 ± 11.9 g/m², $P < 0.001$). Left ventricular mass indexed to body surface area of the older term-born adults did not differ from the young adults born at term ($P > 0.99$). Data shown as box and whisker plot: minimum to maximum.

Table 7.2: Biochemistry and Blood Pressure

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	P Value Ψ	Term-born adults (n = 30)	P Value Υ	P Value Δ
Biochemistry						
Total Cholesterol (mmol/L)	4.89 $\pm$ 1.05	4.23 $\pm$ 0.86	<0.001	5.48 $\pm$ 1.18	0.03	<0.001
HDL-C (mmol/L)	1.53 $\pm$ 0.40	1.47 $\pm$ 0.41	0.54	1.46 $\pm$ 0.39	0.98	>0.99
LDL-C (mmol/L)	2.81 $\pm$ 0.93	2.37 $\pm$ 0.66	<0.001	3.39 $\pm$ 1.00	0.02	<0.001
Triglycerides (mmol/L)	1.12 $\pm$ 0.79	0.87 $\pm$ 0.40	0.02	1.38 $\pm$ 0.91	0.39	0.003
CRP (mmol/L)	2.27 $\pm$ 4.02	1.53 $\pm$ 3.35	0.5	3.99 $\pm$ 7.97	0.12	0.11
Glucose (mmol/L)	5.01 $\pm$ 0.43	4.61 $\pm$ 0.30	<0.001	5.65 $\pm$ 1.60	0.003	<0.001
Insulin (pmol/L)	64.4 $\pm$ 42.8	35.6 $\pm$ 15.9	<0.001	104.4 $\pm$ 89.0	0.006	<0.001
Brachial Blood Pressure (mmHg)						
Systolic	121.3 $\pm$ 10.9	112.9 $\pm$ 10.1	<0.001	121.7 $\pm$ 13.5	>0.99	<0.001
Diastolic	73.0 $\pm$ 7.2	68.8 $\pm$ 7.0	<0.001	73.9 $\pm$ 6.0	>0.99	<0.001
Mean Arterial Pressure	89.1 $\pm$ 7.4	83.5 $\pm$ 7.1	<0.001	89.8 $\pm$ 7.5	>0.99	<0.001
Pulse Pressure	48.4 $\pm$ 9.1	44.1 $\pm$ 8.5	0.001	47.8 $\pm$ 11.3	>0.99	0.12
Aortic Blood Pressure (mmHg)						
Systolic	108.0 $\pm$ 10.0	97.4 $\pm$ 8.8	<0.001	109.1 $\pm$ 10.9	>0.99	<0.001
Diastolic	74.5 $\pm$ 7.4	69.6 $\pm$ 7.7	<0.001	75.3 $\pm$ 7.3	>0.99	<0.001
Mean Arterial Pressure	85.7 $\pm$ 7.7	78.9 $\pm$ 7.6	<0.001	86.5 $\pm$ 8.0	>0.99	<0.001
Pulse Pressure	33.5 $\pm$ 7.1	27.7 $\pm$ 5.6	<0.001	33.8 $\pm$ 7.3	>0.99	<0.001

HDL-C indicates high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; CRP, C-reactive protein.

Values as Mean $\pm$ Standard Deviation unless stated otherwise. *P* values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

mass index: 66.5 $\pm$ 10.9 vs 55.4 $\pm$ 11.4 g/m², *P* < 0.001) (Figure 7.3). Within the preterm group, the more premature their birth the greater their left ventricular mass in young adult life (*r* = -0.22, *P* = 0.03).

Blood pressure was associated with left ventricular mass in all study groups with brachial pulse pressure being the most closely related parameter in the young adults born preterm (*r* = 0.54, *P* < 0.001), young adults born at term (*r* = 0.54, *P* < 0.001), and the older term-born adults (*r* = 0.68, *P* < 0.001). Therefore, as blood pressure in young adulthood was higher in those born preterm, I explored to what extent this accounted for their higher left ventricular mass. Initially, as the older term-born adults had similar blood pressure levels to the preterm group, I compared levels of left ventricular mass between these groups and

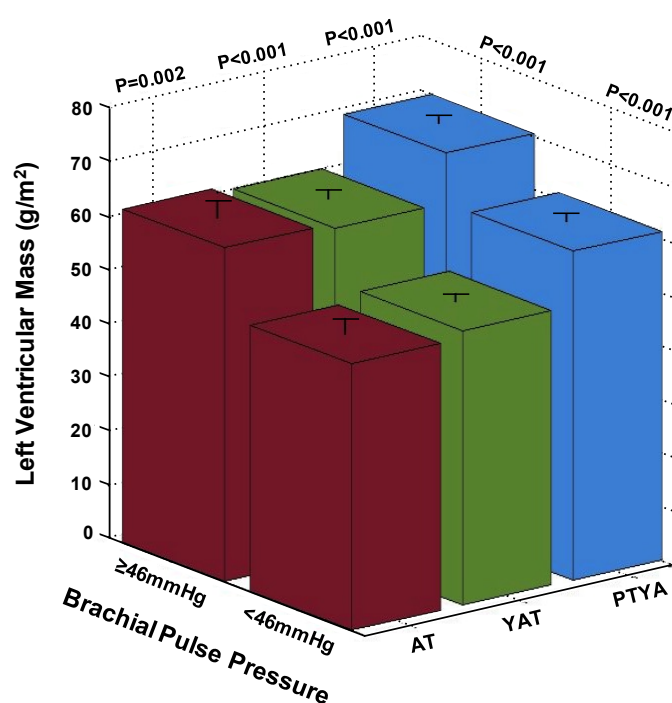


Figure 7.4: Comparison of left ventricular mass indexed to body surface area by equivalent blood pressure groups. The median brachial pulse pressure (46 mmHg) for the entire population was used as a cutoff point to divide each subgroup (PTYA = preterm-born young adults, blue; YAT = term-born young adults, green; AT = term-born adults a decade older, red) into two. Comparison of those with brachial pulse pressure <46mmHg ($n = 44$ preterm-born young adults, $n = 58$ term-born young adults, and $n = 13$ term-born adults a decade older) showed that preterm-born young adults had significantly higher left ventricular mass indexed to body surface area compared to term-born young adults and term-born adults a decade older (61.2 ± 9.8 vs 51.1 ± 10.7 g/m², $P < 0.001$, and vs 49.6 ± 10.3 g/m², $P < 0.001$). Comparison of those with brachial pulse pressure ≥ 46 mmHg ($n = 58$ preterm-born young adults, $n = 44$ term-born young adults, and $n = 17$ term-born adults a decade older) showed that preterm-born young adults had a similar magnitude of elevation of left ventricular mass indexed to body surface area compared to both YAT and AT (70.5 ± 10.0 vs 61.2 ± 9.8 g/m², $P < 0.001$, and vs 62.1 ± 10.4 g/m², $P = 0.003$). Data shown as mean $\pm$ standard error of the mean.

found left ventricular mass and left ventricular mass index were significantly higher in the younger preterm-born adults ($P < 0.001$ and $P < 0.001$) (Figure 7.3) consistent with an impact of preterm birth on left ventricular mass independent of absolute blood pressure levels alone. To ensure the variation in age between these groups did not confound the comparison, I also carried out a complementary analysis based on a comparison of the impact of preterm birth with the study participants stratified into high and low blood pressure groups around the median brachial pulse pressure within the cohort (Figure 7.4). Left ventricular mass index was higher in those in the higher blood pressure group, consistent with an im-

pact of blood pressure on left ventricular mass index. However, individuals born preterm had additional significant increases in left ventricular mass index, irrespective of blood pressure level, compared to both term-born young adults as well as the older adults born term (61.2 ± 9.8 vs 51.1 ± 10.7 g/m², $P < 0.001$, and vs 49.6 ± 10.3 g/m², $P < 0.001$ in those with a pulse pressure < 46 mmHg and 70.5 ± 10.0 vs 61.2 ± 9.8 g/m², $P < 0.001$, and vs 62.1 ± 10.4 g/m², $P = 0.003$ in those with a pulse pressure ≥ 46 mmHg).

Finally, I used a linear regression model to take account of other potential factors related to left ventricular mass index within the preterm-born and term-born young adult groups. Within sets of interrelated variables, such as blood pressure, the variable with the strongest bivariable correlation was included, leaving six independent variables significantly related to left ventricular mass index ($P < 0.05$, Table 7.3) to be included in the model (glucose level, high density lipoprotein cholesterol, waist to hip ratio, sex, brachial pulse pressure, and preterm birth as a dichotomous categorical variable with young adults born at term as '1' and young adults born preterm as '2'). In this model, preterm birth remained an independent predictor of left ventricular mass index ($B = 10.2$ g/m²/group, $P < 0.001$, 95% confidence interval: 7.48 to 13.0).

7.3.3 Geometric Changes of the Preterm Left Ventricle

Variation in left ventricular geometry was then studied. Principal component analysis was used to identify the major modes of variation within the three-dimensional meshes generated from each of the cardiac datasets. All identified modes were numbered in order based on the amount of variation within the population each accounted for (with mode one accounting for the greatest proportion: 29.8%). When the cohort of preterm-born young adults was compared to the term-born individuals of similar age, significant differences were observed between groups in the first six modes, with levels of significance for modes one, two, and five being in the order $P = 1 \times 10^{-11}$ to $P = 1 \times 10^{-13}$ (Figure 7.5). These first six modes accounted for 84.2% of the variance in left ventricular geometry within the study population (Figure 7.6). Four of these modes also reached significance for differences between the

Table 7.3: Relationship of Variables with Left Ventricular Mass Index in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	11.1	3.99	7.11	<0.001
Age	-0.48	-1.31	0.36	0.26
Sex	13.39	10.44	16.34	<0.001
Smoking	-1.70	-6.05	2.66	0.44
Waist:Hip Ratio	47.3	22.6	72.1	<0.001
Total Cholesterol	-0.20	-2.06	1.66	0.83
LDL Cholesterol	0.75	-1.49	2.99	0.51
HDL Cholesterol	-8.13	-12.4	-3.83	<0.001
Insulin	0.001	-0.06	0.06	0.99
Glucose	8.37	4.15	12.6	<0.001
Triglycerides	2.55	-0.38	5.31	0.09
Brachial Systolic BP	0.52	0.38	0.66	<0.001
Brachial Diastolic BP	-0.0031	-0.24	0.23	0.98
Brachial Mean Arterial Pressure	0.35	0.13	0.57	0.002
Brachial Pulse Pressure	0.80	0.64	0.96	<0.001
Aortic Systolic BP	0.40	0.25	0.56	<0.001
Aortic Diastolic BP	0.042	0.18	0.26	0.72
Aortic Mean Arterial Pressure	0.24	0.030	0.45	0.02
Aortic Pulse Pressure	0.92	0.69	0.69	<0.001

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BP, blood pressure.

preterm-born young adults and the older term-born adults, but no modes reached significance when the two term-born cohorts were compared (Figure 7.5). Figure 7.7 demonstrates where the mean of each group lies relative to the other groups in the principal component analysis parametric space. Separation between preterm-born and term-born offspring is also evident when individual subject data is plotted in three dimensions (Figure 7.8).

The models that corresponded with plus or minus two standard deviations of the mean variation for the six modes that differed between individuals born preterm and individuals born term were then visually assessed. The geometric difference each describes are shown in Figure 7.7. Preterm-born individuals had significantly shorter ventricles, increased left ventricular wall thickness, a shift in apex orientation away from the right ventricle, and reduced internal left ventricular cavity diameter (Figure 7.7 and Figure 7.9). Several of these features could be captured by standard clinical left ventricular measures; specifically, left ventricular length, cavity diameters, and wall thickness at end-diastole. Differences in left ventricular volumes and these standard measures were confirmed between preterm-born

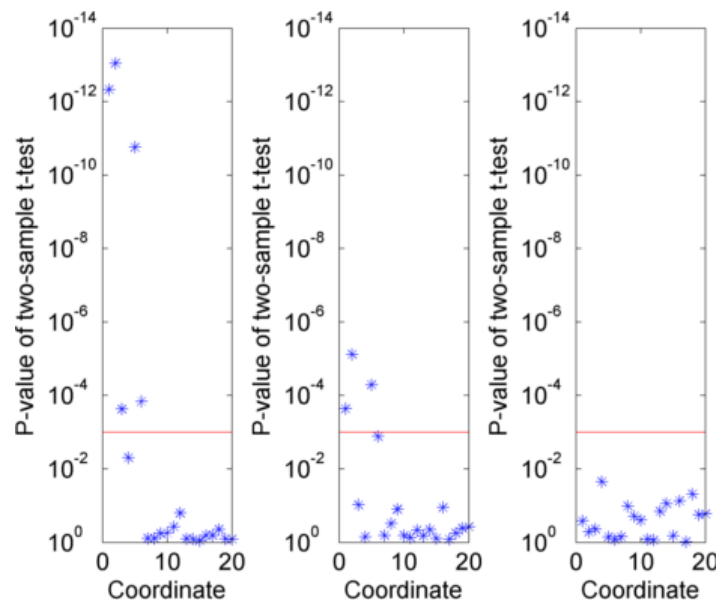


Figure 7.5: Comparison of differences in shape between groups by modes of variation of the principal component analysis. Comparison between groups was performed using a two sided student T test. The red line indicates $P = 0.01$. The panel on the left shows a comparison of the preterm-born young adults (PTYA) and young adults born at term (YAT), demonstrating that the first six modes of variation differed significantly between groups. The middle panel shows a comparison of the preterm-born young adults and older adult term-born (AT) groups, demonstrating that modes one, two, five, and six were significantly different between groups. The panel on the right shows a comparison of the term-born young adults and older term-born adults, demonstrating no significant differences between groups.

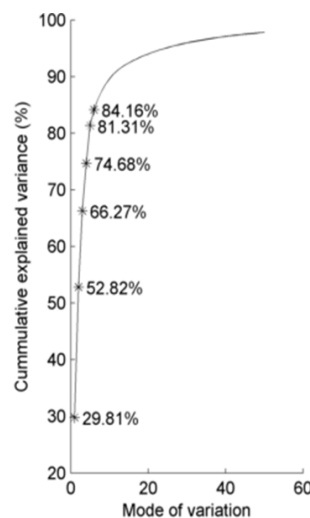


Figure 7.6: Cumulative variance explained by each mode of variation. The first six modes of variation, which differed between the preterm-born young adult and term-born young adult groups, explained 84.2% of the variance in left ventricular geometry within the entire population, with mode one explaining the majority of variance (29.8%).

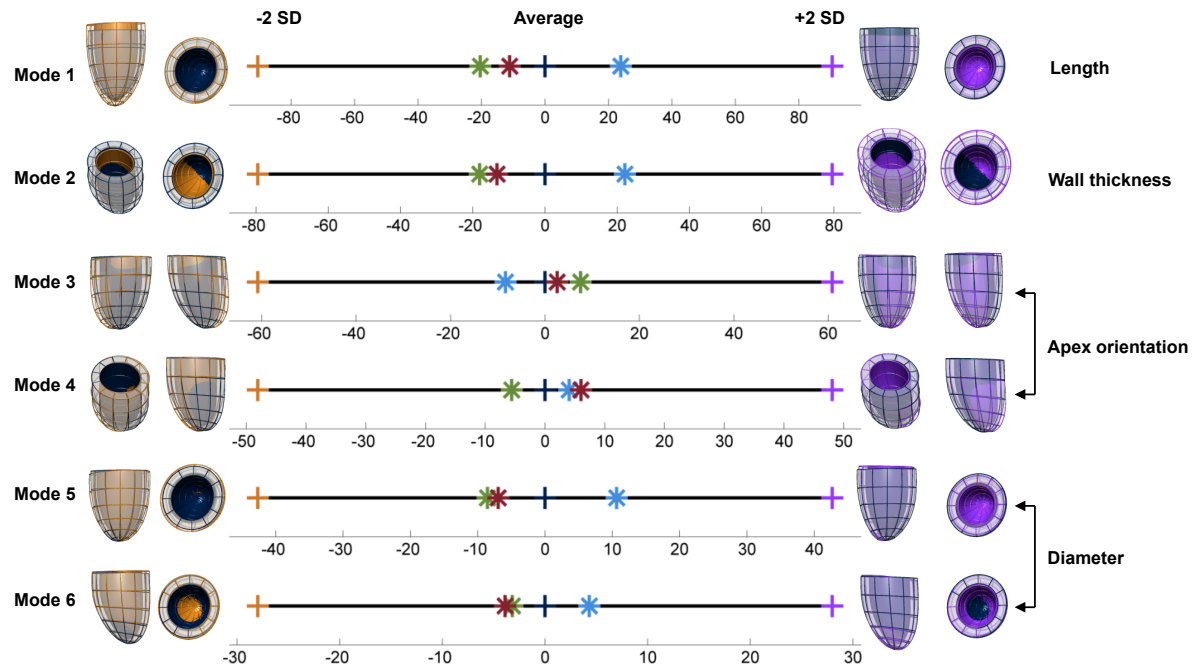


Figure 7.7: The first six independent modes of variation resulting from the principal component analysis. In all six cases, the average is at coordinate zero, and is represented by a dark blue cross and the dark blue left ventricular mesh overlaid to the orange and purple meshes. The orange cross and orange left ventricular mesh represent minus two standard deviations for each mode of variation, and the purple cross and purple left ventricular mesh represent plus two standard deviations for each mode of variation. As illustrated here, visual assessment revealed that the modes of variation represent changes in: one = primarily ventricular length, with a proportion of diameter change; two = wall thickness; three and four = apex orientation; five = primarily diameter, with a proportion of apex position; and six = primarily diameter, with a proportion of length change. The stars represent the principal component analysis coordinates for the average for each group (preterm-born young adults = blue; term-born young adults = green; older adults born term = red), for each mode of variation.

young adults and term-born individuals (Figure 7.10 and Table 7.6). In view of the combination of increased wall thickness with reduced ventricular cavity size, I also calculated relative wall thickness and left ventricular mass relative to end-diastolic volume (left ventricular mass / end-diastolic volume) (Table 7.6). 21 individuals born preterm (20.6%) had a relative wall thickness >0.42 compared to none in either term-born group. Furthermore, those born preterm had increased left ventricular mass / end-diastolic volume compared to both term-born groups ($P < 0.001$, Table 7.6), with an inverse association between gestational age and left ventricular mass / end-diastolic volume ($r = -0.36$, $P < 0.001$).

Using similar methodology to that presented in Figure 7.3, I explored to what extent blood pressure accounted for the increase in left ventricular mass / end-diastolic volume in the young adults born preterm, to ensure that the study conclusions remained consistent.

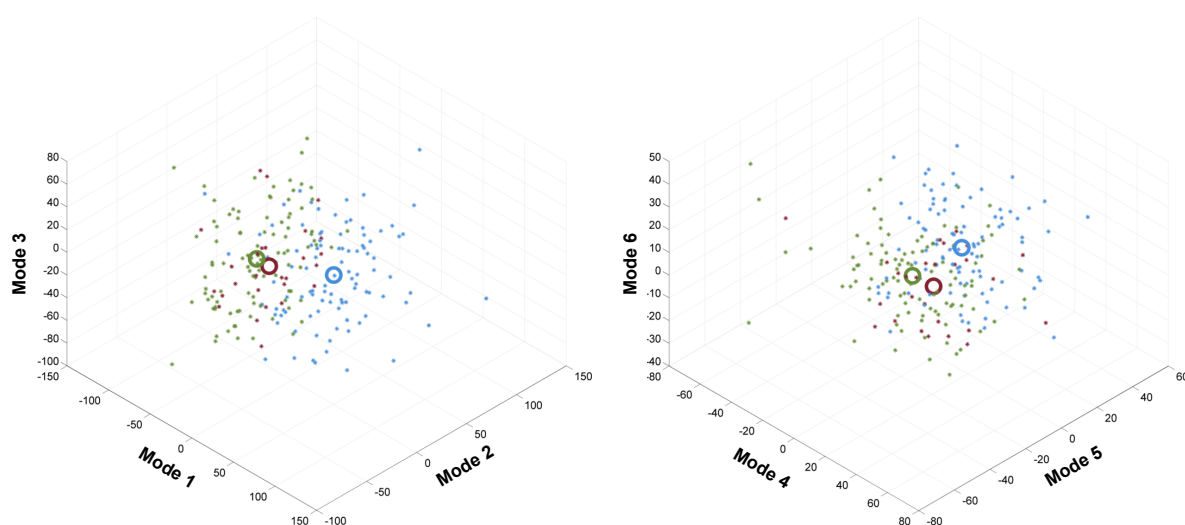


Figure 7.8: Distribution of all subjects in the principal component analysis coordinates. Averages for the groups are indicated by circles (blue = preterm-born young adults; green = term-born young adults; red = older adults born term). The panel on the left represents the first three modes of variation (one, two, and three) and the panel on the right represents the next three modes of variation (four, five, and six).

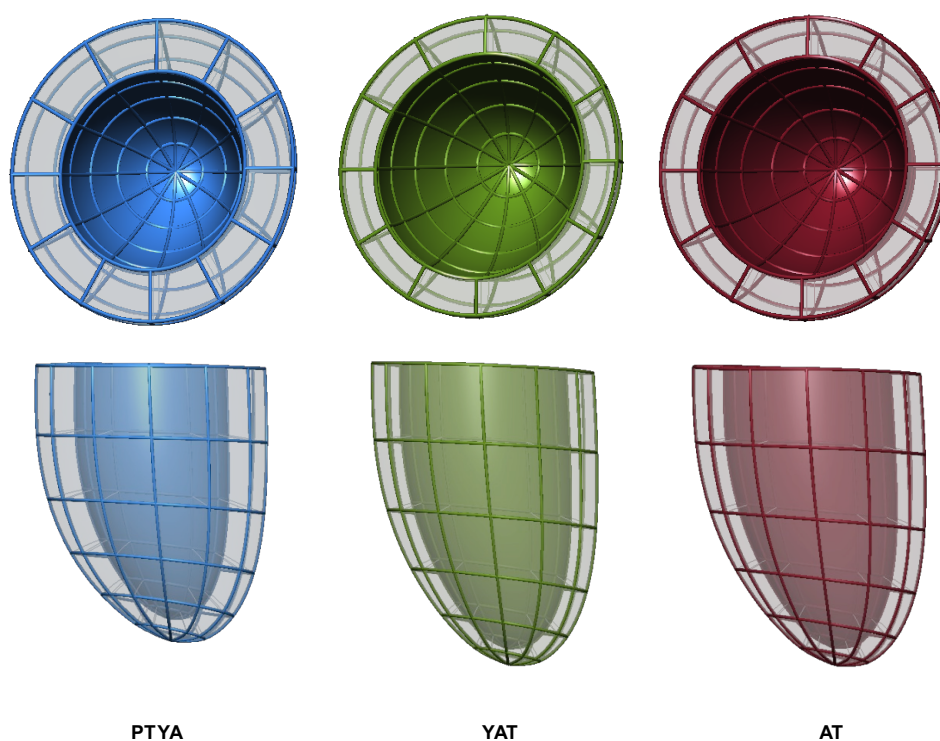


Figure 7.9: Statistical average shape for each group. PTYA, blue = preterm-born young adults; YAT, green = term-born young adults; AT, red = older term-born adults.

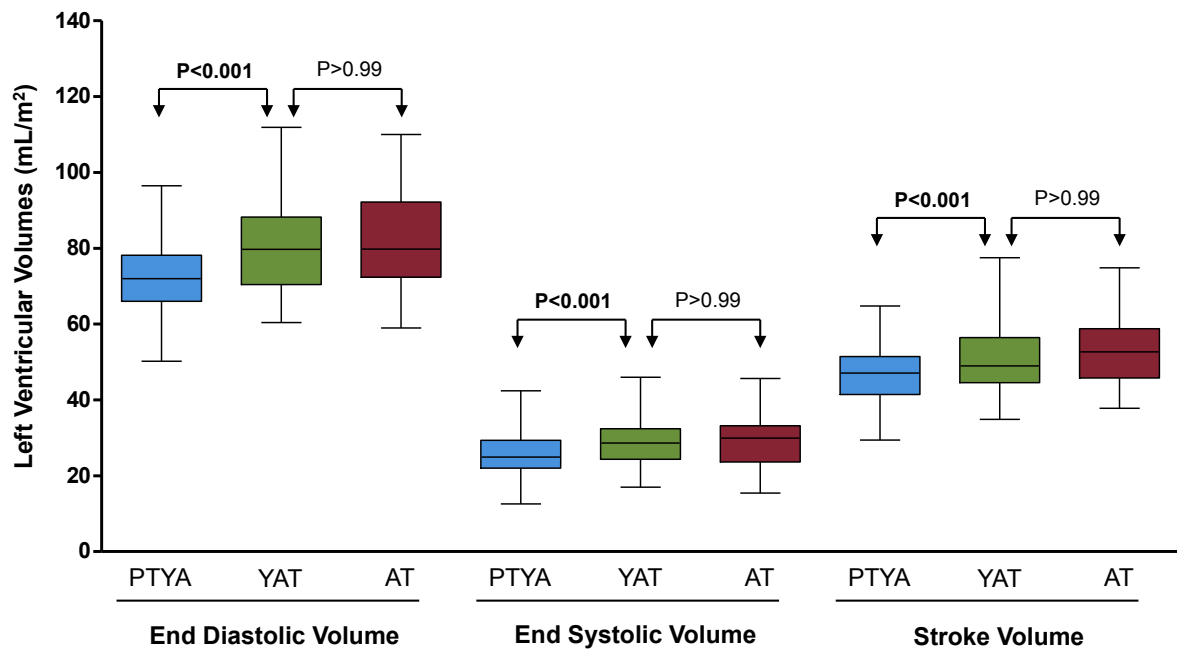


Figure 7.10: Left ventricular volumes. Left ventricular end-diastolic volume indexed to body surface area was reduced in preterm-born young adults (PTYA, blue; $72.2 \pm 9.3 \text{ mL/m}^2$) compared to young adults born at term (YAT, green; $80.2 \pm 11.7 \text{ mL/m}^2$, $P < 0.001$) and adults a decade older born at term (AT, red; $81.5 \pm 12.9 \text{ mL/m}^2$, $P < 0.001$). Similarly, left ventricular systolic volume indexed to body surface area was reduced in preterm-born young adults ($25.7 \pm 5.4 \text{ mL/m}^2$) compared to young adults born at term ($29.1 \pm 6.4 \text{ mL/m}^2$, $P < 0.001$) and adults a decade older born at term ($29.0 \pm 6.4 \text{ mL/m}^2$, $P < 0.001$). Left ventricular stroke volume indexed to body surface area was also reduced in preterm-born young adults ($46.6 \pm 7.4 \text{ mL/m}^2$) compared to young adults born at term ($51.3 \pm 8.9 \text{ mL/m}^2$, $P < 0.001$) and adults a decade older born at term ($52.5 \pm 8.4 \text{ mL/m}^2$, $P < 0.001$). Left ventricular volumes did not differ between the older term-born adults and the young adults born at term ($P > 0.99$). Data shown as box and whisker plot: minimum to maximum.

Similar to with left ventricular mass index, brachial pulse pressure was the most closely related parameter in the young adults born preterm ($r = 0.75$, $P < 0.001$), young adults born at term ($r = 0.73$, $P < 0.001$), and the older term-born adults ($r = 0.90$, $P < 0.001$). Comparison of those with brachial pulse pressure $< 46 \text{ mmHg}$ ($n = 44$ preterm-born young adults, $n = 58$ term-born young adults and $n = 13$ term-born adults a decade older) showed that preterm-born young adults had significantly higher left ventricular mass / end-diastolic volume compared to term-born young adults and term-born adults a decade older ($0.87 \pm 0.13 \text{ g/mL}$ vs $0.68 \pm 0.13 \text{ g/mL}$, $P < 0.001$, and vs $0.64 \pm 0.10 \text{ g/mL}$, $P < 0.001$). Comparison of those with brachial pulse pressure $\geq 46 \text{ mmHg}$ ($n = 58$ preterm-born young adults, $n = 44$ term-born young adults and $n = 17$ term-born adults a decade older) showed that preterm-born young

adults had a similar magnitude of elevation of left ventricular mass / end-diastolic volume compared to both term-born young adults and term-born adults a decade older (0.97 ± 0.14 g/mL vs 0.72 ± 0.11 g/mL, $P < 0.001$, and vs 0.75 ± 0.14 g/mL, $P < 0.001$).

Linear regression models were used to take into account other potential factors related to left ventricular end-diastolic volume index and left ventricular mass / end-diastolic volume within the preterm-born and term-born young adult groups, similar to that used for left ventricular mass index. Similar to the model used for left ventricular mass index, within sets of interrelated variables, such as blood pressure and biochemistry parameters, the variable with the strongest bivariable correlation was included in the model. For left ventricular end-diastolic volume index, this left five independent variables significantly related to left ventricular end-diastolic volume index (sex, high density lipoprotein cholesterol, insulin, aortic diastolic blood pressure, and preterm birth as a dichotomous categorical variable with young adults born at term as '1' and young adults born preterm as '2') to be included in the model ($P < 0.05$, Table 7.4). In this model, preterm birth remained an independent predictor of left ventricular end-diastolic volume index ($B = -5.85$ mL/m²/group, $P < 0.001$, 95% confidence interval: -8.99 to -2.70). For left ventricular mass / end-diastolic volume, five independent variables (sex, total cholesterol, glucose, brachial pulse pressure, and preterm birth) were included in the model ($P < 0.05$, Table 7.5). In this model, preterm birth remained the strongest independent predictor of left ventricular mass / end-diastolic volume ($B = 0.25$ g/mL/group, $P < 0.001$, 95% confidence interval: 0.20 to 0.30).

7.3.4 Altered Left Ventricular Systolic and Diastolic Function

It was then determined whether there were also changes in left ventricular function related to being born preterm. End-systolic volume was reduced in the preterm group and stroke volume was smaller, with the reduction graded according to the degree of prematurity ($r = -0.20$, $P = 0.05$). Although ejection fraction did not differ, myocardial deformation patterns were significantly altered in those born preterm (Table 7.7). Longitudinal peak systolic strain and peak systolic strain rate were reduced compared to both term-born groups (Figure

Table 7.4: Relationship of Variables with Left Ventricular End-Diastolic Volume Index in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	-7.94	-10.9	-4.97	<0.001
Age	-0.046	-0.81	0.72	0.91
Sex	-8.64	-11.6	-5.70	<0.001
Smoking	-2.30	-6.28	1.67	0.25
Waist:Hip Ratio	14.4	-7.98	36.7	0.21
Total Cholesterol	-3.11	-4.68	-1.54	<0.001
LDL Cholesterol	-2.25	-4.21	-0.30	0.02
HDL Cholesterol	-7.19	-11.14	-3.24	<0.001
Insulin	-0.10	-0.15	-0.056	<0.001
Glucose	-2.44	-6.38	1.49	0.22
Triglycerides	-1.36	-3.95	1.24	0.30
Brachial Systolic BP	0.063	-0.078	0.20	0.38
Brachial Diastolic BP	-0.37	-0.58	-0.16	0.001
Brachial Mean Arterial Pressure	-0.18	-0.38	0.026	0.09
Brachial Pulse Pressure	0.35	0.18	0.51	<0.001
Aortic Systolic BP	-0.081	-0.23	0.067	0.28
Aortic Diastolic BP	-0.40	-0.60	-0.21	<0.001
Aortic Mean Arterial Pressure	-0.29	-0.47	-0.10	0.003
Aortic Pulse Pressure	0.33	0.11	0.56	0.004

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BP, blood pressure.

7.11 and Figure 7.12). Furthermore, systolic rotational behaviour varied with reduced basal and apical rotation rates (Table 7.7). Diastolic myocardial relaxation was also reduced with slower longitudinal peak diastolic strain rates compared to term-born individuals of similar age. Interestingly, longitudinal peak diastolic strain rates were reduced in the older adults to a similar degree as measured in those born preterm (Figure 7.11 and Figure 7.12).

A linear regression model was used to take account of other potential factors related to left ventricular global longitudinal peak systolic strain within the preterm-born and term-born young adult groups. Similar to the other regression models, within sets of interrelated variables, such as blood pressure, the variable with the strongest bivariable correlation was included in the model. This left two independent variables significantly related to left ventricular global longitudinal peak systolic strain (aortic pulse pressure and preterm birth as a dichotomous categorical variable with young adults born at term as '1' and young adults born preterm as '2') to be included in the model ($P < 0.05$, Table 7.8). In this model, preterm birth remained an independent predictor of left ventricular global longitudinal peak systolic

Table 7.5: Relationship of Variables with Left Ventricular Mass / End-Diastolic Volume in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	0.24	0.20	0.28	<0.001
Age	-0.0061	-0.018	0.0071	0.38
Sex	-0.0078	-0.13	-0.028	0.003
Smoking	0.0062	-0.059	0.071	0.86
Waist:Hip Ratio	0.42	0.061	0.78	0.02
Total Cholesterol	0.028	0.0021	0.055	0.035
LDL Cholesterol	0.032	0.0010	0.064	<0.001
HDL Cholesterol	-0.029	-0.095	0.038	0.39
Insulin	0.0011	0.00010	0.0021	0.003
Glucose	0.13	0.065	0.19	<0.001
Triglycerides	0.038	-0.0040	0.080	0.08
Brachial Systolic BP	0.0061	0.0032	0.0081	<0.001
Brachial Diastolic BP	0.0032	-0.0011	0.0062	0.14
Brachial Mean Arterial Pressure	0.0052	0.0021	0.0090	0.001
Brachial Pulse Pressure	0.0085	0.0052	0.012	<0.001
Aortic Systolic BP	0.0062	0.0031	0.0083	<0.001
Aortic Diastolic BP	0.0044	0.0014	0.0069	0.02
Aortic Mean Arterial Pressure	0.0063	0.0024	0.0085	<0.001
Aortic Pulse Pressure	0.0072	0.0044	0.010	<0.001

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BP, blood pressure.

strain ($B = 2.81 \text{ \%}/\text{group}$, $P < 0.001$, 95% confidence interval: 1.62 to 3.99).

7.3.5 Preterm Birth Risk Factors and Left Ventricular Geometry and Function

To understand whether preterm birth *per se* or factors linked with being born preterm accounted for the variation in the key geometric (left ventricular mass index, length, and end-diastolic volume) and functional (longitudinal peak systolic strain, peak systolic strain rate and diastolic strain rate) variables that differed in those born preterm, I performed further regression analyses. Firstly, as the preterm-born individuals had been randomised to milk feeding regimes at birth, it was determined by bivariable regression analyses whether the dietary group (as a dichotomous categorical variable with banked breast milk as '1' and formula milk as '2') related to left ventricular structural and functional measures in young adulthood. It was determined that the dietary group did not relate to differences in left ven-

Table 7.6: Left Ventricular Volumes and Dimensions

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	P Value Ψ	Term-born adults (n = 30)	P Value Υ	P Value Δ
ED Volume (mL/m ²)	72.2 ± 9.3	80.2 ± 11.7	<0.001	81.5 ± 12.9	<0.001	>0.99
ES Volume (mL/m ²)	25.7 ± 5.4	29.1 ± 6.4	<0.001	29.0 ± 6.4	0.01	>0.99
Stroke Volume (mL/m ²)	46.6 ± 7.4	51.3 ± 8.9	<0.001	52.5 ± 8.4	<0.001	>0.99
Length (cm)	9.20 ± 0.65	9.81 ± 0.73	<0.001	9.70 ± 0.64	<0.001	>0.99
Luminal Diameter (cm)	5.20 ± 0.47	5.64 ± 0.48	<0.001	5.66 ± 0.34	<0.001	>0.99
External Diameter (cm)	7.33 ± 0.65	7.19 ± 0.64	0.16	7.26 ± 0.43	>0.99	>0.99
Global WT (mm)	9.26 ± 1.40	7.46 ± 1.26	<0.001	7.93 ± 1.26	<0.001	0.07
Anterior WT (mm)	8.21 ± 1.51	6.87 ± 1.51	<0.001	7.39 ± 1.45	0.009	0.15
Anterolateral WT (mm)	8.89 ± 1.74	6.89 ± 1.45	<0.001	7.11 ± 1.50	<0.001	>0.99
Inferolateral WT (mm)	9.40 ± 1.75	7.13 ± 1.53	<0.001	7.52 ± 1.38	<0.001	0.44
Inferior WT (mm)	9.17 ± 1.73	7.22 ± 1.60	<0.001	8.03 ± 1.45	0.003	0.01
Inferoseptal WT (mm)	10.29 ± 1.80	8.56 ± 1.34	<0.001	8.96 ± 1.58	<0.001	0.40
Anteroseptal WT (mm)	9.63 ± 1.79	8.10 ± 1.78	<0.001	8.56 ± 1.68	0.003	0.51
RWT	0.35 ± 0.07	0.26 ± 0.05	<0.001	0.28 ± 0.05	<0.001	0.02
RWT > 0.42, n (%)	21 (20.6)	0 (0)	<0.001	0 (0)	<0.001	>0.99
Mass/ED Volume (g/mL)	0.93 ± 0.14	0.70 ± 0.12	<0.001	0.70 ± 0.13	<0.001	>0.99

ED indicates end-diastole; ES, end-systole; WT, wall thickness; RWT, relative wall thickness.

Values as Mean ± Standard Deviation unless stated otherwise. *P* values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

tricular structural (mass index: $P = 0.81$; length: $P = 0.36$; end-diastolic volume index: $P = 0.93$) or functional (longitudinal peak systolic strain: $P = 0.45$; longitudinal peak systolic strain rate: $P = 0.32$; longitudinal peak diastolic strain rate: $P = 0.34$) measures. I then looked at whether the mode of delivery (as a dichotomous categorical variable with spontaneous delivery as '1' and iatrogenic delivery as '2') related to left ventricular structural and functional measures in young adulthood. It was determined that the mode of delivery did not relate to differences in left structural (mass index: $P = 0.96$; length: $P = 0.91$; end-diastolic volume index: $P = 0.99$) or functional (longitudinal peak systolic strain: $P = 0.25$; longitudinal peak systolic strain rate: $P = 0.26$; longitudinal peak diastolic strain rate: $P = 0.61$) measures.

I therefore went on to perform two regression models for each outcome variable. As the sample size was 102 young adults born preterm, I limited the number of variables included to five key perinatal characteristics (gestational age, birth weight *Z* score, postnatal weight gain in the first two weeks, days of mechanical ventilation, and maternal preeclampsia). Those

Table 7.7: Left Ventricular Systolic and Diastolic Function

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	P Value Ψ	Term-born adults (n = 30)	P Value Υ	P Value Δ
Systolic Function						
Ejection Fraction (%)	64.5 $\pm$ 6.1	64.1 $\pm$ 4.9	>0.99	64.3 $\pm$ 4.8	>0.99	>0.99
<u>Longitudinal</u>						
Strain (%)	-14.8 $\pm$ 3.2	-17.9 $\pm$ 4.1	< 0.001	-17.7 $\pm$ 5.3	0.003	>0.99
Strain Rate (%/s)	-0.90 $\pm$ 0.21	-1.06 $\pm$ 0.31	< 0.001	-1.08 $\pm$ 0.31	0.009	>0.99
Displacement (cm)	3.80 $\pm$ 1.47	4.71 $\pm$ 2.54	0.01	4.23 $\pm$ 1.56	0.68	>0.99
Velocity (cm/s)	2.25 $\pm$ 0.78	3.09 $\pm$ 0.97	< 0.001	3.17 $\pm$ 1.18	< 0.001	>0.99
<u>Circumferential</u>						
Basal Strain (%)	-21.2 $\pm$ 2.9	-21.8 $\pm$ 3.2	0.45	-22.2 $\pm$ 3.4	0.38	>0.99
Basal Strain Rate (%/s)	-1.26 $\pm$ 0.21	-1.28 $\pm$ 0.24	>0.99	-1.32 $\pm$ 0.28	0.63	>0.99
Basal Rotation ($^{\circ}$)	-7.21 $\pm$ 4.59	-8.81 $\pm$ 5.22	0.08	-7.65 $\pm$ 4.12	>0.99	0.88
Basal Rotation Rate ($^{\circ}$ /s)	-46.2 $\pm$ 26.4	-60.2 $\pm$ 35.4	0.006	-52.1 $\pm$ 29.2	>0.99	0.83
Mid Strain (%)	-17.9 $\pm$ 2.2	-17.7 $\pm$ 2.2	>0.99	-18.3 $\pm$ 2.2	>0.99	0.61
Mid Strain Rate (%/s)	-1.06 $\pm$ 0.14	-1.04 $\pm$ 0.19	>0.99	-1.09 $\pm$ 0.18	>0.99	0.97
Apical Strain (%)	-21.6 $\pm$ 3.8	-22.1 $\pm$ 3.8	>0.99	-22.8 $\pm$ 4.0	0.45	>0.99
Apical Strain Rate (%/s)	-1.30 $\pm$ 0.24	-1.25 $\pm$ 0.24	0.39	-1.33 $\pm$ 0.24	>0.99	0.40
Apical Rotation ($^{\circ}$)	7.04 $\pm$ 4.35	7.95 $\pm$ 5.54	0.63	6.13 $\pm$ 4.66	>0.99	0.42
Apical Rotation Rate ($^{\circ}$ /s)	46.3 $\pm$ 26.2	56.1 $\pm$ 30.1	0.05	42.0 $\pm$ 22.8	>0.99	0.04
Net Twist Angle ($^{\circ}$)	14.0 $\pm$ 6.4	16.9 $\pm$ 7.8	0.02	13.5 $\pm$ 6.3	>0.99	0.18
Diastolic Function						
<u>Longitudinal</u>						
Strain Rate (%/s)	0.95 $\pm$ 0.35	1.31 $\pm$ 0.52	< 0.001	1.07 $\pm$ 0.31	0.41	0.01
Velocity (cm/s)	-2.38 $\pm$ 0.81	-3.22 $\pm$ 1.36	< 0.001	-2.60 $\pm$ 1.14	0.84	0.09
<u>Circumferential</u>						
Basal Strain Rate (%/s)	1.42 $\pm$ 0.24	1.62 $\pm$ 0.39	< 0.001	1.55 $\pm$ 0.42	0.11	>0.99
Basal Rotation Rate ($^{\circ}$ /s)	56.7 $\pm$ 37.5	71.4 $\pm$ 40.5	0.03	73.3 $\pm$ 45.7	0.16	>0.99
Mid Strain Rate (%/s)	1.13 $\pm$ 0.18	1.13 $\pm$ 0.19	>0.99	1.18 $\pm$ 0.21	0.59	0.67
Apical Strain Rate (%/s)	1.45 $\pm$ 0.36	1.44 $\pm$ 0.39	>0.99	1.42 $\pm$ 0.34	>0.99	>0.99
Apical Rotation Rate ($^{\circ}$ /s)	-45.5 $\pm$ 27.7	-52.3 $\pm$ 41.8	0.57	-48.0 $\pm$ 35.3	>0.99	>0.99

Values as Mean $\pm$ Standard Deviation unless stated otherwise. *P* values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

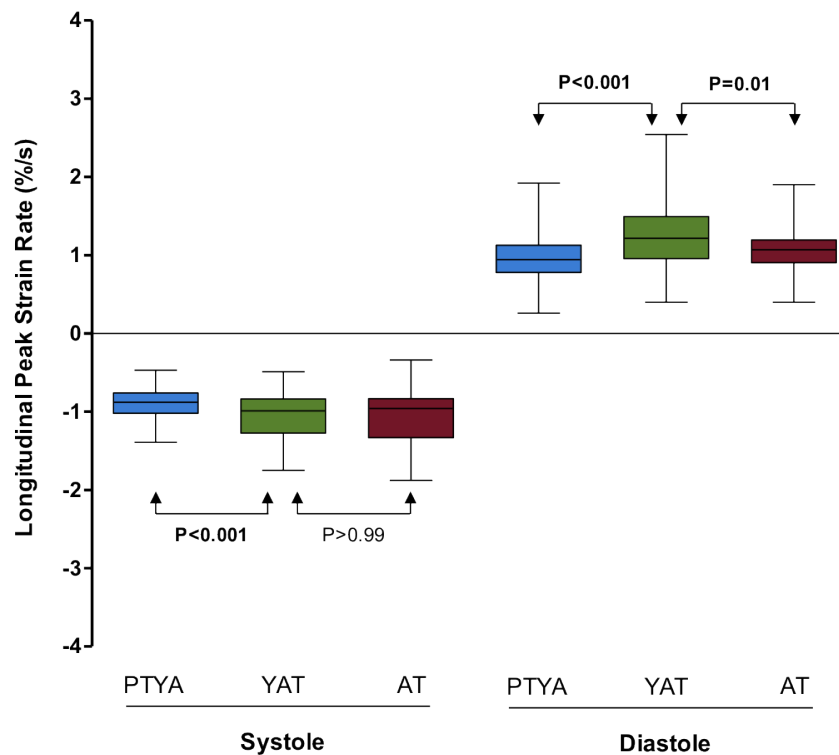


Figure 7.11: Longitudinal peak systolic and diastolic strain rate. Preterm-born young adults (PTYA, blue) have reduced longitudinal peak systolic strain rate (-0.90 ± 0.21 %/s) compared to both the young adults born term (YAT, green; -1.06 ± 0.31 %/s, $P < 0.001$) and the older term-born adults (AT, red; -1.08 ± 0.31 %/s, $P = 0.003$). However, while longitudinal peak diastolic strain rate in the preterm-born young adults was still reduced compared to the term-born young adults (0.95 ± 0.35 %/s vs 1.31 ± 0.52 %/s, $P < 0.001$), it did not differ from the older adults born term (1.07 ± 0.31 %/s, $P = 0.41$). Data shown as box and whisker plot: minimum to maximum.

characteristics that were significant in this model were included in a second model which incorporated potentially relevant cardiovascular risk factors in young adulthood (high density lipoprotein cholesterol, sex, waist-to-hip ratio, glucose, and brachial pulse pressure).

Gestational age was associated with left ventricular structure in the preterm group (Table 7.9). For left ventricular mass index, gestational age was the only independent predictor in model one ($P = 0.02$) and remained an independent predictor in model two ($P = 0.03$). For left ventricular length, gestational age and birth weight Z score were both independent predictors in model one ($P = 0.03$ and $P = 0.04$), but only gestational age remained significant in model two ($P = 0.02$ and $P = 0.11$). For end-diastolic volume index, gestational age approached significance in model one ($P = 0.08$) and was significant in model two ($P = 0.05$).

Interestingly, when functional variables were modeled, maternal preeclampsia, but not

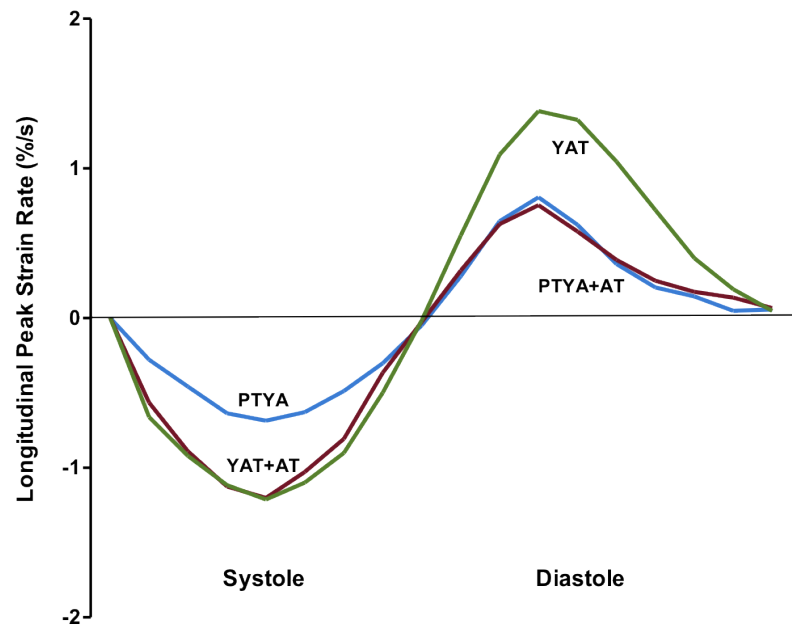


Figure 7.12: Example longitudinal strain rate curves for each group. Young adults born term and older adults born term showed similar longitudinal strain rates in systole, which differed from the preterm-born young adults. In diastole, longitudinal strain rates were similar for the preterm-born young adults and older adults born term, which differed from the young adults born term.

Table 7.8: Relationship of Variables with Left Ventricular Global Peak Longitudinal Systolic Strain in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	3.03	1.96	4.10	<0.001
Age	0.067	-0.21	0.35	0.64
Sex	-0.66	-1.83	0.50	0.26
Smoking	0.17	-1.29	1.63	0.82
Waist:Hip Ratio	1.38	-0.68	0.58	0.74
Total Cholesterol	0.31	-0.29	0.91	0.31
LDL Cholesterol	0.35	-0.38	1.07	0.35
HDL Cholesterol	-0.14	-1.64	1.36	0.85
Insulin	0.0071	-0.010	0.024	0.40
Glucose	1.01	-0.44	2.45	0.17
Triglycerides	0.036	-0.92	0.99	0.94
Brachial Systolic BP	0.069	0.019	0.12	0.008
Brachial Diastolic BP	0.045	-0.033	0.12	0.26
Brachial Mean Arterial Pressure	0.075	0.0010	0.15	0.05
Brachial Pulse Pressure	0.077	0.014	0.14	0.02
Aortic Systolic BP	0.075	0.022	0.13	0.006
Aortic Diastolic BP	0.047	-0.027	0.12	0.21
Aortic Mean Arterial Pressure	0.069	0.0010	0.14	0.05
Aortic Pulse Pressure	0.12	0.040	0.20	0.004

Bolded variables are statistically significant. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BP, blood pressure.

other variables, accounted for a proportion of variation in longitudinal peak systolic strain within the preterm group in model one ($P = 0.05$), and remained significant in model two ($P = 0.05$). Nearly a third ($n = 29$) of the cohort of preterm-born young adults were born to a pregnancy complicated by preeclampsia. The absolute global peak longitudinal systolic strain in those born preterm to a preeclamptic pregnancy was $-13.8 \pm 2.2\%$ compared to $-15.2 \pm 3.4\%$ in those born preterm to normotensive mothers ($P = 0.02$, Figure 7.13). This appeared to be additional to any impact of prematurity on longitudinal peak systolic strain, as individuals born preterm to normotensive pregnancies still showed a significant reduction in longitudinal peak systolic strain compared to term-born individuals of similar age ($P < 0.001$).

Table 7.9: Multivariable Regression Coefficient for Gestational Age in Multiple Regression Models of Key Left Ventricular Parameters

	Model 1				Model 2			
	B (/gestational week)	95% Confidence Interval		P Value	B (/gestational week)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
LV Mass Index (g/m^2)	-0.98	-1.92	-0.21	0.02	-0.93	-1.72	-0.14	0.03
LV Length (cm)*	0.078	0.01	0.154	0.03	0.068	0.011	0.125	0.02
LV End-Diastolic Volume (mL/m^2)	0.68	-0.09	1.44	0.08	0.77	0.02	1.56	0.05

Model 1 independent variables: gestational age, birth weight Z score, postnatal weight gain in the first two weeks, days of ventilation, and maternal preeclampsia.

Model 2 independent variables: gestational age, high density lipoprotein, sex, waist to hip ratio, glucose, and brachial pulse pressure.

*When modeling left ventricular length, birth weight Z score also reached significance in model 1 ($P = 0.04$) and was therefore included in model 2, but did not reach significance ($P = 0.11$).

7.4 Discussion

The findings from this chapter demonstrate for the first time that young adults born preterm have a unique adverse left ventricular structure and function. Left ventricular mass is significantly increased while the left ventricles are shorter, with reduced cardiac volumes and

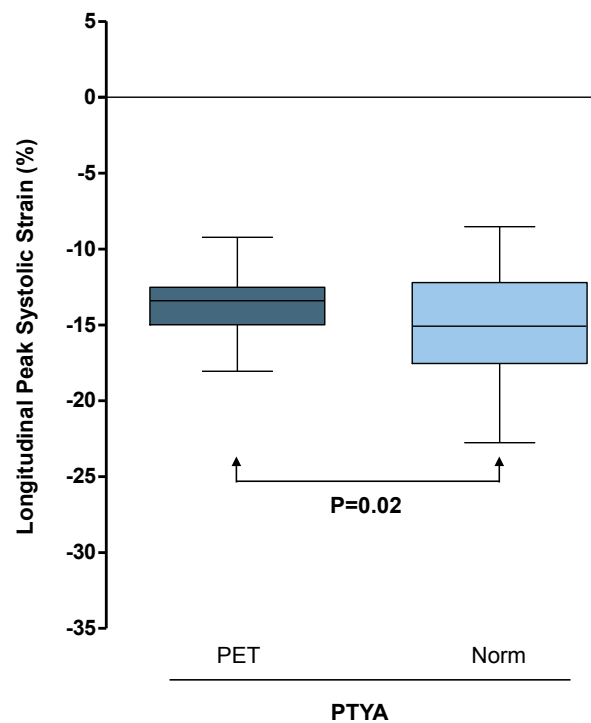


Figure 7.13: Longitudinal peak systolic strain for preterm-born young adults (PTYA). Preterm-born young adults exposed to preeclampsia (PET, darker blue) have reduced longitudinal peak systolic strain compared to preterm-born young adults born to a normotensive pregnancy (Norm, lighter blue) (-13.8 ± 2.2 vs -15.2 ± 3.4 %, $P = 0.02$). Data shown as box and whisker plot: minimum to maximum.

apical displacement. Furthermore, preterm birth is associated with reductions in systolic, diastolic, and rotational function, both in systole and diastole. The variation in left ventricular structure appears to be determined by preterm birth, with the severity of changes graded according to the degree of prematurity, and not by any specific associated perinatal exposures such as preeclampsia, variation in birth weight, exposure to mechanical ventilation or growth. In contrast, maternal preeclampsia had an impact on left ventricular systolic function in addition to that associated with preterm birth.

Increased left ventricular mass index is an independent predictor for cardiovascular morbidity and mortality [232]. The average 19 gram higher left ventricular mass in young adults born preterm is equivalent to that associated with a 9 kg/m^2 increase in body mass index [233], and in longitudinal studies would equate to greater than 50% increased risk of cardiovascular clinical events in later adult life [234; 235]. The major cardiovascular risk previously identified in those born preterm is their higher blood pressure [51], which could have ac-

counted for a proportion of the increased left ventricular mass. However, it was found that for any given level of blood pressure, preterm birth associates with additional significant increases in left ventricular mass and that the increase in left ventricular mass is graded according to the degree of prematurity independent of other perinatal factors. The presence of increased left ventricular mass in those with hypertension or prehypertension is known to have independent prognostic significance [235; 236], and those born preterm appear to have a disproportionate increase in left ventricular mass relative to their blood pressure.

At birth, there is a change in cardiomyocyte development from a fetal to adult arrangement [98; 237], which coincides with an increase in cardiac output and left ventricular end-diastolic pressure [109; 238; 239]. In preterm birth, this occurs before complete *in utero* development and, in lambs born preterm, exposure of a relatively immature heart to the sustained increase in afterload and fluctuating preload of postnatal circulation resulted in accelerated cardiomyocyte hypertrophy while cardiomyocyte number remained static [111; 232]. Consistent with this experimental data, Kozák-Bárány *et al.* [237] found left ventricular mass in humans born preterm increases 56% in the first month postnatally compared to 35% in those born at term. It is possible the increase in those born preterm merely reflected the expected *in utero* cardiac growth rate for this point in development. However, my data are consistent with the rapid increase in neonatal left ventricular mass being a pathological event that persists into adult life.

The decision to use novel computational techniques to build the first atlas of the adult preterm heart provided a valuable tool to capture and analyse three-dimensional variations in left ventricular geometry in addition to changes in mass. The method that was applied was chosen as it, for the first time, allowed the use of a standard 12 slice left ventricular short axis stack rather than full anatomical (isotropic) cardiovascular magnetic resonance scans. This meant some smoothing of the meshes occurred to account for slice gaps and shifts, but is potentially more widely clinically applicable than other mesh creation strategies. The atlas principal component analysis avoided specific presuppositions about likely measures of relevance and the findings demonstrate that it was powerful enough to identify and quantify variation in a range of geometric factors, such as apical position, that would not have been

captured with standard clinical measures. The last trimester is important for cardiac growth and it seems plausible that the short ventricle and reduced cavity size relates to the interruption in development and flow changes seen at preterm birth [237]. Changes in flow patterns within the heart could additionally account for alterations in apical position. However, apical position was also displaced in older adults suggesting other factors may be relevant.

Preterm-born lambs have a five- to seven-fold increase in interstitial, but not perivascular, collagen deposition [111], characteristic of myocardial pressure overload [232]. The inflammatory cascade of preterm labour has also been proposed to lead to myocardial fibrosis [240; 241]. These myocardial patterns would be expected to result in changes in diastolic relaxation, as observed in the preterm-born young adults [242]. The older adults had similar reductions in diastolic function, and it is possible there are changes in extracellular matrix, as well as cardiomyocyte appearance and function, within the preterm group, equivalent to changes observed with ageing.

In addition to diastolic abnormalities, preterm-born young adults showed unique changes in longitudinal, circumferential, and rotational systolic function [243]. In addition, exposure to preeclampsia is associated with further reductions in left ventricular systolic strain. Preeclampsia is indicative of placental pathology and insufficiency, which may not be a feature of other pregnancies that lead to preterm birth. Furthermore, offspring of preeclamptic pregnancies develop with reduced uterine perfusion and relative hypoxia from early in gestation [142]. In newborn pigs, short-term exposure to hypoxaemia leads to sustained reduction in longitudinal peak systolic strain [244]. This is thought to be because longitudinal movement is primarily mediated by subendocardial and subepicardial fibres, which are most susceptible to ischaemia [245; 246]. While animal models of preeclampsia and pregnancy hypoxia have consistently shown cardiac effects in the offspring, this is the first study in humans to confirm a long-term cardiac specific impact of preeclampsia.

As mentioned in Chapter 2, it was not possible to follow-up all those initially recruited to the preterm infant study in the 1980s. Nevertheless, there were no significant differences in demographics or perinatal records between those who took part and the full cohort to suggest any selection bias was introduced (see Table 2.1, Chapter 2). I can be confident of

this, as a major strength of the study is the prospective nature of data collection with very detailed information collected at birth. This also allowed me to study the impact of key perinatal factors on later cardiac structure. Previous work has shown that birth weight is a risk factor for coronary heart disease in later life in term-born individuals [163]. It will be of interest to determine whether the haemodynamic changes seen in small for gestational age term-born individuals also lead to cardiac remodeling patterns similar to those that have been seen in preterm-born young adults. The study size, although the largest cardiac study of preterm-born individuals, meant I remained conservative with the number of factors included in the multiple regression analyses. It is likely that other key parameters that may influence left ventricular development will be of interest to investigators and will require subsequent investigation. At present I have not included left ventricular radial strain results. Reproducibility of radial strain analysis is currently relatively poor [247], which I showed in Table 3.6 in Chapter 3, and further development is needed before results can be confidently reported.

7.5 Conclusion

In summary, this work demonstrates for the first time that individuals born preterm have a unique left ventricular geometry and function. Whether interventions to modify these variations in left ventricular structure and function prevent development of cardiac disease in a growing subgroup of the population will be of future interest. It is known that lifestyle and pharmacological interventions can be beneficial in older people with cardiovascular diseases that have increased left ventricular mass or reduced left ventricular function. Drug interventions are not likely to be suitable for younger people for the prevention of future problems. However, it is known that loss of weight in young obese individuals improves left ventricular size and function. Therefore, it is of interest to know whether lifestyle in young adulthood, or nutrition during the first months of life, may be of relevance to optimise the long-term cardiac health of preterm-born individuals.

8

RIGHT VENTRICLE STRUCTURE AND FUNCTION

This chapter was designed to determine whether preterm-born young adults have a unique right ventricular structure and function. Cardiovascular magnetic resonance was used to compare the young adult preterm-born cohort (*cohort 1*); compared to the young adult term-born cohort (*cohort 2*); and the term-born adults a decade older (*cohort 3*). The data showed that the preterm-born young adults have a unique right ventricular structure and function. These changes are related to gestational age and postnatal lung health, with stronger associations with the degree of prematurity than for the left ventricle. I have published this work in *Circulation* as the first author [162].

8.1 Introduction

The observations made in Chapter 7 that preterm-born young adults have distinct differences in left ventricular size, function, and mass [161] are consistent with earlier animal studies that identified abnormal maturation and accelerated postnatal cardiomyocyte hypertrophy related to preterm birth [111]. Altered cardiomyocytes were also found in right

ventricular tissue [111]. It was therefore hypothesised that preterm birth would be associated with differences in right ventricular size and function in humans.

In addition, it was predicted that preterm birth would have a greater impact on the right ventricle than the left. This is because the immature, preterm right ventricular cardiomyocytes will have needed to undergo more extensive remodeling at birth [111]. During the postnatal period, the right ventricle switches from being a thick walled chamber, that provides 66% of cardiac output into the systemic circulation through the ductus arteriosus, to being the thin walled, crescent shaped chamber that supplies the lower pressure pulmonary circulation [248; 249; 250; 251].

To be able to test these hypotheses, accurate quantification of the complex geometry of the adult right ventricle was required. Cardiovascular magnetic resonance provides the optimal imaging modality for right ventricular assessment with its capability for high resolution, multiplanar images that can be analysed without reliance on geometric assumptions [166]. Standardised guidelines have been developed for accurate characterisation of right ventricular volumes and mass with cardiovascular magnetic resonance [166; 252; 253; 254]. These have been used to study for the first time the long-term impact of preterm birth and related perinatal factors on the right ventricle.

8.2 Methods

8.2.1 Study Population

This chapter includes all 102 young adults born preterm (*cohort 1*), 102 young adults born term frequency matched for age and sex (*cohort 2*), and 30 adults a decade older frequency matched for sex (*cohort 3*). Details of these three cohorts are outlined in Chapter 2.

8.2.2 Study Visit

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity

and blinded analysis. The following methods were used for this chapter as described in detail in Chapter 3:

- 3.2: Cardiovascular Magnetic Resonance

- 3.2.2: Cardiac Imaging

- 3.2.2.1: Cardiac Cine Acquisition

- 3.2.2.2: Right Ventricular Mass and Volumes

- 3.2.2.4: Right Ventricular Dimensions

- 3.2.2.6: Right Ventricular Myocardial Deformation

- 3.4: Anthropometry and Lifestyle Questionnaire
- 3.5: Blood Sampling and Biochemistry
- 3.7: Brachial and Aortic Blood Pressure Measurements

8.2.3 Statistical Analysis

Statistical analysis was carried out using SPSS Version 19 as described in Section 3.9 of Chapter 3. 102 subjects per young adult group provided 80% power at $P = 0.05$ to identify a 0.38 standard deviation difference between young adult groups, equivalent to a 1.34 g/m^2 difference in right ventricular mass and 4.75 mL/m^2 difference in end-diastolic volume, and a 0.58 standard deviation difference between each young adult group and the older adults. Where multiple comparisons were performed between groups, P values were adjusted using the Bonferroni method. Comparisons between preterm-born young adults with term-born young adults were adjusted for age and sex, while comparisons between preterm-born young adults and term-born young adults with the older term-born older adults were adjusted for sex. To determine the relative difference compared to the mean for cardiac measures per gestational week, bivariable regression analyses were performed with the cardiac parameter as the dependent variable and gestational age as the independent variable. The unstandardised regression coefficient (B) was then calculated as a percentage of the

mean of that variable for the preterm group (i.e. for right ventricular mass index, $B = -0.67 \text{ g/m}^2/\text{gestational week}$ and mean = 24.5 g/m^2 , therefore the relative decrease compared to the mean per gestational week was $0.67/24.5 \times 100\% = 2.74\%$).

8.3 Results

8.3.1 Study Population

In adult life, there were no significant differences in number of smokers, personal and family medical history, or lifestyle factors such as socioeconomic status, physical activity or diet ($P > 0.05$). Adults born preterm had a distinct metabolic profile, and brachial blood pressure parameters were similar to individuals a decade older, and significantly higher than term-born young adults (Table 8.1).

8.3.2 Right Ventricular Geometry

Preterm-born young adults had smaller right ventricles with reduced right ventricular end-diastolic volumes compared to both the younger and older groups of term-born adults (Table 8.2). This difference was also demonstrated by significantly reduced right ventricular end-diastolic length and right ventricular internal diameter in the mid-cavity (Table 8.2).

Despite smaller cavities, both the absolute and indexed right ventricular masses were increased in the preterm-born young adults ($24.5 \pm 3.5 \text{ g/m}^2$) compared to the term-born young adults ($20.4 \pm 3.4 \text{ g/m}^2$, $P < 0.001$), who had a similar mean right ventricular mass index to the older term-born adults ($21.4 \pm 3.3 \text{ g/m}^2$, $P > 0.99$) (Figure 8.1). The increase in right ventricular mass index in the preterm-born young adults was inversely related to gestational age ($r = -0.47$, $P < 0.001$). When the preterm-born group was divided by categories of prematurity, there was a significant incremental elevation in right ventricular mass of around 10% between each group (i.e. extremely preterm > very preterm > moderate to late preterm, $P < 0.001$) (Figure 8.1).

Table 8.1: Characteristics of Cohorts

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	P Value Ψ	Term-born adults (n = 30)	P Value Υ	P Value Δ
Demographics & Anthropometrics						
Gestational Age (weeks)	30.3 ± 2.5	39.6 ± 0.9	<0.001	39.8 ± 0.8	<0.001	>0.99
Birth Weight (g)	1297.0 ± 286.8	3460.0 ± 417.0	<0.001	3301.9 ± 423.9	<0.001	0.33
Age (years)	25.1 ± 1.4	25.0 ± 2.6		35.5 ± 1.8		
Males, n (%)	47 (46.1)	47 (46.1)		14 (46.7)		
BMI (kg/m ²)	24.9 ± 5.4	22.9 ± 3.1	0.003	27.1 ± 9.6	0.33	<0.001
Smokers, n (%)	20 (19.6)	20 (19.6)	>0.99	3 (10.0)	0.63	0.63
Biochemistry						
Total Cholesterol (mmol/L)	4.89 ± 1.05	4.23 ± 0.86	<0.001	5.48 ± 1.18	0.03	<0.001
HDL-C (mmol/L)	1.53 ± 0.40	1.47 ± 0.41	0.54	1.46 ± 0.39	0.98	>0.99
LDL-C (mmol/L)	2.81 ± 0.93	2.37 ± 0.66	<0.001	3.39 ± 1.00	0.02	<0.001
Triglycerides (mmol/L)	1.12 ± 0.79	0.87 ± 0.40	0.02	1.38 ± 0.91	0.39	0.003
Glucose (mmol/L)	5.01 ± 0.43	4.61 ± 0.30	<0.001	5.65 ± 1.60	0.003	<0.001
Insulin (pmol/L)	64.4 ± 42.8	35.6 ± 15.9	<0.001	104.4 ± 89.0	0.006	<0.001
Blood Pressure (mmHg)						
Systolic	121.3 ± 10.9	112.9 ± 10.1	<0.001	121.7 ± 13.5	>0.99	<0.001
Diastolic	73.0 ± 7.2	68.8 ± 7.0	<0.001	73.9 ± 6.0	>0.99	<0.001
Mean Arterial Pressure	89.1 ± 7.4	83.5 ± 7.1	<0.001	89.8 ± 7.5	>0.99	<0.001
Pulse Pressure	48.4 ± 9.1	44.1 ± 8.5	0.001	47.8 ± 11.3	>0.99	0.12

BMI indicates body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

Values as Mean ± Standard Deviation unless stated otherwise. P values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

Two separate linear regression models were used to take account of other potential factors related to right ventricular end-diastolic volume index and right ventricular mass index, comparing preterm-born and term-born young adult groups. Within sets of interrelated variables, such as blood pressure and biochemistry, the variable with the strongest correlation was included. For right ventricular end-diastolic volume, six independent variables were included in the model (sex, waist to hip ratio, high density lipoprotein cholesterol, insulin, aortic diastolic blood pressure, and preterm birth as a dichotomous categorical variable with the term-born young adults as '1' and the preterm-born young adults as '2') (Table

Table 8.2: Right Ventricular Volumes and Dimensions

	Preterm-born young adults (n = 102)	Term-born young adults (n = 102)	<i>P</i> Value Ψ	Term-born adults (n = 30)	<i>P</i> Value Υ	<i>P</i> Value Δ
ED Volume (mL/m ²)	79.8 ± 13.2	88.5 ± 11.8	<0.001	87.9 ± 16.6	0.01	>0.99
ES Volume (mL/m ²)	34.1 ± 7.2	35.6 ± 7.7	0.35	34.9 ± 9.7	>0.99	>0.99
Stroke Volume (mL/m ²)	45.8 ± 10.5	52.9 ± 7.2	<0.001	53.0 ± 9.4	0.003	>0.99
ED Length (cm)	8.47 ± 0.81	8.97 ± 0.76	<0.001	8.97 ± 0.76	0.006	>0.99
Internal Diameter 1 (cm)	3.08 ± 0.52	3.16 ± 0.51	0.68	3.14 ± 0.57	>0.99	>0.99
Internal Diameter 2 (cm)	4.28 ± 0.52	4.63 ± 0.55	<0.001	4.60 ± 0.56	0.007	>0.99

ED indicates end-diastolic; ES, end-systolic; Internal Diameter 1, the internal diameter measurement within the basal third of the right ventricle; Internal Diameter 2, the internal diameter measurement at the level of the right ventricular papillary muscles.

Values as Mean ± Standard Deviation unless stated otherwise. *P* values were adjusted using the Bonferroni method.

Ψ = Preterm-born young adults vs term-born young adults. Comparisons adjusted for age and sex.

Υ = Preterm-born young adults vs term-born adults. Comparisons adjusted for sex.

Δ = Term-born young adults vs term-born adults. Comparisons adjusted for sex.

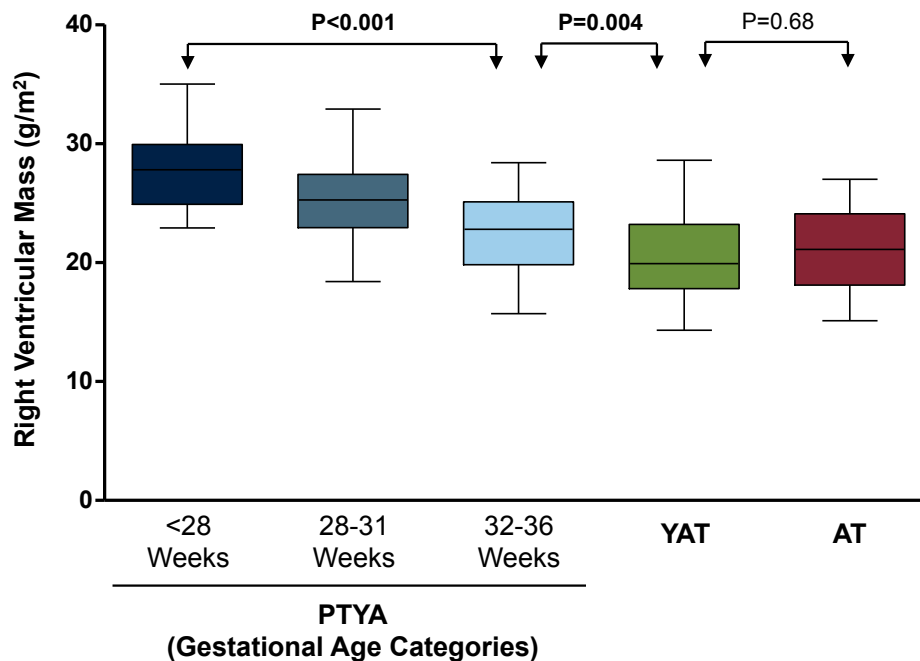


Figure 8.1: Elevated right ventricular mass in preterm-born young adults. Preterm-born young adults (PTYA) were divided by gestational age category. Right ventricular mass index was higher in the more preterm gestational age categories (ANOVA $P < 0.001$). Right ventricular mass index tended to be greater in the extremely preterm group (<28 weeks, $n = 14$; 27.1 ± 3.3 g/m²) than the very preterm group (28 to 31 weeks, $n = 56$; 25.1 ± 3.1 g/m², $P = 0.14$), which was significantly greater than in the moderate to late preterm group (32 to 36 weeks, $n = 32$; 22.5 ± 3.2 g/m², $P < 0.001$). Right ventricular mass index was increased in the preterm-born young adults (24.5 ± 3.5 g/m²) compared to both the term-born young adults (YAT, green; 20.4 ± 3.4 g/m², $P < 0.001$) and term-born adults a decade older (AT, red; 21.4 ± 3.3 g/m², $P < 0.001$). Data shown as box and whisker plot: minimum to maximum.

8.3). In this model, preterm birth remained an independent predictor of right ventricular end-diastolic volume index ($B = -5.71 \text{ mL/m}^2/\text{group}$, $P = 0.002$, 95% confidence interval: -9.28 to -2.15). For right ventricular mass index, six independent variables were included in the model (sex, smoking, high density lipoprotein cholesterol, glucose, aortic pulse pressure, and preterm birth) (Table 8.4). Preterm birth once again remained an independent predictor of right ventricular mass index in this model ($B = 3.87 \text{ g/m}^2/\text{group}$, $P < 0.001$, 95% confidence interval: 2.73 to 5.02).

Table 8.3: Relationship of Variables with Right Ventricular End-Diastolic Volume Index in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	8.71	5.21	12.2	0.002
Age	0.15	-0.74	1.04	0.73
Sex	-11.52	-14.9	-8.17	<0.001
Smoking	-1.96	-6.62	2.69	0.41
Waist:Hip Ratio	36.0	10.2	61.7	0.006
Total Cholesterol	-2.71	-4.57	-0.84	0.005
LDL Cholesterol	-1.85	-4.15	0.45	0.11
HDL Cholesterol	-8.91	-13.5	-4.31	<0.001
Insulin	-0.13	-0.18	-0.078	<0.001
Glucose	-5.06	-9.61	-0.50	0.03
Triglycerides	1.38	-1.65	4.42	0.37
Brachial Systolic BP	-0.0031	-0.67	0.16	0.98
Brachial Diastolic BP	-0.46	-0.71	0.22	<0.001
Brachial Mean Arterial Pressure	-0.28	-0.52	-0.047	0.02
Brachial Pulse Pressure	0.25	-0.015	0.52	0.06
Aortic Systolic BP	-0.18	-0.35	-0.0061	0.04
Aortic Diastolic BP	-0.52	-0.74	-0.29	<0.001
Aortic Mean Arterial Pressure	-0.41	-0.62	-0.19	<0.001
Aortic Pulse Pressure	0.31	0.11	0.51	0.003

Bolded variables are statistically significant. LDL indicates low density lipoprotein; HDL, high density lipoprotein; BP, blood pressure.

8.3.3 Right Ventricular Function

Stroke volume was reduced in preterm-born young adults compared to both term-born young adults ($P < 0.001$) and older term-born adults ($P = 0.003$) (Table 8.2). Similarly, ejection fraction was significantly reduced in preterm-born young adults ($57.0 \pm 7.6\%$) compared to term-born young adults ($60.0 \pm 5.3\%$, $P = 0.006$) and term-born adults a decade older

Table 8.4: Relationship of Variables with Right Ventricular Mass Index in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	4.12	3.15	5.09	<0.001
Age	0.053	-0.22	0.32	0.70
Sex	-1.90	-3.03	-0.84	<0.001
Smoking	1.44	0.036	2.85	0.04
Waist:Hip Ratio	3.04	-4.93	11.01	0.45
Total Cholesterol	-0.018	-0.60	0.56	0.95
LDL Cholesterol	0.072	-0.63	0.78	0.84
HDL Cholesterol	-1.68	-3.11	-0.24	0.02
Insulin	0.004	-0.013	0.021	0.65
Glucose	1.97	0.60	3.35	0.005
Triglycerides	0.23	-0.70	1.15	0.63
Brachial Systolic BP	0.13	0.086	0.18	<0.001
Brachial Diastolic BP	0.074	-0.021	0.15	0.06
Brachial Mean Arterial Pressure	0.14	0.066	0.21	<0.001
Brachial Pulse Pressure	0.16	0.016	0.21	<0.001
Aortic Systolic BP	0.13	0.077	0.18	<0.001
Aortic Diastolic BP	0.048	-0.024	0.12	0.19
Aortic Mean Arterial Pressure	0.099	0.032	0.17	0.004
Aortic Pulse Pressure	0.24	0.17	0.31	<0.001

Bolded variables are statistically significant. LDL indicates low density lipoprotein; HDL, high density lipoprotein; BP, blood pressure.

($60.3 \pm 5.3\%$, $P = 0.04$) (Figure 8.2). Although only a 3% absolute difference in mean ejection fraction between groups, there was a greater spread of values in the group of young adults born preterm such that 21% of preterm-born individuals had an ejection fraction below the lower limit of normality defined by the young adult control group. Indeed, six out of the 102 preterm-born individuals (5.88%) had values that fell below the clinically accepted normal range ($<45\%$) and would be defined as having mild right ventricular systolic dysfunction (range: 41.7 to 44.6%). Furthermore, 15.7% of the preterm-born young adults fell below the 95% confidence interval for right ventricular ejection fraction defined by Maceira *et al.* [255], with none of the term-born individuals falling below this interval. In a linear regression model for right ventricular ejection fraction, only two independent variables were included in the model (aortic pulse pressure and preterm birth as a dichotomous categorical variable with the term-born young adults as '1' and the preterm-born young adults as '2') as they were the only two variables that reached significance in bivariable regression with ejection fraction (Table 8.5). Preterm birth remained an independent predictor of ejection fraction

($B = -2.46\%/group$, $P = 0.02$, 95% confidence interval: -4.49 to -0.44).

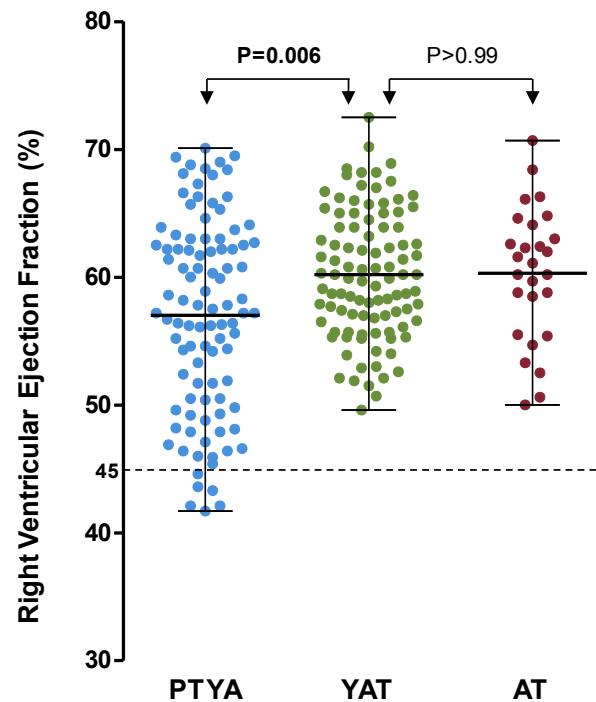


Figure 8.2: Reduced ejection fraction in preterm-born young adults. Ejection fraction was significantly reduced in preterm-born young adults (PTYA, blue; $57.0 \pm 7.6\%$) compared to term-born young adults (YAT, green; $60.0 \pm 5.3\%$, $P = 0.006$) and term-born adults a decade older (AT, red; $60.3 \pm 5.3\%$, $P = 0.04$). The dotted line indicates the lower range of normal right ventricular ejection fraction (45%). Six out of the 102 preterm-born individuals (5.88%) had an ejection fraction $<45\%$. Data shown as individual values with mean and range: minimum to maximum.

In agreement with the reduction in right ventricular ejection fraction, right ventricular shortening was reduced in preterm-born young adults ($21.4 \pm 4.7\%$) compared to both term-born groups (young adults: $25.5 \pm 4.1\%$, $P < 0.001$; older adults: $25.1 \pm 4.9\%$, $P < 0.001$). Furthermore, longitudinal peak systolic strain and peak systolic strain rate were reduced in preterm-born young adults (strain: $-17.5 \pm 3.4\%$; strain rate: $-1.03 \pm 0.23\%/s$) compared to the term-born young adults (strain: $-20.5 \pm 3.3\%$, $P < 0.001$; strain rate: $-1.31 \pm 0.29\%/s$, $P < 0.001$) and the older term-born adults (strain: $-19.8 \pm 2.9\%$, $P = 0.002$; strain rate: $-1.23 \pm 0.33\%/s$, $P < 0.001$) (Figure 8.3, Panels A and B). Interestingly, peak diastolic strain rate was also reduced in preterm-born young adults ($1.09 \pm 0.30\%/s$) compared to the term-born

Table 8.5: Relationship of Variables with Right Ventricular Ejection Fraction in Preterm-Born and Term-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Preterm Birth	-2.94	-4.78	-1.11	0.002
Age	0.25	-0.20	0.70	0.27
Sex	1.23	-0.65	3.10	0.20
Smoking	0.61	-1.76	2.97	0.61
Waist:Hip Ratio	2.11	-11.2	15.4	0.76
Total Cholesterol	0.20	-0.77	1.17	0.68
LDL Cholesterol	0.32	-0.85	1.50	0.59
HDL Cholesterol	-1.29	-3.71	1.13	0.29
Insulin	0.021	-0.0061	0.049	0.13
Glucose	-0.53	-2.87	1.80	0.65
Triglycerides	0.25	-1.29	1.79	0.75
Brachial Systolic BP	-0.016	-0.099	0.068	0.71
Brachial Diastolic BP	0.037	-0.091	0.17	0.57
Brachial Mean Arterial Pressure	0.074	-0.021	0.15	0.06
Brachial Pulse Pressure	0.074	-0.021	0.15	0.06
Aortic Systolic BP	-0.032	-0.12	0.055	0.47
Aortic Diastolic BP	0.060	-0.060	0.18	0.33
Aortic Mean Arterial Pressure	0.018	-0.095	0.13	0.75
Aortic Pulse Pressure	-0.16	-0.29	-0.022	0.02

Bolded variables are statistically significant. LDL indicates low density lipoprotein; HDL, high density lipoprotein; BP, blood pressure.

young adults ($1.28 \pm 0.43\%/s$, $P = 0.001$), who had similar rates to the older adult group (1.22 ± 0.32 , $P > 0.99$).

8.3.4 Comparisons Between the Right and Left Ventricles

The changes observed in right ventricular size and function in preterm-born young adults were then compared to those previously reported in the same individuals for the left ventricle [161]. Right ventricular dimensions and volumes were very closely related to the equivalent left ventricular measures (end-diastolic volume: $r = 0.73$, $P < 0.001$; length: $r = 0.85$, $P < 0.001$); consistent with preterm birth being associated with global changes in myocardial dimensions. There was a more moderate agreement for mass ($r = 0.41$, $P < 0.001$), and the amount of variation in ventricular mass accounted for by preterm birth was greater for the right ventricle than for the left ventricle. For each week shorter the gestation, there was a 2.74% relative increase in right ventricular mass, compared to a 1.47% increase in left ven-

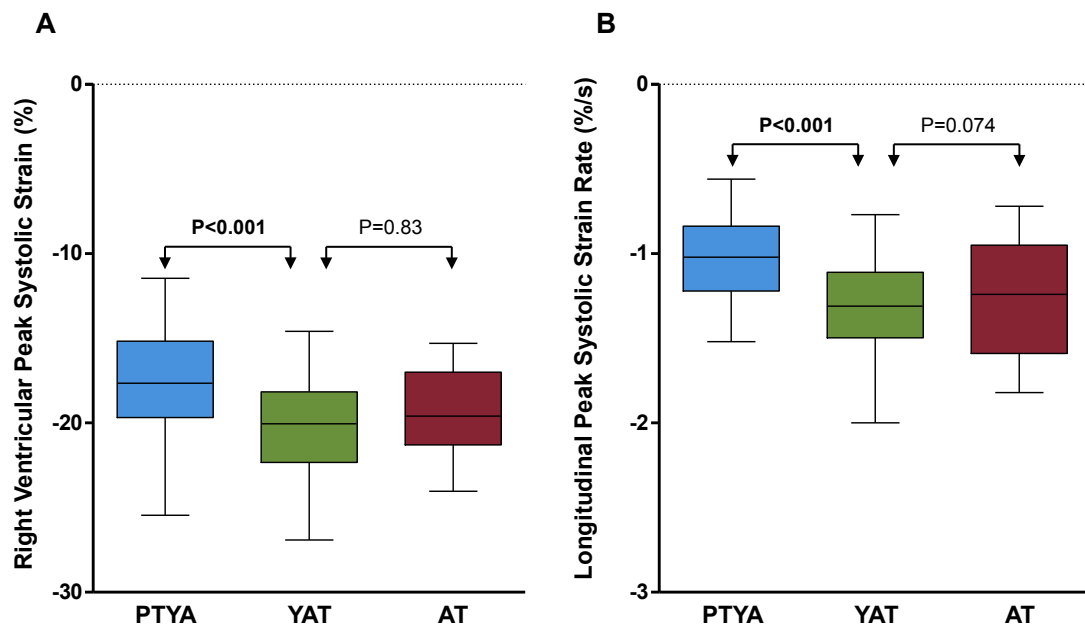


Figure 8.3: Reduced peak systolic strain and strain rate in preterm-born young adults. **A**, Longitudinal peak systolic strain was reduced in preterm-born young adults ($-17.5 \pm 3.4\%$) compared to the term-born young adults ($-20.5 \pm 3.3\%$, $P < 0.001$) and the older term-born adults ($-19.8 \pm 2.9\%$, $P = 0.002$). **B**, Longitudinal peak systolic rate was reduced in preterm-born young adults ($-1.03 \pm 0.23\%/s$) compared to the term-born young adults ($-1.31 \pm 0.29\%/s$, $P < 0.001$) and the older term-born adults ($-1.23 \pm 0.33\%/s$, $P < 0.001$). Data shown as box and whisker plot: minimum to maximum.

tricular mass (Figure 8.4). Stroke volume was similar between ventricles (45.8 ± 10.5 vs 46.6 ± 7.4 mL/m², $P = 0.53$), and there was a moderate agreement in systolic ejection fraction between left and right ventricles ($r = 0.40$, $P < 0.001$). Although preterm birth had been associated with changes in myocardial deformation in the left ventricle, there were no differences in ejection fraction. In contrast, there was a significant 2.51% relative decline in right ventricular ejection fraction in adult life for each week shorter the gestation of the individual; consistent with a greater impact of preterm birth on the right ventricle than the left ventricle.

8.3.5 Perinatal Risk Factors and the Right Ventricle

Bivariable regression analyses were then performed to identify key perinatal and adult factors related to right ventricular mass index, end-diastolic volume index, and ejection fraction (Table 8.6). As the preterm-born individuals had been randomised to milk feeding regimes

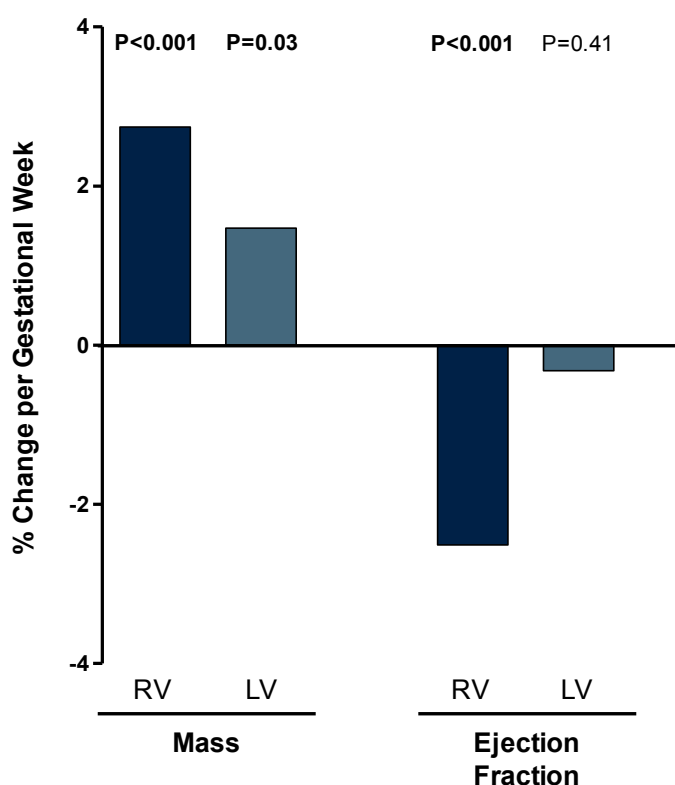


Figure 8.4: Percent change per gestational week in cardiac measures in the preterm-born group. For each week shorter the gestation, there was a 2.74% ($P < 0.001$) relative increase in right ventricular mass index in young adulthood, compared to a 1.47% ($P = 0.03$) increase in left ventricular mass index. For ejection fraction, there was a significant 2.51% ($P < 0.001$) relative decline in right ventricular ejection fraction in adult life for each week shorter the gestation of the individual, with no significant decline for the left ventricle ($P = 0.41$).

at birth, it was determined by bivariable regression analyses whether the dietary group (as a dichotomous categorical variable with banked breast milk as ‘1’ and formula milk as ‘2’) related to right ventricular structural and functional measures in young adulthood. It was determined that the dietary group did not relate to differences in right ventricular measures (Table 8.6).

The results of these independent bivariable regression analyses were used to select variables for multivariable regression analyses. As the sample size was 102 individuals, the number of variables was limited in each model to the five strongest related in the bivariable regression analyses. Of perinatal factors, gestational age, birth weight Z score, postnatal days of ventilation, antenatal glucocorticoid exposure, and presence of a clinically significant patent ductus arteriosus in the neonatal period were included in the first model. Any

Table 8.6: Bivariable Regression Coefficient for Perinatal and Adult Risk Factors and Key Right Ventricular Parameters

	Mass Index		End-Diastolic Volume Index		Ejection Fraction	
	Coefficient (B)	P Value	Coefficient (B)	P Value	Coefficient (B)	P Value
Perinatal Factors						
Gestational Age	-0.67	<0.001	1.39	0.01	1.43	<0.001
Birth Weight Z Score	0.70	0.02	-1.63	0.15	-1.92	0.002
Mode of Delivery	-0.60	0.44	0.97	0.23	2.70	0.34
Ventilation	0.19	<0.001	-0.18	0.33	-0.25	0.02
Patent Ductus Arteriosus	1.05	0.23	-7.47	0.02	-3.16	0.09
Apgar Score (1 min)	-0.24	0.14	0.10	0.87	0.59	0.08
Apgar Score (5 mins)	-0.29	0.09	-0.59	0.36	0.40	0.28
Postnatal Infections	0.56	0.76	0.72	0.92	-1.92	0.37
Maternal Preeclampsia	-0.99	0.21	2.48	0.40	1.11	0.52
Antenatal Steroid Exposure	1.90	0.04	1.23	0.72	-3.65	0.07
Received Postnatal Intravenous Lipids	0.027	0.51	-0.16	0.28	-0.057	0.52
Trial Diet Group	0.38	0.60	-0.75	0.46	-1.10	0.69
Maternal Smoking (1st Trimester)	0.015	0.76	0.29	0.11	-0.055	0.60
Maternal Smoking (2nd & 3rd Trimesters)	0.071	0.28	0.17	0.50	-0.051	0.72
Adult Factors						
Smoking	0.74	0.10	-1.38	0.68	-0.73	0.71
Waist:Hip Ratio	1.58	0.72	45.6	0.005	9.12	0.34
Sex	-1.27	0.07	-11.7	<0.001	0.29	0.86
Total Cholesterol	-0.62	0.08	-0.27	0.84	0.33	0.67
LDL Cholesterol	-0.68	0.09	0.16	0.92	0.34	0.70
HDL Cholesterol	-1.52	0.11	-7.41	0.06	-2.95	0.15
Insulin	-0.019	0.03	-0.065	0.05	0.044	0.02
Glucose	-0.041	0.96	2.00	0.55	1.43	0.46
Triglycerides	-0.31	0.52	2.95	0.08	0.53	0.61
Brachial SBP	0.10	0.002	0.17	0.17	0.092	0.20
Brachial DBP	0.089	0.073	-0.11	0.55	0.17	0.11
Brachial MAP	0.099	0.05	0.045	0.80	0.17	0.09
Brachial PP	0.083	0.03	0.31	0.03	0.017	0.84
Aortic SBP	0.072	0.05	0.14	0.31	0.11	0.15
Aortic DBP	0.041	0.41	-0.22	0.22	-0.073	0.51
Aortic MAP	0.066	0.16	-0.064	0.72	0.23	0.03
Aortic PP	0.13	0.002	0.54	0.004	0.26	0.01

Bolded *P* values are statistically significant (<0.05). Coefficient (B) indicates the unstandardised bivariable regression coefficient; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.

Table 8.7: Multivariable Regression Coefficient for Gestational Age in Multiple Regression Models of Key Right Ventricular Parameters

	Model 1				Model 2			
	B (/gestational week)	95% Confidence Interval		P Value	B (/gestational week)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
RV Mass Index (g/m ²)*	-0.52	-0.88	-0.16	0.003	-0.48	-0.79	-0.17	0.003
RV ED Volume Index (mL/m ²)	1.33	-0.15	2.81	0.08	1.80	0.83	2.77	<0.001
RV Ejection Fraction (%)	1.17	0.37	1.96	0.005	1.31	0.70	1.92	<0.001

Model 1 independent variables: gestational age, birth weight Z score, days of ventilation, antenatal glucocorticoid exposure, and clinically significant patent ductus arteriosus.

Model 2 independent variables: gestational age, sex, waist-to-hip ratio, insulin, and aortic pulse pressure.

RV indicates right ventricle; ED, end-diastolic.

*When modeling right ventricular mass index, ventilation also reached significance in model 1 ($P = 0.03$) and was therefore included in model 2, nearly reaching significance ($P = 0.06$).

significant independent characteristics were then carried over to a second model that incorporated four adult factors related to right ventricular indices: waist-to-hip ratio, sex, insulin, and aortic pulse pressure. For right ventricular mass index, both gestational age and ventilation were independent predictors in model one ($P = 0.003$ and $P = 0.03$), with ventilation just losing significance in model two ($P = 0.003$ and $P = 0.06$) (Table 8.7). For right ventricular end-diastolic volume index, gestational age was the only variable that nearly reached significance in model one ($P = 0.08$) and became significant in model two ($P < 0.001$). Gestational age was the only independent predictor in model one ($P = 0.003$) for right ventricular ejection fraction, and remained significant in model two ($P < 0.001$).

8.4 Discussion

Young adults born preterm have reduced right ventricular ejection fraction with nearly 6% having systolic function below the clinically accepted lower limit of normal. They also have smaller right ventricles but increased right ventricular mass, similar to changes reported in the left ventricle. Although changes in both the left and right ventricle are graded by degree of prematurity, consistent with a global impact of preterm birth on the myocardium, the

variation in right ventricular parameters is greater, with twice the relative change in mass per gestational week compared to the left ventricle.

Right ventricular systolic function is of independent and additive prognostic value in chronic heart failure and is one of the most powerful predictors of mortality in left heart failure [256]. Therefore, changes in right ventricular structure and function may not only serve as a sensitive marker of subclinical pulmonary or left ventricular disease, but may also directly contribute to the onset of clinical heart failure [257]. It is plausible that right ventricular pathophysiological changes, present from so early in life, may have a greater impact on cardiac function in later life [232; 256]. It is known that, over time, right ventricular dysfunction can proceed to ventricular dilatation in order to maintain stroke volume [258]. This induces a negative effect on left ventricular diastolic function, that, in turn, leads to further reductions in right ventricular function related to increased myocardial oxygen demands and reduced myocardial perfusion [258]. Data on the relevance of the right ventricle to disease development in the general population is sparse, in particular for young populations. Nevertheless, the Multi-Ethnic Study of Atherosclerosis found that clinical cardiovascular disease-free participants with increased right ventricular mass have a significant increased risk of heart failure and cardiovascular death, independent of left ventricular mass [257].

A proportion of the variation in mass appeared to relate to requirements for postnatal ventilation. Respiratory disease and need for mechanical ventilation are major complications of preterm birth [135; 136], and given the close interaction between the pulmonary circulation and the right heart, this observation is plausible. Extreme changes in intrathoracic pressure and lung volume can lead to increased right atrial pressure, with reduced venous return and subsequent reductions in right ventricular preload and cardiac output [137]. In addition, hyperinflation can result in alveolar distension and subsequent alveolar vessel compression, which would increase pulmonary vascular resistance and right ventricular afterload. The increased right ventricular mass therefore may represent an early persistent adaptation to this increased intraluminal pressure [138]. Alternatively, the underlying clinical indication for ventilation, such as respiratory distress or infection, may have led to changes in the pulmonary vasculature sufficient to have a haemodynamic impact on the right ventricle.

Related, long-term changes in lung function may also have a continued influence on right ventricular structure. A persistent patent ductus arteriosus during early neonatal life could also have had an impact on right ventricular afterload. In bivariable regression, the presence of a clinically significant patent ductus arteriosus in the neonatal period was significantly related to right ventricular end-diastolic volume. However, the incidence tended to be higher in those born more preterm, and this factor did not survive multiple regression analyses. I can also be relatively confident, based on the aortic magnetic resonance images, that these individuals did not have a persistent ductus in later life. Unfortunately, data on lung function or measures of right ventricular systolic pressures in later life were not available in this cohort but will be of interest in future investigations.

Nevertheless, the key finding from the analysis is that other perinatal or adult factors do not adequately account for the strong, graded associations between gestational age and right ventricular structure and function. The pattern of changes are similar to those seen in the left ventricle, consistent with a global impact of preterm birth on the myocardium. The last trimester is particularly relevant for global cardiac growth [110] and, in sheep, cardiomyocytes of both right and left ventricles exhibit similar morphological changes after preterm birth [111]. However, the variation in structure and function accounted for by gestational age is greater for the right ventricle, which may relate to its more substantial remodeling around the time of birth [251]. This complex remodeling would have been of interest to study with computational modeling techniques, as previously performed for the left ventricle [161], but the complexity of the right ventricle has also meant it has remained technically more difficult to perform using the approaches currently available. Inaccuracies in quantification of the right ventricle could account for the greater variation in the right ventricle. However, the values for right ventricular parameters are comparable to age-matched values in other studies [255; 259; 260]. I also used a very detailed approach for identification of borders for determining right ventricular volumes and mass, and thus the reproducibility for these measurements was very good, as demonstrated in Table 3.3 in Chapter 3. Furthermore, comparisons against normal ranges were derived using identical techniques in the term-born groups. Right ventricular myocardial deformation analysis is not as well validated but the

reproducibility for the presented measures was acceptable (Table 3.7, Chapter 3). Furthermore, the findings of reduced myocardial systolic strain are consistent with the significant reductions in systolic ejection fraction observed, based on volumetric cardiovascular magnetic resonance measures. Interestingly, myocardial feature tracking analysis also allowed for the assessment of right ventricular diastolic function, and it was determined that individuals born preterm had significant impairment. Although of uncertain clinical relevance, changes in right ventricular diastolic function have been found in those with mild to moderate hypertension [261]. The presence of slower myocardial relaxation in both ventricles in the group of young adults born preterm is also consistent with a global impact of preterm birth on the myocardium.

8.5 Conclusion

In summary, young adults born preterm have smaller right ventricles with increased mass. Furthermore, the relative impact of preterm birth is greater on the right ventricle than previously reported for the left ventricle. The reduction in right ventricular systolic function is such that 21% of young adults born preterm have ejection fractions below the lower limit observed in adults born at term. The findings of a reduction in cardiac size are strikingly similar to previous observations of a generalised reduction in size of other parts of the vascular tree in individuals born preterm [262]. A better understanding of the mechanisms underlying these changes in cardiovascular structure and function, and their clinical impact on later cardiac disease development, will help determine possible pathways to prevent future disease in the growing cohort of adults born preterm.

9

ANTIANGIOGENIC FACTORS, CAPILLARY DENSITY, AND BLOOD PRESSURE

This chapter was designed to determine whether preterm-born young adults have altered levels of circulating proangiogenic and antiangiogenic factors, as well as whether exposure to maternal preeclampsia has an additional impact. To do this, I compared the preterm-born young adults (*cohort 1*) to the term-born young adults (*cohort 2*). Preterm-born young adults have an elevation in the levels of circulating antiangiogenic factors soluble endoglin (sENG) and soluble endothelial growth factor receptor 1 (VEGFR-1 or sFlt-1). The increase in antiangiogenic factors relates to a reduction in capillary density in preterm-born young adults, which also relates to elevated resting brachial blood pressure and 24 hour ambulatory blood pressure levels.

9.1 Introduction

It has been demonstrated that preterm birth results in altered angiogenic properties in endothelial colony forming cells from cord blood, with a shift towards an antiangiogenic state and impaired capillary vasculogenesis [77]. Preterm birth has also been associated with ab-

normal retinal vascularisation [78; 79] and a reduction in functional and structural dermal capillary density [80]. Furthermore, preeclampsia, an antecedent to 20 to 30% of all preterm births [10; 140; 141], is characterised by an altered angiogenic state in the mother [147], and thus the offspring develop in an environment of increased levels of circulating antiangiogenic factors. Offspring of preeclamptic pregnancies have also been shown to have a reduction in capillary density [263], which may be linked to changes in circulating antiangiogenic factors. The microvasculature is the greatest contributor to vascular resistance and thus is the likely site of any haemodynamically important disturbance in vascular structure and function [264]. While hypertension is characterised by increased peripheral vascular resistance, capillary rarefaction has also been shown to be inversely related to blood pressure even in normotensive individuals [265].

Therefore, I have investigated for the first time whether preterm birth is associated with changes in levels of the circulating antiangiogenic factors soluble endoglin (sENG) and soluble endothelial growth factor receptor 1 (VEGFR-1 or sFlt-1) and the proangiogenic factor vascular endothelial growth factor (VEGF-A) in young adulthood, and whether exposure to maternal preeclampsia has an additional impact. Furthermore, I determined whether changes in these circulating biomarkers related to previously observed capillary rarefaction and blood pressure elevation in individuals born preterm.

9.2 Methods

9.2.1 Study Population

This chapter includes all 102 young adults born preterm (*cohort 1*) and 102 young adults born term frequency matched for age and sex (*cohort 2*) for comparison of the circulating proangiogenic factor VEGF-A and the antiangiogenic factors (sFlt-1 and sENG), as well as resting brachial blood pressure measurements. A subgroup of 30 preterm-born individuals had functional and structural capillary density measures done and 24 hour ambulatory blood pressure monitoring. The results were compared to a subgroup of the term-born

young adult group ($n = 60$), frequency matched for age and sex. Details of the cohorts are outlined in Chapter 2 and cohort characteristics are provided in Tables 9.2 and 9.5.

9.2.2 Study Visit

All study participants attended the study in the early morning following a 12 hour overnight fast. All data were coded with subject and study specific IDs (e.g. EVS001) to ensure anonymity and blinded analysis. The following methods were used for this chapter for all subjects as described in detail in Chapter 3:

- 3.4: Anthropometry and Lifestyle Questionnaire
- 3.5: Blood Sampling and Biochemistry
- 3.6: Enzyme-Linked Immunosorbent Assays
- 3.7: Resting Brachial Blood Pressure Measurements

In addition, the following methods were used for the subgroup of preterm-born ($n = 30$) and term-born ($n = 60$) young adults:

- 3.8: Ambulatory Blood Pressure Monitoring
- 3.3: Intravital Capillary Video Microscopy Acquisition
- 4.2.4: Intravital Capillary Video Microscopy Analysis

9.2.3 Statistical Analysis

Statistical analysis was carried out using SPSS Version 19 as described in Section 3.9 of Chapter 3. 102 subjects per group provided 80% power at $P = 0.05$ to identify a 0.38 standard deviation difference between young adult groups. 30 subjects in the preterm-born group and 60 subjects in the term-born group provided 80% power at $P = 0.05$ to identify a 0.62 standard deviation difference between young adult groups. Comparisons between preterm-born young adults with term-born young adults were adjusted for age and sex.

9.3 Results

9.3.1 Study Population

The full preterm-born group of young adults ($n = 102$) and the subgroup of preterm-born young adults ($n = 30$) were representative of the original preterm cohort, with similar perinatal characteristics (Table 9.1). In adult life, there were no significant differences between groups in number of smokers, personal and family medical history, or lifestyle factors such as socioeconomic status, physical activity or diet ($P > 0.05$). Young adults born preterm had a distinct metabolic profile and significantly higher brachial blood pressure levels at rest than term-born young adult controls (Table 9.2).

Table 9.1: Comparison of Key Perinatal Characteristics Between Birth Cohort and Follow-Up Cohort and Subgroup of Preterm-Born Individuals

	Original cohort ($n = 926$)	Follow-up cohort ($n = 102$)	P Value Ψ	Follow-up subgroup ($n = 30$)	P Value Υ	P Value Δ
Males, n (%)	463 (50.0)	47 (46.1)	0.48	15 (50.0)	>0.99	0.71
Gestational age (weeks)	30.8 ± 2.8	30.3 ± 2.5	0.30	30.5 ± 2.7	0.56	0.71
Birth weight (grams)	1369.8 ± 315.5	1297.0 ± 286.8	0.03	1295.6 ± 304.5	0.21	0.98
Birth weight Z score	-0.81 ± 1.32	-0.84 ± 1.21	0.83	-0.86 ± 1.43	0.84	0.94
Small for gestational age, n (%)	310 (33.5)	32 (31.4)	0.67	12 (40)	0.46	0.38
Maternal smoking during pregnancy, n (%)	299 (32.3)	25 (24.5)	0.11	7 (23.3)	0.30	0.90
Paternal smoking during pregnancy, n (%)	224 (24.2)	31 (30.4)	0.17	8 (26.7)	0.76	0.69
Maternal preeclampsia, n (%)	259 (28.0)	29 (28.4)	0.92	10 (33.3)	0.52	0.61
Ventilation (days)	4.65 ± 9.84	4.14 ± 7.43	0.61	5.57 ± 10.2	0.61	0.40
Number of individuals ventilated, n (%)	476 (51.4)	55 (53.9)	0.63	17 (56.7)	0.57	0.79

Values as Mean $\pm$ Standard Deviation unless stated otherwise.

Ψ = Original cohort vs follow-up cohort.

Υ = Original cohort vs follow-up subgroup.

Δ = Follow-up cohort vs follow-up subgroup.

Table 9.2: Characteristics of Cohorts

	Preterm-born young adults (n=102)	Term-born young adults (n=102)	P Value
Demographics & Anthropometrics			
Gestational Age (wks)	30.3 ± 2.5	39.6 ± 0.9	<0.001
Birth weight (g)	1297.0 ± 286.8	3460.0 ± 417.0	<0.001
Age (years)	25.1 ± 1.4	25.0 ± 2.6	
Males, n (%)	47 (46.1)	47 (46.1)	
BMI (kg/m ²)	24.9 ± 5.4	22.9 ± 3.1	0.001
Smokers, n (%)	20 (19.6)	20 (19.6)	>0.99
Biochemistry			
Total Cholesterol (mmol/L)	4.89 ± 1.05	4.23 ± 0.86	<0.001
HDL-C (mmol/L)	1.53 ± 0.40	1.47 ± 0.41	0.17
LDL-C (mmol/L)	2.81 ± 0.93	2.37 ± 0.66	<0.001
Triglycerides (mmol/L)	1.12 ± 0.79	0.87 ± 0.40	0.005
Glucose (mmol/L)	5.01 ± 0.43	4.61 ± 0.30	<0.001
Insulin (pmol/L)	64.4 ± 42.8	35.6 ± 15.9	<0.001
Blood Pressure (mmHg)			
Systolic	121.3 ± 10.9	112.9 ± 10.1	<0.001
Diastolic	73.0 ± 7.2	68.8 ± 7.0	<0.001
Mean Arterial Pressure	89.1 ± 7.4	83.5 ± 7.1	<0.001
Pulse Pressure	48.4 ± 9.1	44.1 ± 8.5	0.001

BMI indicates body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Values as Mean ± Standard Deviation unless stated otherwise. *P* values were adjusted for age and sex.

9.3.2 Increased Levels of Circulating Antiangiogenic Factors

Preterm-born young adults had increased sFlt-1 levels (88.1 ± 19.0 pg/mL) compared to the term-born young adults (73.0 ± 15.3 pg/mL, $P < 0.001$) (Figure 9.1, Panel A), with no differences in VEGF-A levels (139.5 ± 71.3 vs. 148.5 ± 65.9 pg/mL, $P = 0.85$). sENG levels were also elevated in individuals born preterm (5.64 ± 1.03 ng/mL) compared to those born term (4.06 ± 0.85 ng/mL, $P < 0.001$) (Figure 9.1, Panel B).

Two separate linear regression models were used to investigate key perinatal and adult factors that may have accounted for the elevation in sFlt-1 and sENG levels in the preterm-born group of young adults. For sFlt-1, three independent variables reached significance (age, gestational age, and maternal preeclampsia as a dichotomous categorical variable with those born to a normotensive pregnancy as '1' and to a preeclamptic pregnancy as '2') (Table 9.3). In this model, both gestational age (-1.72 pg/mL/gestational week, $P = 0.02$, 95% confidence interval: -3.20 to -0.23) and maternal preeclampsia (14.4 pg/mL/group, $P = 0.001$,

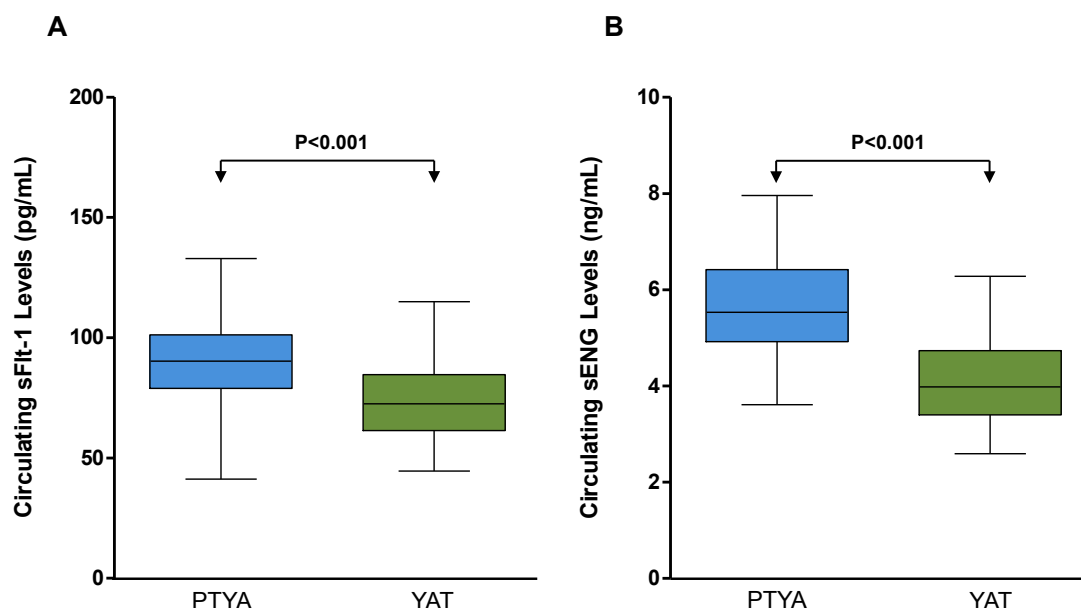


Figure 9.1: Elevated circulating antiangiogenic factors in preterm-born young adults. **A**, Preterm-born young adults (PTYA, blue) had increased sFlt-1 levels (88.1 ± 19.0 pg/mL) compared to the term-born young adults (YAT, green; 73.0 ± 15.3 pg/mL, $P < 0.001$). **B**, sENG levels were also elevated in the PTYA group compared to the YAT group (5.64 ± 1.03 ng/mL vs. 4.06 ± 0.85 ng/mL, $P < 0.001$). Data shown as box and whisker plot: minimum to maximum.

95% confidence interval: 6.31 to 22.4) remained as independent predictors. For sENG, gestational age was the only statistically significant predictor using bivariable regression (-0.12 ng/mL/gestational week, $P = 0.006$, 95% confidence interval: -0.20 to -0.034) (Table 9.4).

The absolute sFlt-1 level in young adults born preterm to a preeclamptic pregnancy was 97.7 ± 15.9 pg/mL compared to 84.2 ± 18.9 pg/mL in those born preterm to a normotensive pregnancy ($P = 0.002$, Figure 9.2). This appeared to be additional to any impact of preterm birth on sFlt-1 levels, as individuals born preterm to normotensive pregnancies still showed a significant elevation in sFlt-1 levels compared to term-born young adults (73.0 ± 15.3 pg/mL, $P < 0.001$).

9.3.3 Capillary Density and the Rise in Circulating Antioangiogenic Factors

To test the hypothesis that the elevation in circulating levels of sENG and sFLT-1 in young adulthood in individuals born preterm related to changes in functional and structural capil-

Table 9.3: Relationship of Variables with Soluble Endothelial Growth Factor Receptor 1 in Preterm-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Age	3.56	0.79	6.33	0.02
Sex	-4.12	-12.0	3.70	0.30
Smoking	-4.11	-14.0	5.74	0.41
Waist:Hip	9.89	-38.1	57.1	0.68
BMI	-0.12	-0.85	0.61	0.74
Total Cholesterol	2.18	-1.65	6.01	0.26
LDL Cholesterol	3.09	-1.23	7.40	0.16
HDL Cholesterol	-6.47	-16.6	3.67	0.21
Insulin	-0.044	-0.14	0.051	0.36
Glucose	-5.60	-15.1	3.92	0.25
Triglycerides	2.06	-3.06	7.18	0.43
Gestational Age	-1.11	-2.70	-0.49	0.05
Maternal Preeclampsia	13.6	5.32	21.8	0.002
Birthweight Z Score	-0.50	-3.79	2.78	0.76
Ventilation	0.51	-0.015	1.04	0.16
Postnatal Weight Gain	-1.66	-56.8	53.5	0.95
Trial Diet Group	-3.51	-11.3	4.31	0.38
Mode of Delivery	5.32	-2.97	13.6	0.21

Bolded variables are statistically significant. BMI indicates body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 9.4: Relationship of Variables with Soluble Endoglin in Preterm-Born Young Adults using Bivariable Regression

Variable	Unstandardised Coefficient (B)	95% Confidence Interval		P Value
		Lower Bound	Upper Bound	
Age	0.025	-0.13	0.18	0.75
Sex	-0.14	-0.57	0.28	0.51
Smoking	-0.0072	-0.54	0.53	0.98
Waist:Hip	-0.10	-2.70	2.49	0.94
BMI	0.018	-0.021	0.057	0.35
Total Cholesterol	0.061	-0.15	0.27	0.56
LDL Cholesterol	0.13	-0.11	0.36	0.29
HDL Cholesterol	-0.40	-0.95	0.14	0.15
Insulin	0.0021	-0.0030	0.0074	0.43
Glucose	0.14	-0.37	0.66	0.58
Triglycerides	0.052	-0.23	0.33	0.71
Gestational Age	-0.12	-0.20	-0.034	0.006
Maternal Preeclampsia	0.21	-0.26	0.68	0.37
Birthweight Z Score	0.13	-0.041	0.31	0.13
Ventilation	0.024	-0.0051	0.052	0.10
Postnatal Weight Gain	-2.47	-5.40	0.47	0.10
Trial Diet Group	-0.12	-0.55	0.30	0.56
Mode of Delivery	-0.014	-0.47	0.44	0.95

Bolded variables are statistically significant. BMI indicates body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

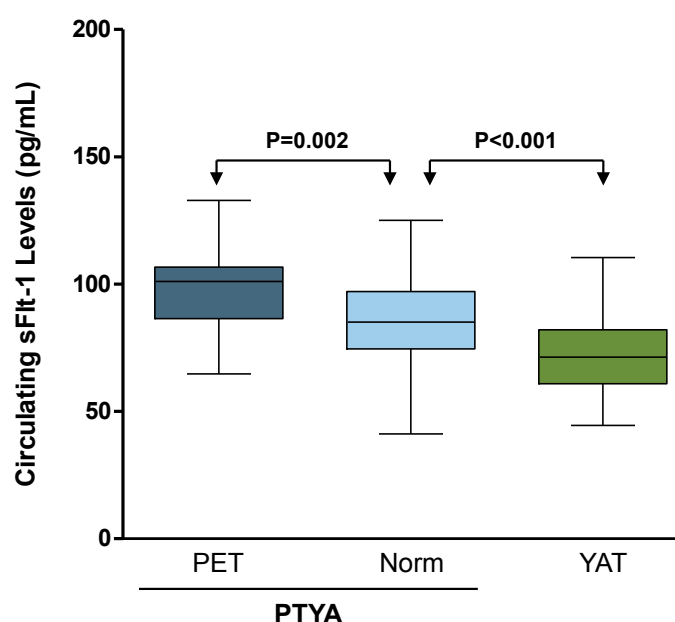


Figure 9.2: Elevated circulating sFlt-1 levels with exposure to maternal preeclampsia. sFlt-1 level in those born preterm (PTYA) to a preeclamptic pregnancy (PET, darker blue) was 97.7 ± 15.9 pg/mL compared to 84.2 ± 18.9 pg/mL in those born preterm to normotensive mothers (Norm, lighter blue; $P = 0.002$). Individuals born preterm to normotensive pregnancies still showed a significant elevation in sFlt-1 levels compared to term-born young adults (YAT, green; 73.0 ± 15.3 pg/mL, $P < 0.001$). Data shown as box and whisker plot: minimum to maximum.

lary density, capillary measures were first compared between the preterm-born and term-born groups. Similar to the full cohorts, the subgroups of preterm-born and term-born young adults did not differ in adult life in number of smokers, personal and family medical history, or lifestyle factors such as socioeconomic status, physical activity or diet ($P > 0.50$). Young adults born preterm had a distinct metabolic profile and significantly higher brachial blood pressure levels at rest than term-born young adult controls (Table 9.5).

Preterm-born young adults showed a reduction in baseline and venous occlusion capillary densities (93.5 ± 10.6 capillaries/mm² and 101.8 ± 12.7 capillaries/mm², respectively) compared to the term-born young adults (105.2 ± 10.6 capillaries/mm², $P < 0.001$ and 115.9 ± 11.8 capillaries/mm², $P < 0.001$ respectively) (Figure 9.3). Furthermore, there was a small but statistically significant difference in percent increase in capillary density with venous occlusion ($8.75 \pm 3.51\%$ vs. $10.2 \pm 2.8\%$, $P = 0.05$). Within the group of young adults born preterm, gestational age was significantly related to baseline ($r = 0.43$, $P = 0.04$) and venous occlusion ($r = 0.43$, $P = 0.02$) capillary density measures, and showed a trend towards significance in

Table 9.5: Characteristics of Subgroups

	Preterm-born young adults (n = 30)	Term-born young adults (n = 60)	P Value
Demographics & Anthropometrics			
Gestational Age (wks)	30.5 ± 2.7	39.6 ± 0.8	
Birth weight (g)	1295.6 ± 304.5	3411.2 ± 319.0	
Age (years)	26.6 ± 1.0	26.2 ± 1.9	
Males, n (%)	15 (50.0)	30 (50.0)	
BMI (kg/m ²)	26.3 ± 7.2	23.0 ± 3.3	0.006
Smokers, n (%)	5 (16.7)	10 (16.7)	>0.99
Biochemistry			
Total Cholesterol (mmol/L)	4.79 ± 0.77	4.23 ± 0.86	0.009
HDL-C (mmol/L)	1.47 ± 0.38	1.46 ± 0.42	0.85
LDL-C (mmol/L)	2.69 ± 0.82	2.39 ± 0.67	0.12
Triglycerides (mmol/L)	1.10 ± 0.64	0.86 ± 0.33	0.03
Glucose (mmol/L)	4.70 ± 0.35	4.60 ± 0.31	0.001
Insulin (pmol/L)	64.4 ± 42.8	35.6 ± 15.9	<0.001

BMI indicates body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Values as Mean ± Standard Deviation unless stated otherwise. *P* values were adjusted for age and sex.

percent increase in capillary density with venous occlusion ($r = 0.33$, $P = 0.08$). There were no significant associations between maternal preeclampsia and capillary density measures in the preterm group ($P > 0.50$). Interestingly, sENG levels were inversely related to baseline ($r = -0.57$, $P = 0.02$) and venous occlusion ($r = -0.65$, $P < 0.001$) capillary density measures in the preterm group, as well as with percent increase in capillary density with venous occlusion ($r = -0.47$, $P = 0.02$). There were no significant associations between sFlt-1 levels and capillary density measures in the group of young adults born preterm ($P > 0.05$). There were also no associations between sENG and sFlt-1 levels with capillary density measures in the group of young adults born term ($P > 0.05$).

9.4 Antiangiogenesis, Capillary Rarefaction, and Elevated Blood Pressure

In order to investigate the relationship between measures of capillary density and blood pressure, the 24 hour ambulatory blood pressure levels were first compared between the

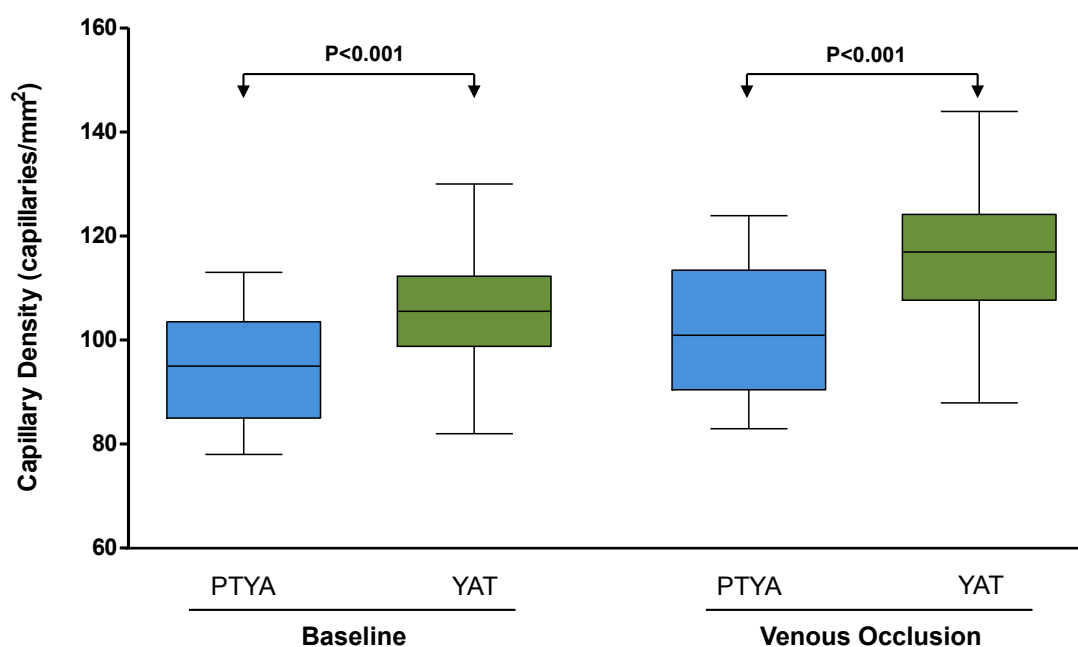


Figure 9.3: Reduced functional and structural capillary density in preterm-born young adults. Preterm-born young adults (PTYA, blue) showed a reduction in baseline and venous occlusion capillary densities (93.5 ± 10.6 capillaries/mm² and 101.8 ± 12.7 capillaries/mm², respectively) compared to the term-born young adults (YAT, green; 105.2 ± 10.6 capillaries/mm², $P < 0.001$ and 115.9 ± 11.8 capillaries/mm², $P < 0.001$ respectively). Data shown as box and whisker plot: minimum to maximum.

preterm-born and term-born groups. Table 9.6 shows that blood pressure levels were higher at night (sleep), during the day (awake), and for the full 24 hour period (full) for all parameters when comparing the preterm-born young adults with the term-born young adults.

Both baseline and venous occlusion capillary density measures were closely related to 24 hour ambulatory blood pressure levels in the preterm-born subgroup, with the strongest associations with sleep and full 24 hour blood pressure levels (Table 9.7 and 9.8). Furthermore, both sENG and sFlt-1 levels were positively related to many of the 24 hour blood pressure parameters in the preterm-born subgroup of young adults (Table 9.9 and 9.10). There were no associations between sENG and sFlt-1 levels with 24 hour blood pressure parameters ($P > 0.05$) in the term-born young adult group. In the full cohort of preterm-born individuals ($n = 102$), sENG showed the strongest association with resting brachial systolic blood pressure ($r = 0.30$, $P = 0.003$) while sFlt-1 showed the strongest association with resting brachial mean arterial pressure ($r = 0.26$, $P = 0.01$). In the full cohort of young adults born term (n

Table 9.6: 24 Hour Ambulatory Blood Pressure Levels

	Preterm-born young adults (n = 30)	Term-born young adults (n = 60)	P Value
Sleep			
Systolic Blood Pressure (mmHg)	110.3 ± 9.2	101.1 ± 8.1	<0.001
Diastolic Blood Pressure (mmHg)	59.0 ± 6.9	56.4 ± 4.7	0.04
Mean Arterial Pressure (mmHg)	75.6 ± 7.0	71.0 ± 5.0	0.001
Pulse Pressure (mmHg)	51.4 ± 7.4	44.7 ± 7.0	<0.001
Awake			
Systolic Blood Pressure (mmHg)	126.3 ± 6.5	116.6 ± 8.3	<0.001
Diastolic Blood Pressure (mmHg)	72.9 ± 5.2	70.3 ± 5.9	0.05
Mean Arterial Pressure (mmHg)	90.5 ± 4.4	85.7 ± 6.1	0.001
Pulse Pressure (mmHg)	53.4 ± 7.6	46.3 ± 6.1	<0.001
Full			
Systolic Blood Pressure (mmHg)	123.3 ± 6.4	112.8 ± 7.5	<0.001
Diastolic Blood Pressure (mmHg)	69.6 ± 4.3	67.1 ± 4.9	0.02
Mean Arterial Pressure (mmHg)	87.3 ± 4.1	82.1 ± 5.2	<0.001
Pulse Pressure (mmHg)	53.7 ± 6.9	45.7 ± 5.8	<0.001

Values as Mean ± Standard Deviation unless stated otherwise. *P* values were adjusted for age and sex.

= 102), neither circulating sENG or sFlt-1 were associated with any of the resting brachial blood pressure parameters ($P > 0.05$).

Table 9.7: Bivariable Correlations Between Functional Capillary Density and 24 Hour Ambulatory Blood Pressure Levels

	Pearson Correlation Coefficient (r)	P Value
Sleep		
Systolic Blood Pressure	-0.68	<0.001
Diastolic Blood Pressure	-0.33	0.11
Mean Arterial Pressure	-0.53	0.008
Pulse Pressure	-0.53	0.007
Awake		
Systolic Blood Pressure	-0.44	0.03
Diastolic Blood Pressure	-0.085	0.69
Mean Arterial Pressure	-0.29	0.15
Pulse Pressure	-0.32	0.12
Full		
Systolic Blood Pressure	-0.69	<0.001
Diastolic Blood Pressure	-0.25	0.23
Mean Arterial Pressure	-0.53	0.006
Pulse Pressure	-0.49	0.01

Table 9.8: Bivariable Correlations Between Structural Capillary Density and 24 Hour Ambulatory Blood Pressure Levels in Preterm-Born Young Adults

	Pearson Correlation Coefficient (r)	P Value
Sleep		
Systolic Blood Pressure	-0.66	<0.001
Diastolic Blood Pressure	-0.31	0.15
Mean Arterial Pressure	-0.50	0.01
Pulse Pressure	-0.53	0.008
Awake		
Systolic Blood Pressure	-0.39	0.05
Diastolic Blood Pressure	-0.018	0.93
Mean Arterial Pressure	-0.18	0.38
Pulse Pressure	-0.34	0.09
Full		
Systolic Blood Pressure	-0.64	0.001
Diastolic Blood Pressure	-0.17	0.42
Mean Arterial Pressure	-0.45	0.03
Pulse Pressure	-0.49	0.01

Table 9.9: Bivariable Correlations Between Soluble Endoglin and 24 Hour Ambulatory Blood Pressure Levels in Preterm-Born Young Adults

	Pearson Correlation Coefficient (r)	P Value
Sleep		
Systolic Blood Pressure	0.42	0.06
Diastolic Blood Pressure	0.44	0.05
Mean Arterial Pressure	0.46	0.04
Pulse Pressure	0.11	0.64
Awake		
Systolic Blood Pressure	0.43	0.05
Diastolic Blood Pressure	0.24	0.28
Mean Arterial Pressure	0.40	0.07
Pulse Pressure	0.21	0.34
Full		
Systolic Blood Pressure	0.56	0.007
Diastolic Blood Pressure	0.31	0.17
Mean Arterial Pressure	0.50	0.02
Pulse Pressure	0.33	0.14

Table 9.10: Bivariable Correlations Between Soluble Vascular Endothelial Growth Factor Receptor 1 and 24 Hour Ambulatory Blood Pressure Levels in Preterm-Born Young Adults

	Pearson Correlation Coefficient (r)	P Value
Sleep		
Systolic Blood Pressure	0.069	0.77
Diastolic Blood Pressure	0.21	0.36
Mean Arterial Pressure	0.17	0.47
Pulse Pressure	0.14	0.56
Awake		
Systolic Blood Pressure	0.18	0.43
Diastolic Blood Pressure	0.44	0.04
Mean Arterial Blood Pressure	0.44	0.04
Pulse Pressure	0.14	0.52
Full		
Systolic Blood Pressure	0.023	0.92
Diastolic Blood Pressure	0.43	0.05
Mean Arterial Pressure	0.36	0.10
Pulse Pressure	0.25	0.26

9.5 Discussion

Preterm-born individuals have increased sFlt-1 and sENG levels in young adulthood, which are both inversely related to gestational age. Being born preterm to a preeclamptic pregnancy relates to an additional, significant elevation in sFlt-1 levels compared to being born preterm to a normotensive pregnancy. However, exposure to maternal preeclampsia does not relate to additional changes in sENG levels. VEGF-A levels did not differ between preterm-born and term-born young adults, suggesting an imbalance between circulating proangiogenic and antiangiogenic factors in young adults born preterm. The increase in antiangiogenic factors relates to the increase in resting brachial blood pressure and 24 hour ambulatory brachial blood pressure levels in the group of individuals born preterm. Furthermore, functional and structural capillary density are decreased in the preterm-born group, which is related to gestational age and inversely related to circulating levels of sENG, as well as both resting and 24 hour blood pressure levels. The fact that the associations between these parameters are unique to the preterm-born group and that they are correlated in a dose-response fashion with the degree of prematurity suggests potential causal mechanisms.

Preterm birth is a pathophysiological event, often comprising a combination of stress,

hypoxia, infection, and inflammation [10; 13; 77]. Such changes may contribute to an altered angiogenic state and impaired capillary vasculogenesis and angiogenesis [77; 80]. Ligi *et al.* [77] demonstrated in endothelial colony forming cells isolated from the mononuclear cell fraction obtained from cord blood that preterm birth is related to impaired angiogenesis both *in vitro* and *in vivo*. Gene profiling provided a mechanistic link between preterm birth and altered angiogenesis, indicating a shift towards increased expression of genes with antiangiogenic functions. To my knowledge, the work presented in this chapter is the first demonstration that circulating levels of antiangiogenic factors are elevated in later life in individuals born preterm, with an additional impact of being born to a preeclamptic pregnancy.

The positive relationship between circulating levels of antiangiogenic factors and blood pressure in the preterm group may be due to changes in the microvasculature. A reduction in the number of capillaries or ability to recruit capillaries results in inadequate circulation at the capillary level, which can lead to increased peripheral vascular resistance, known to contribute to hypertension [266]. It is also possible that an increase in blood pressure contributes to capillary rarefaction, as it has been demonstrated that experimental elevation of blood pressure causes an increase in the generation of reactive oxygen species in endothelial cells [267; 268], which could negatively affect microvascular integrity. Nevertheless, capillary rarefaction is of central importance in essential hypertension [265; 266], and it has been found in normotensive individuals at high risk of developing hypertension [265], suggesting that capillary rarefaction antedates the onset of blood pressure elevation.

The second and third trimester of pregnancy is a period of very rapid fetal growth. As such, the accelerated development of organs requires a rapidly expanding capillary network, and interruption of this process may prevent normal capillary remodeling. However, D'Souza *et al.* [269] showed that a group of low birth weight infants (mix of preterm-born and term-born infants) had an increase in capillary density compared to normal birth weight infants. They also showed that infants who were exposed to maternal hypertension during pregnancy had a reduction in capillary density, unless they were born preterm, in which case they had an increase [263]. Though human and animal studies have demonstrated capillary

rarefaction in preterm-born individuals [78; 270], D'Souza *et al.* [269] suggest that the rarefaction occurs postnatally rather than *in utero*. Though this requires further investigation in a better defined group of preterm-born infants, it is possible that, if the elevation in circulating antiangiogenic factors that I observed in young adulthood has been sustained since early life, this elevation may contribute to a continuous, slow regression of capillary density over time. It is also possible that a reduction in capillary density results in reduced tissue perfusion and hypoxia, altering levels of angiogenic and antiangiogenic factors [271; 272; 273]. Therefore, the observed increase in antiangiogenic factors and reduced capillary density may be a bidirectional pathway that worsens over time. This may explain why Bonamy *et al.* [80] showed, in children aged seven to 12 years, that those born preterm had a 6.94% reduction in resting (functional) capillary density and a 5.27% reduction in capillary density post venous occlusion (structural), whereas I have demonstrated that the functional and structural capillary density in this preterm-born cohort of individuals aged 25 to 28 years is reduced by 11.1% and 12.2% compared to term-born controls, respectively. While Bonamy *et al.* [80] failed to show any association between capillary density and blood pressure in childhood, it has been demonstrated by several studies that the difference in blood pressure between preterm-born and term-born individuals increases over time [9]. While there are almost certainly several mechanisms contributing to the elevation in blood pressure, the data in young adulthood suggests that the reduction in capillary density becomes of increasing importance to the progression of blood pressure elevation with ageing in individuals born preterm.

Kvehaugen *et al.* [274] previously showed that circulating levels of sFlt-1 were not elevated in children born to a preeclamptic pregnancy. However, the group of preeclamptic offspring they studied was a mix of term-born and preterm-born individuals. All of the young adults born to a preeclamptic pregnancy in my study were born less than 34 weeks gestation, with 48.3% born extremely or very preterm (less than 32 weeks gestation). Therefore, it is possible that the effect is of greater importance with very early onset preeclampsia. Unfortunately, it was not possible to determine with statistical certainty whether individuals born preterm to preeclamptic pregnancies showed an additional reduction in capillary density

compared to preterm-born individuals born to normotensive pregnancies, as only a third of individuals in the subgroup of preterm-born young adults with capillary density measures were born to a preeclamptic pregnancy. However, there is no trend towards a reduction in capillary density in those exposed to maternal preeclampsia, nor is there any association between sFlt-1 and capillary density levels. Rather, the strong association is with sENG levels, which are elevated in all preterm-born individuals regardless of exposure to maternal preeclampsia, with the greatest determinant being gestational age.

9.6 Conclusion

This is the first demonstration of a heightened antiangiogenic state in later life in individuals who were born preterm. These findings may indicate a potential mechanistic link between an imbalance in proangiogenic and antiangiogenic factors, capillary density, and blood pressure elevation in preterm-born young adults (Figure 9.4). Further longitudinal research with repeated measures throughout childhood, adolescence, and into young adulthood would help to solidify this hypothesis. Nevertheless, this may present a novel target for future prevention strategies to delay the development of hypertension in individuals born preterm.

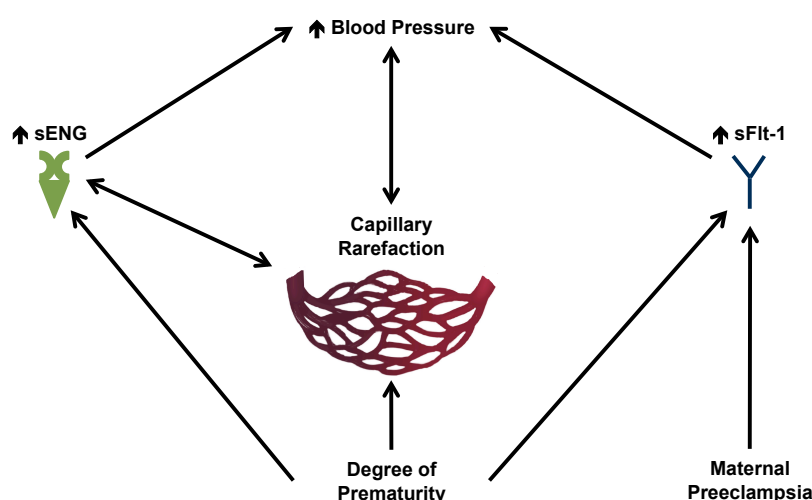


Figure 9.4: Relationship between circulating antiangiogenic factors, capillary density, and blood pressure in preterm-born young adults. Soluble endoglin (sENG), soluble vascular endothelial growth factor receptor 1 (sFlt-1), and blood pressure were all elevated, while capillary density was decreased in the group of young adults born preterm. Arrows indicate significant associations between parameters ($P < 0.05$), with potential directions of causality.

10

CONCLUSIONS

In conclusion, this work has provided a more holistic and well-defined understanding of cardiovascular changes in young adults born preterm. Furthermore, key perinatal factors that may contribute to these distinct differences were identified. This was achieved by conducting detailed cardiovascular phenotyping of a cohort of preterm-born young adults, with uniquely detailed prospective data collection from birth into adolescence, as well as performing similar investigations on two groups of term-born individuals to define normal ranges for comparisons.

The first objective of this thesis was to determine methods to be used for cardiovascular phenotyping of preterm-born and term-born individuals, which would then be able to satisfy the remaining experimental objectives of this thesis. In Chapter 3, the various methods were presented that were used to phenotype the three cohorts of individuals described in Chapter 2. The selection of methods was such that it allowed for detailed structural and functional cardiovascular imaging measurements, as well as detailed blood pressure measurements, anthropometric and demographic data collection, and blood sampling. The analysis of the imaging data was also shown in Chapters 3 and 4 to be highly reproducible. It was demonstrated throughout this thesis that a unique feature of this work was the comprehensive data collection in young adulthood, which was complemented by the large amount of

data collected since recruitment at birth in the cohort of preterm-born individuals.

The use of cardiovascular magnetic resonance proved especially effective in meeting a number of objectives of this thesis. Firstly, it allowed for detailed aortic imaging in order to determine regional aortic pulse wave velocity and aortic distensibility, which allowed for the establishment of the long-term impact of perinatal interventions, relevant to growth and development, on the aorta. This objective was met by utilising the perinatal data collected in the preterm-born group to design two independent nested case-control studies. It was determined in Chapter 5 that postnatal intravenous lipid exposure in preterm-born infants results in an increase in total cholesterol levels at that time, which relates to increased global aortic stiffness in young adulthood, most notably in the abdominal aorta [159]. In addition, the elevation of total cholesterol levels with intravenous lipid exposure in early postnatal life was demonstrated to be an independent predictor of reduced left ventricular apical circumferential peak systolic strain in young adulthood. The second nested case-control study presented in Chapter 6 revealed that preterm-born young adults exposed to antenatal glucocorticoids have a selective increase in aortic stiffness in the arch of the aorta compared to preterm-born young adults not exposed to antenatal glucocorticoids [160]. Furthermore, antenatal glucocorticoid exposure is associated with altered glucose metabolism in adolescence and young adulthood. However, this change in glucose metabolism does not explain the increased aortic arch stiffness in individuals exposed to antenatal glucocorticoids. These two studies demonstrate the ability of cardiovascular magnetic resonance to disentangle the unique influences of different exposures on regional changes of the aorta.

The next objectives were to define left ventricular and right ventricular changes in preterm-born young adults. Using cardiovascular magnetic resonance and computational atlas formation, it was first revealed in Chapter 7 that preterm-born young adults have increased left ventricular mass, out of proportion to blood pressure, and a unique three-dimensional left ventricular geometry, including a reduction in length and volumes [161]. Furthermore, preterm birth is associated with reductions in left ventricular systolic, diastolic, and rotational function. Once again, the detailed perinatal data collected in the preterm-born group of young adults proved to be especially important when determining key risk factors related

to left ventricular differences. This data made it possible to determine that the degree of prematurity, and not other perinatal exposures, is the strongest predictor of left ventricular geometric changes, while exposure to maternal preeclampsia has an impact on left ventricular systolic function in addition to that associated with preterm birth. Similar changes were observed for the right ventricle (Chapter 8), including an increase in right ventricular mass with a reduction in right ventricular size [162]. Although changes in both the left and right ventricle are graded by degree of prematurity, consistent with a global impact of preterm birth on the myocardium, the variation in right ventricular parameters is greater, with twice the relative change in mass compared to the left ventricle. Unique to the right ventricle, young adults born preterm also have a reduced right ventricular ejection fraction with nearly 6% having systolic function below the clinically accepted lower limit of normal.

Following this, the focus shifted towards meeting the final experimental objective of this thesis, which was to determine whether preterm birth relates to changes in circulating antiangiogenic and angiogenic factors in young adulthood, and whether these changes relate to the previously reported capillary rarefaction and elevated blood pressure levels seen in individuals born preterm. As part of meeting this objective, I established an analysis method for capillary density using Image Pro Plus software to reduce analysis time and improve reproducibility, which was presented in Chapter 4. It was demonstrated in Chapter 9 using enzyme-linked immunosorbent assays that preterm-born young adults have an elevation in circulating levels of the antiangiogenic factors soluble endoglin (sENG) and soluble vascular endothelial growth factor receptor 1 (sFlt-1). Furthermore, exposure to maternal preeclampsia relates to an additional elevation in sFlt-1 levels. Interestingly, the increase in sENG levels relates to the observed reduction in both functional and structural capillary density and elevated blood pressure levels in individuals born preterm.

It would have been of interest to investigate in greater detail whether there were differences between males and females for the investigations performed for this thesis. However, due to the size of the preterm-born cohort and magnitude of the differences observed compared to the groups of individuals born term, it was not possible to conduct such analyses with certainty, as this would have resulted in a high probability of type II errors. Larger stud-

ies will provide the opportunity to investigate differences in cardiovascular structure and function between males and females born preterm. A potential limitation of the cohort of preterm-born young adults that were used for the investigations presented throughout this thesis is that it was a mixed population of individuals with different aetiologies of preterm birth and a large range of perinatal complications and exposures. However, this also allowed for investigation into the potential influence of different perinatal characteristics related to being born preterm on long-term alterations in cardiovascular structure and function, which would not have been possible in a more homogeneous population of preterm-born individuals.

Overall, this thesis has strengthened the current literature and our understanding of the long-term cardiovascular risk of being born preterm. As summarised in Figure 10.1, the work carried out for this thesis has demonstrated that specific perinatal events in preterm-born individuals are important determinants of long-term changes of different components of the cardiovascular system. As 10% of births are preterm, any adverse health impact of this unusual development pattern on cardiovascular development is relevant to a large population of adults. Of interest is that the perinatal interventions affected all preterm-born individuals who were exposed to them, regardless of gestational age. Moreover, the alterations in left and right ventricular structure and function extended across the gestational age range to include moderate to late preterm-born young adults, as did the heightened antiangiogenic state and capillary rarefaction. The public health implications of this are therefore of considerable magnitude given that 80% of preterm births occur between 32 and 36 weeks gestation [12]. The most obvious clinical implication of the findings from this thesis, in combination with previous work, is that preterm-born individuals, regardless of gestational age, require continued follow-up and close monitoring of cardiovascular risk. Knowledge of perinatal characteristics and complications are also important as the work in this thesis has demonstrated the localised effects of specific perinatal events on long-term cardiovascular changes in preterm-born individuals. While the ultimate goal is to prevent the progression of cardiovascular disease in this growing subgroup of individuals, to do this, further research is required to better understand the underlying mechanisms driving the observed changes in

cardiovascular structure and function. In addition, follow-up of these individuals into later life will allow for the quantification of cardiovascular disease progression over time.

10.1 Future Work

The final objective of this thesis was to discuss and propose future research to further explore the underlying pathophysiology of the findings from this work. One area requiring further research is for a more well-characterised study to take place in preterm-born neonates to investigate some of these cardiovascular changes in early life. Currently within our research group, a neonatal pilot study of this nature is taking place, which includes a mix of term-born infants, preterm-born infants, and infants born to a pregnancy complicated by preeclampsia. All of these infants are seen in the first couple days following birth, with questionnaire data and medical records collected for both the mothers and their offspring. Furthermore, blood samples and height and weight measurements are collected for both the mothers and infants, with microvascular imaging and echocardiography measurements done on the infants. All measurements are also repeated three months after birth.

To build on the findings of altered circulating antiangiogenic factors relating to capillary rarefaction and elevated blood pressure levels in preterm-born young adults presented in Chapter 9, the plan is to perform the same analyses in younger cohorts to better understand how these changes progress over time. Data will be used from the neonatal pilot study as well as a study of children who were born to preeclamptic pregnancies, which includes a mix of term-born and preterm-born children and controls. In addition to this, a large body of work within our research group has focused on the collection of umbilical cords from preeclamptic pregnancies (preterm and term pregnancies) and controls (preterm and term pregnancies) for isolation of human umbilical vein endothelial cells. DNA microarrays have been used to create a gene expression database. This therefore allows for the opportunity in the future to examine the data under a filter of angiogenic and vascular structure and function related pathways between groups.

In Chapters 7 and 8, it was demonstrated using cardiovascular magnetic resonance and

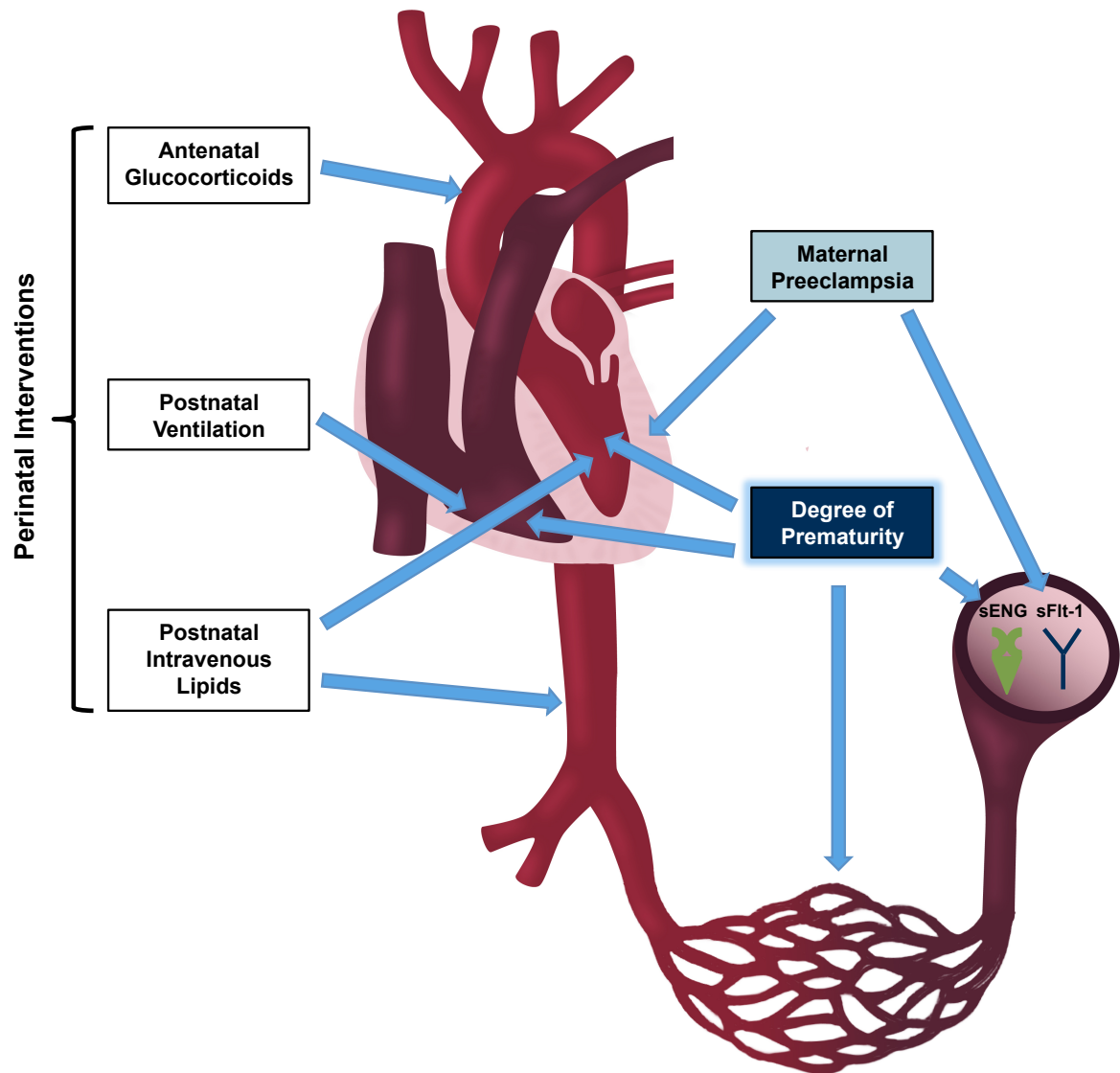


Figure 10.1: Summary of key perinatal factors related to cardiovascular changes in preterm-born young adults. The work in this thesis has demonstrated that antenatal glucocorticoid exposure has a localised impact on the arch of the aorta, while postnatal intravenous lipids have the greatest impact on the abdominal aorta. Though intravenous lipids also relate to a reduction in left ventricular apical circumferential peak systolic strain, the distinct differences between individuals born preterm and term-born controls in left ventricular structure and function are most closely related to the degree of prematurity and being born to a pregnancy complicated by preeclampsia. Similarly, for the right ventricle, the unique structural and functional changes between preterm-born and term-born individuals are most closely related to the degree of prematurity, with a proportion of the variation in right ventricular mass related to requirements for postnatal ventilation. The observed increase in the circulating antiangiogenic factors sENG and sFlt-1 and reduction in functional and structural capillary density in the young adults born preterm are most closely related to the degree of prematurity. Being born to a pregnancy complicated by preeclampsia associates with an additional elevation in circulating sFlt-1 levels compared to young adults born preterm to a normotensive pregnancy.

computational atlas techniques that preterm-born young adults have altered cardiac geometric shapes and function compared to term-born young adults of equivalent age and sex, as well as older term-born adults with increased cardiovascular risk factors [161]. This is a particular area that I have focused on taking forward given its potential clinical implications. Perinatal modification may be ideal, but development of targeted prevention in early adulthood is of equal or even greater importance given the millions of individuals worldwide who are already at this stage of life. I have now designed a new study to build on these observations and to test the hypothesis that altered cardiac structure in preterm-born adults leads to proportional reductions in exercise capacity. Furthermore, advanced cardiovascular magnetic resonance imaging techniques will be used to identify myocardial tissue characteristics underlying the increased myocardial size, as well as to determine whether changes deteriorate over time. I have received ethical approval for the study and the project has recently been submitted for a project grant that my supervisor and I wrote.

10.1.1 Further Questions Regarding Cardiac Changes

Preterm-born young adults have previously been noted to have a reduced exercise capacity, which could not be explained by variation in pulmonary structure or function [275]. Why this occurs has remained unclear. It is of further interest to test the hypothesis that the changes observed in preterm-born young adults in left and right ventricular structure and function lead to proportional changes in maximal exercise capacity [276; 277]; an independent predictor of cardiovascular morbidity and mortality [278; 279; 280].

In addition, further imaging will be undertaken, in combination with sample collection, to try to identify the key structural differences that contribute to the increase in myocardial mass and reduced cardiac function. Experimental animal data would suggest the cardiac changes in individuals born preterm may predominantly relate to myocardial hypertrophy during the first days of life. However, further possible pathways have been implicated, that may be amenable to alternative preventive interventions. Preterm-born infants are known to have abnormal ectopic fat deposition [69] and, using proton spectroscopy in adults and

children, techniques have recently been established in Oxford for quantification of myocardial liposis that would allow for the determination of whether offspring born preterm have alterations in myocardial lipid deposition. Furthermore, experimental data highlight that tissue from preterm-born offspring have altered mitochondrial energetics [110]. This could lead to changes in exercise capacity independent of structural changes, and may be relevant to myocardial hypertrophy in those born preterm. Therefore, magnetic resonance spectroscopy will also be used to determine whether preterm-born adults exhibit alterations in myocardial energetics. Additionally, some characteristics of the diastolic functional impairment observed in the preterm-born young adults are characteristic of ageing related changes seen with increased fibrosis. It is now possible to quantify degree of diffuse fibrosis using T1-mapping cardiovascular magnetic resonance [281].

Finally, the current cardiovascular data on the preterm-born individuals come from a single time point. It is possible the proportional increase in right and left ventricular mass observed is fixed during the neonatal period. Alternatively, preterm-born individuals may be predisposed to disproportionately increase their left ventricular mass over time. As such, prospective measures of left and right ventricular structure will be of value.

10.1.1.1 Original Hypotheses

This study will address three specific hypotheses (Figure 10.2):

- Hypothesis 1 - Reduced exercise capacity in preterm-born adults is proportional to baseline differences in cardiac structure and function, which relate to abnormalities in myocardial metabolism;
- Hypothesis 2 - Increased cardiac mass in preterm-born adults relates to increased myocyte mass rather than increased fibrosis or lipid deposition;
- Hypothesis 3 - Preterm-born adults have a disproportionate increase in ventricular mass over time.

10.1.1.2 Expected Value of Results

A better understanding of the mechanisms underlying changes in left ventricular and right ventricular structure and function, relevant to later cardiac disease development, will help determine possible pathways to prevent future disease. Changes in functional exercise capacity are known to be a strong independent predictor of cardiovascular outcomes and have already been noted to be reduced in preterm-born individuals. This study will provide a better understanding of how baseline cardiovascular structure and function influence the response to physiological stress in these young individuals, as well as how these differences relate to underlying myocardial structure. The results are expected to highlight novel pathways relevant to cardiovascular disease progression, of particular relevance to those born preterm. Ultimately, these can then be translated into the design of interventions to modify cardiovascular physiology and prevent development of disease in a growing subgroup of the population.

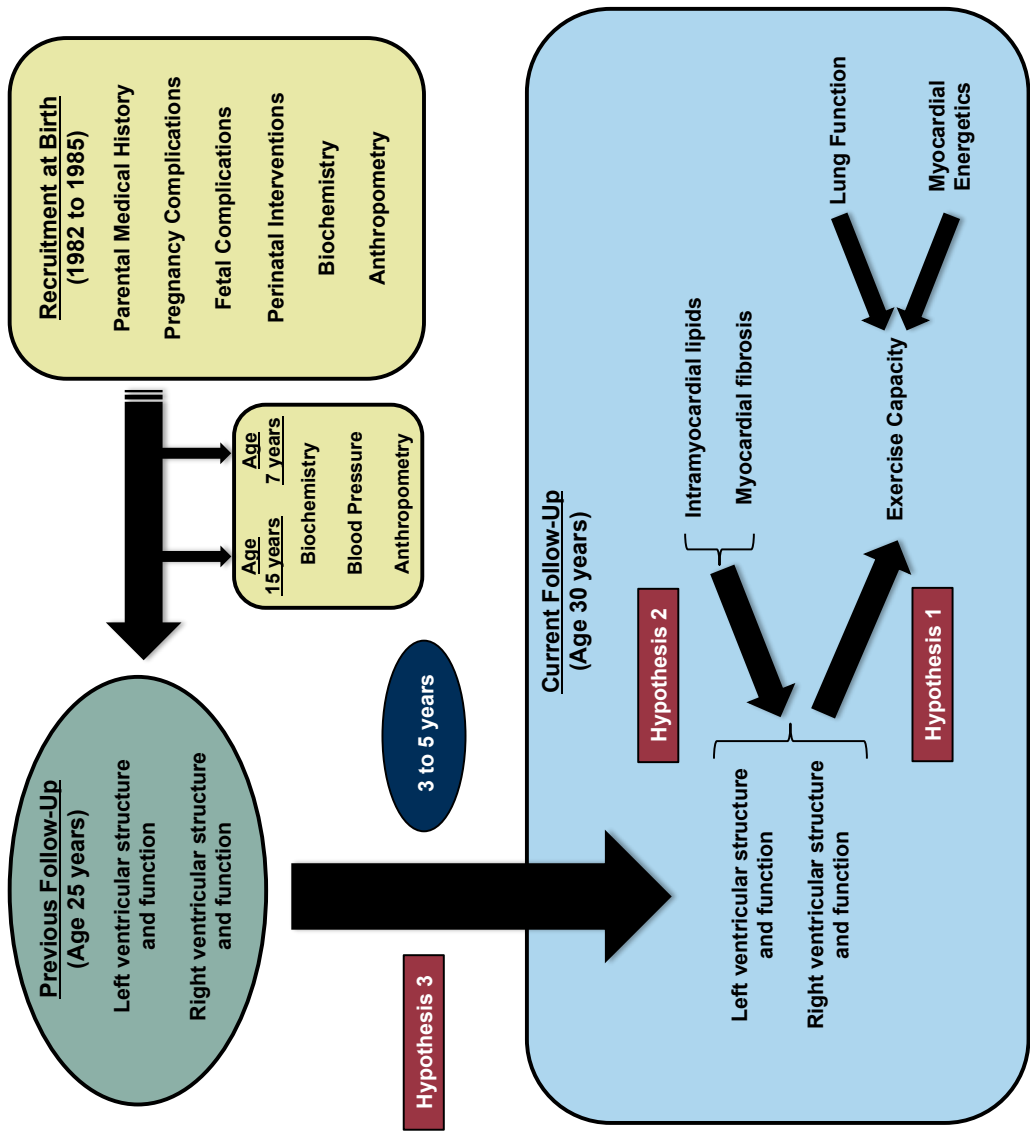


Figure 10.2: Cardiovascular mechanisms to be investigated in the preterm-born cohort. The study will investigate 3 hypotheses related to the cardiac changes in preterm-born adults: (1) reduced exercise capacity in preterm-born adults is proportional to baseline differences in cardiac structure and function, which relate to abnormalities in myocardial metabolism; (2) increased cardiac mass in preterm-born adults relates to increased myocyte mass rather than increased fibrosis or lipid deposition; and (3) preterm-born adults have a disproportionate increase in ventricular mass over time.

LIST OF REFERENCES

- [1] Townsend, N., Wickramasinghe, K., Bhatnagar, P., Smolina, K., Nichols, M., Leal, J., Luengo-Fernandez, R. and Rayner, M.: Coronary heart disease statistics. *London: British Heart Foundation*, 2012.
- [2] Louey, S. and Thornburg, K.L.: The prenatal environment and later cardiovascular disease. *Early Human Development*, vol. 81, no. 9, pp. 745–751, 2005.
- [3] Barker, D.J., Winter, P.D., Osmond, C., Margetts, B. and Simmonds, S.J.: Weight in infancy and death from ischaemic heart disease. *The Lancet*, vol. 2, no. 8663, pp. 577–580, 1989.
- [4] Barker, D.J.: Fetal origins of coronary heart disease. *British Medical Journal*, vol. 311, no. 6998, pp. 171–174, 1995.
- [5] Barker, D.J.: Fetal programming of coronary heart disease. *Trends in Endocrinology and Metabolism*, vol. 13, no. 9, pp. 364–368, 2002.
- [6] Singhal, A., Kattenhorn, M., Cole, T.J., Deanfield, J. and Lucas, A.: Preterm birth, vascular function, and risk factors for atherosclerosis. *The Lancet*, vol. 358, no. 9288, pp. 1159–1160, 2001.
- [7] Dalziel, S.R., Parag, V., Rodgers, A. and Harding, J.E.: Cardiovascular risk factors at age 30 following pre-term birth. *International Journal of Epidemiology*, vol. 36, no. 4, pp. 907–915, 2007.
- [8] Irving, R.J., Belton, N.R., Elton, R.A. and Walker, B.R.: Adult cardiovascular risk factors in premature babies. *The Lancet*, vol. 355, no. 9221, pp. 2135–2136, 2000.
- [9] Norman, M.: Preterm birth—an emerging risk factor for adult hypertension? *Seminars in Perinatology*, vol. 34, no. 3, pp. 183–187, 2010.

- [10] Goldenberg, R.L., Culhane, J.F., Iams, J.D. and Romero, R.: Epidemiology and causes of preterm birth. *The Lancet*, vol. 371, no. 9606, pp. 75–84, 2008.
- [11] Beck, S., Wojdyla, D., Say, L., Betran, A.P., Merialdi, M., Requejo, J.H., Rubens, C., Menon, R. and Look, P.F.V.: The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. *Bulletin of the World Health Organization*, vol. 88, no. 1, pp. 31–38, 2010.
- [12] Ananth, C.V. and Vintzileos, A.M.: Epidemiology of preterm birth and its clinical subtypes. *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 19, no. 12, pp. 773–782, 2006.
- [13] Gotsch, F., Romero, R., Erez, O., Vaisbuch, E., Kusanovic, J., Mazaki-Tovi, S., Kim, S., Hassan, S. and Yeo, L.: The preterm parturition syndrome and its implications for understanding the biology, risk assessment, diagnosis, treatment and prevention of preterm birth. *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 22, no. Suppl 2, pp. 5–23, 2009.
- [14] Tucker, J.M., Goldenberg, R.L., Davis, R.O., Copper, R.L., Winkler, C.L. and Hauth, J.C.: Etiologies of preterm birth in an indigent population: is prevention a logical expectation? *Obstetrics & Gynecology*, vol. 77, no. 3, pp. 343–347, 1991.
- [15] Romero, R., Espinoza, J., Kusanovic, J.P., Gotsch, F., Hassan, S., Erez, O., Chaiworapongsa, T. and Mazar, M.: The preterm parturition syndrome. *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 113, no. Suppl 3, pp. 17–42, 2006.
- [16] Conde-Agudelo, A., Rosas-Bermúdez, A. and Kafury-Goeta, A.C.: Birth spacing and risk of adverse perinatal outcomes. *The Journal of the American Medical Association*, vol. 295, no. 15, pp. 1809–1823, 2006.
- [17] Smith, G.C., Pell, J.P. and Dobbie, R.: Interpregnancy interval and risk of preterm birth and neonatal death: retrospective cohort study. *British Medical Journal*, vol. 327, no. 7410, p. 313, 2003.
- [18] Tamura, T., Goldenberg, R.L., Freeberg, L.E., Cliver, S.P., Cutter, G.R. and Hoffman, H.J.: Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. *The American Journal of Clinical Nutrition*, vol. 56, no. 2, pp. 365–370, 1992.

- [19] Hendler, I., Goldenberg, R.L., Mercer, B.M., Iams, J.D., Meis, P.J., Moawad, A.H., MacPherson, C.A., Caritis, S.N., Miodovnik, M., Menard, K.M., Thurnau, G. and Sorokin, Y.: The preterm prediction study: Association between maternal body mass index and spontaneous and indicated preterm birth. *American Journal of Obstetrics and Gynecology*, vol. 192, no. 3, pp. 882–886, 2005.
- [20] Scholl, T.O.: Iron status during pregnancy: setting the stage for mother and infant. *The American Journal of Clinical Nutrition*, vol. 81, no. 5, pp. 1218S–1222S, 2005.
- [21] Mercer, B.M., Goldenberg, R.L., Moawad, A.H., Meis, P.J., Iams, J.D., Das, A.F., Caritis, S.N., Miodovnik, M., Menard, M.K., Thurnau, G.R., Dombrowski, M.P., Roberts, J.M. and McNellis, D.: The preterm prediction study: effect of gestational age and cause of preterm birth on subsequent obstetric outcome. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *American Journal of Obstetrics and Gynecology*, vol. 181, no. 5 Pt 1, pp. 1216–1221, 1999.
- [22] Copper, R.L., Goldenberg, R.L., Das, A., Elder, N., Swain, M., Norman, G., Ramsey, R., Cotroneo, P., Collins, B., Johnson, F., Jones, P. and Meier, A.: The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *American Journal of Obstetrics and Gynecology*, vol. 175, no. 5, pp. 1286–1292, 1996.
- [23] Lobel, M., Dunkel-Schetter, C. and Scrimshaw, S.C.: Prenatal maternal stress and prematurity: a prospective study of socioeconomically disadvantaged women. *Health Psychology*, vol. 11, no. 1, pp. 32–40, 1992.
- [24] Andres, R.L. and Day, M.: Perinatal complications associated with maternal tobacco use. *Seminars in Neonatology*, vol. 5, no. 3, pp. 231–241, 2000.
- [25] Cnattingius, S.: The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine & Tobacco Research*, vol. 6, no. Suppl 2, pp. 125–140, 2004.
- [26] Knox, I.C. and Hoerner, J.K.: The role of infection in premature rupture of the membranes. *American Journal of Obstetrics and Gynecology*, vol. 173, no. 3, Part 1, pp. 951–951, 1995.

- [27] Goldenberg, R.L., Hauth, J.C. and Andrews, W.W.: Intrauterine infection and preterm delivery. *New England Journal of Medicine*, vol. 342, no. 20, pp. 1500–1507, 2000.
- [28] Porter, T.F., Fraser, A.M., Hunter, C.Y., Ward, R.H. and Varner, M.W.: The risk of preterm birth across generations. *Obstetrics & Gynecology*, vol. 90, no. 1, pp. 63–67, 1997.
- [29] Engel, S.A.M., Erichsen, H.C., Savitz, D.A., Thorp, J., Chanock, S.J. and Olshan, A.F.: Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms. *Epidemiology*, vol. 16, no. 4, pp. 469–477, 2005.
- [30] Menon, R., Velez, D.R., Simhan, H., Ryckman, K., Jiang, L., Thorsen, P., Vogel, I., Jacobsson, B., Merialdi, M., Williams, S.M. and Fortunato, S.J.: Multilocus interactions at maternal tumor necrosis factor- α , tumor necrosis factor receptors, interleukin-6 and interleukin-6 receptor genes predict spontaneous preterm labor in European-American women. *American Journal of Obstetrics and Gynecology*, vol. 194, no. 6, pp. 1616–1624, 2006.
- [31] Crider, K.S., Whitehead, N. and Buus, R.M.: Genetic variation associated with preterm birth: a HuGE review. *Genetics in Medicine*, vol. 7, no. 9, pp. 593–604, 2005.
- [32] Barker, D.J. and Osmond, C.: Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *The Lancet*, vol. 1, no. 8489, pp. 1077–1081, 1986.
- [33] Barker, D.J., Osmond, C., Simmonds, S.J. and Wield, G.A.: The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *British Medical Journal*, vol. 306, no. 6875, pp. 422–426, 1993.
- [34] Lucas, A.: Role of nutritional programming in determining adult morbidity. *Archives of Disease in Childhood*, vol. 71, no. 4, pp. 288–290, 1994.
- [35] Lucas, A.: Programming by early nutrition: an experimental approach. *The Journal of Nutrition*, vol. 128, no. 2, pp. 401S–406S, 1998.
- [36] Snoeck, A., Remacle, C., Reusens, B. and Hoet, J.J.: Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biology of the Neonate*, vol. 57, no. 2, pp. 107–118, 1990.

- [37] Lucas, A., Morley, R., Cole, T.J., Lucas, P.J., Gore, S.M., Crowle, P., Pearce, R., Boon, A.J. and Powell, R.: Early diet in preterm babies and developmental status at 18 months. *The Lancet*, vol. 335, no. 8704, pp. 1477–1481, 1990.
- [38] Abitbol, C.L. and Rodriguez, M.M.: The long-term renal and cardiovascular consequences of prematurity. *Nature Reviews Nephrology*, vol. 8, no. 5, pp. 265–274, 2012.
- [39] Johansson, S., Iliadou, A., Bergvall, N., Tuvemo, T., Norman, M. and Cnattingius, S.: Risk of high blood pressure among young men increases with the degree of immaturity at birth. *Circulation*, vol. 112, no. 22, pp. 3430–3436, 2005.
- [40] Crump, C., Winkleby, M.A., Sundquist, K. and Sundquist, J.: Risk of hypertension among young adults who were born preterm: a Swedish national study of 636,000 births. *American Journal of Epidemiology*, vol. 173, no. 7, pp. 797–803, 2011.
- [41] Bonamy, A.K.E., Bendito, A., Martin, H., Andolf, E., Sedin, G. and Norman, M.: Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. *Pediatric Research*, vol. 58, no. 5, pp. 845–849, 2005.
- [42] Kistner, A., Celsi, G., Vanpée, M. and Jacobson, S.H.: Increased systolic daily ambulatory blood pressure in adult women born preterm. *Pediatric Nephrology*, vol. 20, no. 2, pp. 232–233, 2005.
- [43] Evensen, K.A.I., Steinshamn, S., Tjonna, A.E., Stolen, T., Hoydal, M.A., Wisloff, U., Brubakk, A. and Vik, T.: Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Human Development*, vol. 85, no. 4, pp. 239–245, 2009.
- [44] Rotteveel, J., van Weissenbruch, M.M., Twisk, J.W.R. and Delemarre-Van de Waal, H.A.: Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. *Pediatrics*, vol. 122, no. 2, pp. 313–321, 2008.
- [45] Hovi, P., Andersson, S., Eriksson, J.G., Järvenpää, A.L., Strang-Karlsson, S., Mäkitie, O. and Kajantie, E.: Glucose regulation in young adults with very low birth weight. *New England Journal of Medicine*, vol. 356, no. 20, pp. 2053–2063, 2007.
- [46] Keijzer-Veen, M.G., Finken, M.J.J., Nauta, J., Dekker, F.W., Hille, E.T.M., Frölich, M., Wit, J.M., van der Heijden, A.J. and Dutch POPS-19 Collaborative Study Group: Is blood pressure in-

- creased 19 years after intrauterine growth restriction and preterm birth? A prospective follow-up study in the Netherlands. *Pediatrics*, vol. 116, no. 3, pp. 725–731, 2005.
- [47] Hack, M., Schluchter, M., Cartar, L. and Rahman, M.: Blood pressure among very low birth weight (<1.5 kg) young adults. *Pediatric Research*, vol. 58, no. 4, pp. 677–684, 2005.
- [48] Doyle, L.W., Faber, B., Callanan, C. and Morley, R.: Blood pressure in late adolescence and very low birth weight. *Pediatrics*, vol. 111, no. 2, pp. 252–257, 2003.
- [49] Pharoah, P., Stevenson, C. and West, C.: Association of blood pressure in adolescence with birthweight. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 79, no. 2, pp. F114–F118, 1998.
- [50] Vohr, B.R., Allan, W., Katz, K.H., Schneider, K.C. and Ment, L.R.: Early predictors of hypertension in prematurely born adolescents. *Acta Pædiatrica*, vol. 99, no. 12, pp. 1812–1818, 2010.
- [51] Lazdam, M., de la Horra, A., Pitcher, A., Mannie, Z., Diesch, J., Trevitt, C., Kylintireas, I., Contractor, H., Singhal, A., Lucas, A., Neubauer, S., Kharbanda, R., Alp, N., Kelly, B. and Leeson, P.: Elevated blood pressure in offspring born premature to hypertensive pregnancy: is endothelial dysfunction the underlying vascular mechanism? *Hypertension*, vol. 56, no. 1, pp. 159–165, 2010.
- [52] Hovi, P., Andersson, S., Räikkönen, K., Strang-Karlsson, S., Järvenpää, A.L., Eriksson, J.G., Pesonen, A.K., Heinonen, K., Pyhälä, R. and Kajantie, E.: Ambulatory blood pressure in young adults with very low birth weight. *The Journal of Pediatrics*, vol. 156, no. 1, pp. 54–59.e1, 2010.
- [53] Chen, X. and Wang, Y.: Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. *Circulation*, vol. 117, no. 25, pp. 3171–3180, 2008.
- [54] Law, M., Wald, N. and Morris, J.: Lowering blood pressure to prevent myocardial infarction and stroke: a new preventive strategy. *Health Technology Assessment*, vol. 7, no. 31, pp. 1–94, 2003.
- [55] Alberti, K.G., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P., Loria, C.M. and Smith, S.C.: Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Fed-

- eration; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, vol. 120, no. 16, pp. 1640–1645, 2009.
- [56] Hofman, P.L., Regan, F., Jackson, W.E., Jefferies, C., Knight, D.B., Robinson, E.M. and Cutfield, W.S.: Premature birth and later insulin resistance. *New England Journal of Medicine*, vol. 351, no. 21, pp. 2179–2186, 2004.
- [57] Mitanchez-Mokhtari, D., Lahlou, N., Kieffer, F., Magny, J.F., Roger, M. and Voyer, M.: Both relative insulin resistance and defective islet β -cell processing of proinsulin are responsible for transient hyperglycemia in extremely preterm infants. *Pediatrics*, vol. 113, no. 3, pp. 537–541, 2004.
- [58] Plagemann, A., Harder, T., Rake, A., Voits, M., Fink, H., Rohde, W. and Dörner, G.: Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergetic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats. *Brain Research*, vol. 836, no. 1-2, pp. 146–155, 1999.
- [59] Gournay, V., Drouin, E. and Rozé, J.C.: Development of baroreflex control of heart rate in preterm and full term infants. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 86, no. 3, pp. F151–F154, 2002.
- [60] Barker, D.J.P., Osmond, C., Golding, J., Kuh, D. and Wadsworth, M.E.J.: Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *British Medical Journal*, vol. 298, no. 6673, pp. 564–567, 1989.
- [61] Taittonen, L., Nuutinen, M., Turtinen, J. and Uhari, M.: Prenatal and postnatal factors in predicting later blood pressure among children: cardiovascular risk in young Finns. *Pediatric Research*, vol. 40, no. 4, pp. 627–632, 1996.
- [62] Singhal, A., Cole, T.J. and Lucas, A.: Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *The Lancet*, vol. 357, no. 9254, pp. 413–419, 2001.
- [63] Hack, M., Schluchter, M., Cartar, L., Rahman, M., Cuttler, L. and Borawski, E.: Growth of very low birth weight infants to age 20 years. *Pediatrics*, vol. 112, no. 1, pp. e30–e38, 2003.

- [64] Ericson, A. and Källén, B.: Very low birthweight boys at the age of 19. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 78, no. 3, pp. F171–F174, 1998.
- [65] Cooke, R.W.I.: Health, lifestyle, and quality of life for young adults born very preterm. *Archives of Disease in Childhood*, vol. 89, no. 3, pp. 201–206, 2004.
- [66] Lucas, A., Fewtrell, M.S. and Cole, T.J.: Fetal origins of adult disease - the hypothesis revisited. *British Medical Journal*, vol. 319, no. 7204, pp. 245–249, 1999.
- [67] Regan, F.M., Cutfield, W.S., Jefferies, C., Robinson, E. and Hofman, P.L.: The impact of early nutrition in premature infants on later childhood insulin sensitivity and growth. *Pediatrics*, vol. 118, no. 5, pp. 1943–1949, 2006.
- [68] Thomas, E., Uthaya, S., Vasu, V., McCarthy, J.P., McEwan, P., Hamilton, G., Bell, J.D. and Modi, N.: Neonatal intrahepatocellular lipid. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 93, no. 5, pp. F382–F383, 2008.
- [69] Uthaya, S., Thomas, E.L., Hamilton, G., Dore, C.J., Bell, J. and Modi, N.: Altered adiposity after extremely preterm birth. *Pediatric Research*, vol. 57, no. 2, pp. 211–215, 2005.
- [70] Wajchenberg, B.L., Giannella-Neto, D., da Silva, M.E. and Santos, R.F.: Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. *Hormone and Metabolic Research*, vol. 34, no. 11/12, pp. 616–621, 2002.
- [71] Singhal, A., Farooqi, I.S., O’Rahilly, S., Cole, T., Fewtrell, M. and Lucas, A.: Early nutrition and leptin concentrations in later life. *The American Journal of Clinical Nutrition*, vol. 75, no. 6, pp. 993–999, 2002.
- [72] Santos, F. and Dean, W.: Epigenetic reprogramming during early development in mammals. *Reproduction*, vol. 127, no. 6, pp. 643–651, 2004.
- [73] Van den Veyver, I.B.: Genetic effects of methylation diets. *Annual Review of Nutrition*, vol. 22, pp. 255–282, 2002.
- [74] Waterland, R.A. and Garza, C.: Potential mechanisms of metabolic imprinting that lead to chronic disease. *The American Journal of Clinical Nutrition*, vol. 69, no. 2, pp. 179–197, 1999.

- [75] Waterland, R.A. and Jirtle, R.L.: Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition*, vol. 20, no. 1, pp. 63–68, 2004.
- [76] Beck, L. and D'Amore, P.A.: Vascular development: cellular and molecular regulation. *The FASEB Journal*, vol. 11, no. 5, pp. 365–373, 1997.
- [77] Ligi, I., Simoncini, S., Tellier, E., Vassallo, P.F., Sabatier, F., Guillet, B., Lamy, E., Sarlon, G., Quemener, C., Bikfalvi, A., Marcelli, M., Pascal, A., Dizier, B., Simeoni, U., Dignat-George, F. and Anfosso, F.: A switch toward angiostatic gene expression impairs the angiogenic properties of endothelial progenitor cells in low birth weight preterm infants. *Blood*, vol. 118, no. 6, pp. 1699–1709, 2011.
- [78] Kistner, A., Jacobson, L., Jacobson, S.H., Svensson, E. and Hellstrom, A.: Low gestational age associated with abnormal retinal vascularization and increased blood pressure in adult women. *Pediatric Research*, vol. 51, no. 6, pp. 675–680, 2002.
- [79] Hellstrom, A., Hard, A.L., Niklasson, A., Svensson, E. and Jacobsson, B.: Abnormal retinal vascularisation in preterm children as a general vascular phenomenon. *The Lancet*, vol. 352, no. 9143, p. 1827, 1998.
- [80] Bonamy, A.K.E., Martin, H., Jörneskog, G. and Norman, M.: Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *Journal of Internal Medicine*, vol. 262, no. 6, pp. 635–642, 2007.
- [81] Kelleher, C.M., McLean, S.E., Mecham, R.P. and Gerald, P.S.: Vascular extracellular matrix and aortic development. Current Topics in Developmental Biology. *Academic Press*, vol. 62, pp. 153–188, 2004.
- [82] Wells, S.M. and Walter, E.J.: Changes in the mechanical properties and residual strain of elastic tissue in the developing fetal aorta. *Annals of Biomedical Engineering*, vol. 38, no. 2, pp. 345–356, 2010.
- [83] Cliff, W.J.: The aortic tunica media in growing rats studied with the electron microscope. *Laboratory Investigation*, vol. 17, no. 6, pp. 599–615, 1967.

- [84] Berry, C.L., Looker, T. and Germain, J.: The growth and development of the rat aorta. I. Morphological aspects. *Journal of Anatomy*, vol. 113, no. Pt 1, pp. 1–16, 1972.
- [85] Wolinsky, H. and Glagov, S.: A lamellar unit of aortic medial structure and function in mammals. *Circulation Research*, vol. 20, no. 1, pp. 99–111, 1967.
- [86] Belz, G.G.: Elastic properties and Windkessel function of the human aorta. *Cardiovascular Drugs and Therapy*, vol. 9, no. 1, pp. 73–83, 1995.
- [87] Schubert, U., Muller, M., Edstedt Bonamy, A.K., Abdul-Khaliq, H. and Norman, M.: Aortic growth arrest after preterm birth: a lasting structural change of the vascular tree. *Journal of Developmental Origins of Health and Disease*, vol. 2, no. 4, pp. 218–225, 2010.
- [88] Martyn, C.N. and Greenwald, S.E.: Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. *The Lancet*, vol. 350, no. 9082, pp. 953–955, 1997.
- [89] Davis, E.C.: Stability of elastin in the developing mouse aorta: a quantitative radioautographic study. *Histochemistry*, vol. 100, no. 1, pp. 17–26, 1993.
- [90] Labella, F.S. and Lindsay, W.G.: The structure of human aortic elastin as influenced by age. *Journal of Gerontology*, vol. 18, pp. 111–118, 1963.
- [91] Struijk, P.C., Mathews, V.J., Loupas, T., Stewart, P.A., Clark, E.B., Steegers, E.A. and Wladimiroff, J.W.: Blood pressure estimation in the human fetal descending aorta. *Ultrasound in Obstetrics and Gynecology*, vol. 32, no. 5, pp. 673–681, 2008.
- [92] Bendeck, M.P., Keeley, F.W. and Langille, B.L.: Perinatal accumulation of arterial wall constituents: relation to hemodynamic changes at birth. *American Journal of Physiology*, vol. 267, no. 6 Pt 2, pp. H2268–H2279, 1994.
- [93] Guyton, J.R. and Hartley, C.J.: Flow restriction of one carotid artery in juvenile rats inhibits growth of arterial diameter. *American Journal of Physiology*, vol. 248, no. 4 Pt 2, pp. H540–H546, 1985.
- [94] Coyle, P.: Interruption of the middle cerebral artery in 10-day-old rat alters the normal development of distal collaterals. *The Anatomical Record*, vol. 212, no. 2, pp. 179–182, 1985.

- [95] Tauzin, L., Rossi, P., Giusano, B., Gaudart, J., Boussuges, A., Fraisse, A. and Simeoni, U.: Characteristics of arterial stiffness in very low birth weight premature infants. *Pediatric Research*, vol. 60, no. 5, pp. 592–596, 2006.
- [96] Oren, A., Vos, L.E., Bos, W.J., Safar, M.E., Uiterwaal, C.S., Gorissen, W.H., Grobbee, D.E. and Bots, M.L.: Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? *American Journal of Hypertension*, vol. 16, no. 1, pp. 76–79, 2003.
- [97] Cheung, Y.F., Wong, K.Y., Lam, B.C.C. and Tsoi, N.S.: Relation of arterial stiffness with gestational age and birth weight. *Archives of Disease in Childhood*, vol. 89, no. 3, pp. 217–221, 2004.
- [98] Rudolph, A.M.: Myocardial growth before and after birth: clinical implications. *Acta Paediatrica*, vol. 89, no. 2, pp. 129–133, 2000.
- [99] Manasek, F.J.: Mitosis in developing cardiac muscle. *The Journal of Cell Biology*, vol. 37, no. 1, pp. 191–195, 1968.
- [100] Polinger, I.S.: Growth and DNA synthesis in embryonic chick heart cells, in vivo and in vitro. *Experimental Cell Research*, vol. 76, no. 2, pp. 253–262, 1973.
- [101] Oparil, S.: Pathogenesis of ventricular hypertrophy. *Journal of the American College of Cardiology*, vol. 5, no. 6 Suppl, pp. 57B–65B, 1985.
- [102] Sandritter, W. and Scomazzoni, G.: Deoxyribonucleic acid content (Feulgen photometry) and dry weight (interference microscopy) of normal and hypertrophic heart muscle fibers. *Nature*, vol. 202, pp. 100–101, 1964.
- [103] Recavarren, S. and Aria-Stella, J.: Growth and development of the ventricular myocardium from birth to adult life. *Br Heart J*, vol. 26, no. 2, pp. 187–192, 1964.
- [104] Neffgen, J.F. and Korecky, B.: Cellular hyperplasia and hypertrophy in cardiomegalies induced by anemia in young adult rats. *Circulation Research*, vol. 30, no. 1, pp. 104–113, 1972.
- [105] Anatskaya, O.V., Sidorenko, N.V., Beyer, T.V. and Vinogradov, A.E.: Neonatal cardiomyocyte ploidy reveals critical windows of heart development. *International Journal of Cardiology*, vol. 141, no. 1, pp. 81–91, 2009.

- [106] Adler, C.P. and Friedburg, H.: Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. *Journal of Molecular and Cellular Cardiology*, vol. 18, no. 1, pp. 39–53, 1986.
- [107] Brodsky, V., Sarkisov, D.S., Arefyeva, A.M. and Panova, N.W.: DNA and protein relations in cardiomyocytes. Growth reserve in cardiac muscle. *European Journal of Histochemistry*, vol. 37, no. 3, pp. 199–206, 1993.
- [108] Brodsky, V., Sarkisov, D.S., Arefyeva, A.M., Panova, N.W. and Gvasava, I.G.: Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values. *Virchows Arch - The European Journal of Pathology*, vol. 424, no. 4, pp. 429–435, 1994.
- [109] Gessner, I., Krovetz, L.J., Benson, R.W., Prystowsky, H., Stenger, V. and Eitzman, D.V.: Hemodynamic adaptations in the newborn infant. *Pediatrics*, vol. 36, no. 5, pp. 752–762, 1965.
- [110] Kluckow, M.: Low systemic blood flow and pathophysiology of the preterm transitional circulation. *Early Human Development*, vol. 81, no. 5, pp. 429–437, 2005.
- [111] Bensley, J.G., Stacy, V.K., De Matteo, R., Harding, R. and Black, M.J.: Cardiac remodelling as a result of pre-term birth: implications for future cardiovascular disease. *European Heart Journal*, vol. 31, no. 16, pp. 2058–2066, 2010.
- [112] Innis, S.M.: Essential fatty acids in growth and development. *Progress in Lipid Research*, vol. 30, no. 1, pp. 39–103, 1991.
- [113] Callow, J., Samra, J.S. and Frayn, K.N.: Effect of infusion of a triacylglycerol emulsion on low-density lipoprotein composition and oxidizability. *Atherosclerosis*, vol. 137, no. 1, pp. 115–123, 1998.
- [114] Griffin, E., Breckenridge, W.C., Kuksis, A., Bryan, M.H. and Angel, A.: Appearance and characterization of lipoprotein x during continuous intralipid infusions in the neonate. *The Journal of Clinical Investigation*, vol. 64, no. 6, pp. 1703–1712, 1979.
- [115] Kerner, J.A. and Poole, R.L.: The use of IV fat in neonates. *Nutrition in Clinical Practice*, vol. 21, no. 4, pp. 374–380, 2006.

- [116] Faggiotto, A., Ross, R. and Harker, L.: Studies of hypercholesterolaemia in the non-human primate: changes that lead to fatty streak formation. *Arteriosclerosis*, vol. 4, no. 4, pp. 323–340, 1984.
- [117] Ross, R.: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, vol. 362, no. 6423, pp. 801–809, 1993.
- [118] Ross, R. and Harker, L.: Hyperlipidemia and atherosclerosis. *Science*, vol. 193, no. 4258, pp. 1094–1100, 1976.
- [119] Stary, H.C.: Evolution and progression of atherosclerotic lesions in coronary arteries in children and young adults. *Arteriosclerosis*, vol. 99, no. Suppl I, pp. I19–32, 1989.
- [120] Napoli, C., D’Armiento, F.P., Mancini, F.P., Witztum, J.L., Palumbo, G. and Palinski, W.: 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. *The Journal of Clinical Investigation*, vol. 100, no. 11, pp. 2680–2690, 1997.
- [121] Napoli, C., Glass, C.K., Witztum, J.L., Deutsch, R., D’Armiento, F.P. and Palinski, W.: Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. *The Lancet*, vol. 354, no. 9186, pp. 1234–1241, 1999.
- [122] Huang, Y., Walker, K.E., Hanley, E., Narula, J., Houser, S.R. and Tulenko, T.N.: Cardiac systolic and diastolic dysfunction after a cholesterol-rich diet. *Circulation*, vol. 109, no. 1, pp. 97–102, 2004.
- [123] Rubinstein, J., Pelosi, A., Vedre, A., Kotaru, P. and Abela, G.: Hypercholesterolemia and myocardial function evaluated via tissue doppler imaging. *Cardiovascular Ultrasound*, vol. 7, no. 1, p. 56, 2009.
- [124] National Institutes of Health. Effect of corticosteroids for fetal maturation on perinatal outcomes. *National Institutes of Health Consensus Statement*, vol. 12, no. 2, pp. 1–24, 1994.

- [125] Lawson, E.E.: Antenatal corticosteroids - too much of a good thing? *The Journal of the American Medical Association*, vol. 286, no. 13, pp. 1628–1630, 2001.
- [126] Roberts, D. and Dalziel, S.R.: Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database of Systematic Reviews*, vol. 19, no. 3, p. CD004454, 2006.
- [127] Surbek, D., Drack, G., Irion, O., Nelle, M., Huang, D. and Hoesli, I.: Antenatal corticosteroids for fetal lung maturation in threatened preterm delivery: indications and administration. *Archives of Gynecology and Obstetrics*, vol. 286, no. 2, pp. 277–281, 2012.
- [128] Murphy, K.E., Hannah, M.E., Willan, A.R., Hewson, S.A., Ohlsson, A., Kelly, E.N., Matthews, S.G., Asztalos, E., Ross, S., Delisle, M.F., Amankwah, K., Guselle, P., Gafni, A., Lee, S.K., Armson, B.A. and MACS Collaborative Group: Multiple courses of antenatal corticosteroids for preterm birth (MACS): a randomised controlled trial. *The Lancet*, vol. 372, no. 9656, pp. 2143–2151, 2008.
- [129] Crowther, C.A., McKinlay, C.J.D., Middleton, P. and Harding, J.E.: Repeat doses of prenatal corticosteroids for women at risk of preterm birth for preventing neonatal respiratory disease. *Cochrane Database of Systematic Reviews*, vol. 6, p. CD003935, 2011.
- [130] Dodic, M., Abouantoun, T., O'Connor, A., Wintour, E.M. and Moritz, K.M.: Programming effects of short prenatal exposure to dexamethasone in sheep. *Hypertension*, vol. 40, no. 5, pp. 729–734, 2002.
- [131] Dalziel, S.R., Walker, N.K., Parag, V., Mantell, C., Rea, H.H., Rodgers, A. and Harding, J.E.: Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. *The Lancet*, vol. 365, no. 9474, pp. 1856–1862, 2005.
- [132] Bensley, J.G., De Matteo, R., Harding, R. and Black, M.J.: Preterm birth with antenatal corticosteroid administration has injurious and persistent effects on the structure and composition of the aorta and pulmonary artery. *Pediatric Research*, vol. 71, no. 2, pp. 150–155, 2012.
- [133] Rudolph, A., Roman, C. and Gournay, V.: Perinatal myocardial dna and protein changes in the lamb: Effect of cortisol in the fetus. *Pediatric Research*, vol. 46, no. 2, pp. 141–146, 1999.

- [134] Chacko, S.: DNA synthesis, mitosis, and differentiation in cardiac myogenesis. *Developmental Biology*, vol. 35, no. 1, pp. 1–18, 1973.
- [135] Brown, M.K. and DiBlasi, R.M.: Mechanical ventilation of the premature neonate. *Respiratory Care*, vol. 56, no. 9, pp. 1298–1311, 2011.
- [136] Shekerdemian, L. and Desmond, B.: Cardiovascular effects of mechanical ventilation. *Archives of Disease in Childhood*, vol. 80, no. 5, pp. 475–480, 1999.
- [137] Marini, J.J., Culver, B.H. and Butler, J.: Mechanical effect of lung distention with positive pressure on cardiac function. *The American Review of Respiratory Disease*, vol. 124, no. 4, pp. 382–386, 1981.
- [138] Bogaard, H.J., Abe, K., Vonk Noordegraaf, A. and Voelkel, N.E.: The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest*, vol. 135, no. 3, pp. 794–804, 2009.
- [139] Brown, M.A., Lindheimer, M.D., de Swiet, M., Van Assche, A. and Moutquin, J.M.: The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertension in Pregnancy*, vol. 20, no. 1, pp. IX–XIV, 2001.
- [140] Duley, L.: The global impact of pre-eclampsia and eclampsia. *Seminars in Perinatology*, vol. 33, no. 3, pp. 130–137, 2009.
- [141] Henderson, J.J., McWilliam, O.A., Newnham, J.P. and Pennell, C.E.: Preterm birth aetiology 2004-2008. Maternal factors associated with three phenotypes: spontaneous preterm labour, preterm pre-labour rupture of membranes and medically indicated preterm birth. *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 25, no. 6, pp. 642–647, 2012.
- [142] Davis, E.F., Newton, L., Lewandowski, A.J., Lazdam, M., Kelly, B.A., Kyriakou, T. and Leeson, P.: Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. *Clinical Science*, vol. 123, no. 2, pp. 53–72, 2012.
- [143] Lazdam, M., de la Horra, A., Diesch, J., Kenworthy, Y., Davis, E., Lewandowski, A.J., Szmigielski, C., Shore, A., Mackillop, L., Kharbada, R., Alp, N., Redman, C., Kelly, B. and Leeson, P.:

- Unique blood pressure characteristics in mother and offspring after early onset preeclampsia. *Hypertension*, vol. 60, no. 5, pp. 1338–1345, 2012.
- [144] Redman, C.W. and Sargent, I.L.: Placental stress and pre-eclampsia: a revised view. *Placenta*, vol. 30, no. Suppl A, pp. S38–S42, 2009.
- [145] Rodie, V.A., Freeman, D.J., Sattar, N. and Greer, I.A.: Pre-eclampsia and cardiovascular disease: metabolic syndrome of pregnancy? *Atherosclerosis*, vol. 175, no. 2, pp. 189–202, 2004.
- [146] Poston, L.: Endothelial dysfunction in pre-eclampsia. *Pharmacological Reports*, vol. 58, no. Suppl, pp. 69–74, 2006.
- [147] Lazdam, M., Davis, E.F., Lewandowski, A.J., Worton, S.A., Kenworthy, Y., Kelly, B. and Leeson, P.: Prevention of vascular dysfunction after preeclampsia: a potential long-term outcome measure and an emerging goal for treatment. *Journal of Pregnancy*, vol. 2012, no. 704146, 2012.
- [148] Furuya, M., Kurasawa, K., Nagahama, K., Kawachi, K., Nozawa, A., Takahashi, T. and Aoki, I.: Disrupted balance of angiogenic and antiangiogenic signalings in preeclampsia. *Journal of Pregnancy*, vol. 2011, p. 123717, 2011.
- [149] Kajantie, E., Eriksson, J.G., Osmond, C., Thornburg, K. and Barker, D.J.: Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. *Stroke*, vol. 40, no. 4, pp. 1176–1180, 2009.
- [150] Touwslager, R.N., Houben, A.J., Gielen, M., Zeegers, M.P., Stehouwer, C.D., Zimmermann, L.J., Kessels, A.G., Gerver, W.J., Blanco, C.E. and Mulder, A.L.: Endothelial vasodilatation in newborns is related to body size and maternal hypertension. *Journal of Hypertension*, vol. 30, no. 1, pp. 124–131, 2012.
- [151] Tong, W., Xue, Q., Li, Y. and Zhang, L.: Maternal hypoxia alters matrix metalloproteinase expression patterns and causes cardiac remodeling in fetal and neonatal rats. *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 301, no. 5, pp. H2113–2121, 2011.
- [152] Xu, Y., Williams, S.J., O'Brien, D. and Davidge, S.T.: Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in

- adult male offspring. *The Journal of the Federation of American Societies for Experimental Biology*, vol. 20, no. 8, pp. 1251–1253, 2006.
- [153] Rueda-Clausen, C.F., Morton, J.S., Lopaschuk, G.D. and Davidge, S.T.: Long-term effects of intrauterine growth restriction on cardiac metabolism and susceptibility to ischaemia/reperfusion. *Cardiovascular Research*, vol. 90, no. 2, pp. 285–294, 2011.
- [154] Li, G., Xiao, Y., Estrella, J.L., Ducsay, C.A., Gilbert, R.D. and Zhang, L.: Effect of fetal hypoxia on heart susceptibility to ischemia and reperfusion injury in the adult rat. *Journal of the Society of Gynecologic Investigation*, vol. 10, no. 5, pp. 265–274, 2003.
- [155] Himmelmann, A., Svensson, A. and Hansson, L.: Five-year follow-up of blood pressure and left ventricular mass in children with different maternal histories of hypertension: the hypertension in pregnancy offspring study. *Journal of Hypertension*, vol. 12, no. 1, pp. 89–95, 1994.
- [156] Fugelseth, D., Ramstad, H.B., Kvehaugen, A.S., Nestaas, E., Stoylen, A. and Staff, A.C.: Myocardial function in offspring 5–8 years after pregnancy complicated by preeclampsia. *Early Human Development*, vol. 87, no. 8, pp. 531–535, 2011.
- [157] Singhal, A., Cole, T.J., Fewtrell, M. and Lucas, A.: Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *The Lancet*, vol. 363, no. 9421, pp. 1571–1578, 2004.
- [158] Lucas, A., Morley, R., Cole, T.J., Lister, G. and Leeson-Payne, C.: Breast milk and subsequent intelligence quotient in children born preterm. *The Lancet*, vol. 339, no. 8788, pp. 261–4, 1992.
- [159] Lewandowski, A.J., Lazdam, M., Davis, E., Kyliantiras, I., Diesch, J., Francis, J., Neubauer, S., Singhal, A., Lucas, A., Kelly, B. and Leeson, P.: Short-term exposure to exogenous lipids in premature infants and long-term changes in aortic and cardiac function. *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 9, pp. 2125–35, 2011.
- [160] Kelly, B.A., Lewandowski, A.J., Worton, S.A., Davis, E.F., Lazdam, M., Francis, J., Neubauer, S., Lucas, A., Singhal, A. and Leeson, P.: Antenatal glucocorticoid exposure and long-term alterations in aortic function and glucose metabolism. *Pediatrics*, vol. 129, no. 5, pp. e1282–e1290, 2012.

- [161] Lewandowski, A.J., Augustine, D., Lamata, P., Davis, E.F., Lazdam, M., Francis, J., McCormick, K., Wilkinson, A., Singhal, A., Lucas, A., Smith, N., Neubauer, S. and Leeson, P.: Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry, and function. *Circulation*, vol. 127, no. 2, pp. 197–206, 2013.
- [162] Lewandowski, A.J., Bradlow, W.M., Augustine, D., Davis, E.F., Francis, J., Singhal, A., Lucas, A., Neubauer, S., McCormick, K. and Leeson, P.: Right ventricular systolic dysfunction in young adults born preterm. *Circulation*, vol. 128, no. 7, pp. 713–720, 2013.
- [163] Leeson, C.P.M., Kattenhorn, M., Morley, R., Lucas, A. and Deanfield, J.E.: Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation*, vol. 103, no. 9, pp. 1264–1268, 2001.
- [164] Grotenhuis, H.B., Westenberg, J.J.M., Steendijk, P., van der Geest, R.J., Ottenkamp, J., Bax, J.J., Jukema, J.W. and de Roos, A.: Validation and reproducibility of aortic pulse wave velocity as assessed with velocity-encoded MRI. *Journal of Magnetic Resonance Imaging*, vol. 30, no. 3, pp. 521–526, 2009.
- [165] Karamitsos, T., Hudsmith, L., Selvanayagam, J., Neubauer, S. and Francis, J.M.: Operator induced variability in left ventricular measurements with cardiovascular magnetic resonance is improved after training. *Journal of Cardiovascular Magnetic Resonance*, vol. 9, no. 5, pp. 777–783, 2007.
- [166] Bradlow, W.M., Hughes, M.L., Keenan, N.G., Bucciarelli-Ducci, C., Assomull, R., Gibbs, J.S.R. and Mohiaddin, R.H.: Measuring the heart in pulmonary arterial hypertension (PAH): implications for trial study size. *Journal of Magnetic Resonance Imaging*, vol. 31, no. 1, pp. 117–124, 2010.
- [167] Leeson, C.P., Robinson, M., Francis, J.M., Robson, M.D., Channon, K.M., Neubauer, S. and Wiesmann, F.: Cardiovascular magnetic resonance imaging for non-invasive assessment of vascular function: validation against ultrasound. *Journal of Cardiovascular Magnetic Resonance*, vol. 8, no. 2, pp. 381 – 387, 2006.
- [168] Augustine, D., Lewandowski, A.J., Lazdam, M., Rai, A., Francis, J., Myerson, S., Noble, A., Becher, H., Neubauer, S., Petersen, S.E. and Leeson, P.: Global and regional left ventricular myocardial

- deformation measures by magnetic resonance feature tracking in healthy volunteers: comparison with tagging and relevance of gender. *Journal of Cardiovascular Magnetic Resonance*, vol. 15, no. 1, p. 8, 2013.
- [169] Noon, J.P., Walker, B.R., Webb, D.J., Shore, A.C., Holton, D.W., Edwards, H.V. and Watt, G.C.: Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *Journal of Clinical Investigation*, vol. 99, no. 8, pp. 1873–1879, 1997.
- [170] Friedewald, W.T., Levy, R.I. and Fredrickson, D.S.: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, vol. 18, no. 6, pp. 499–502, 1972.
- [171] Ferrara, N.: Role of vascular endothelial growth factor in regulation of physiological angiogenesis. *American Journal of Physiology - Cell Physiology*, vol. 280, no. 6, pp. C1358–C1366, 2001.
- [172] Jung, J.J., Tiwari, A., Inamdar, S.M., Thomas, C.P., Goel, A. and Choudhury, A.: Secretion of soluble vascular endothelial growth factor receptor 1 (sVEGFR1/sFlt1) requires Arf1, Arf6, and Rab11 GTPases., journal = PLoS One, volume = 7, number = 9, pages = e44572, year = 2012.
- [173] Giannattasio, C., Mangoni, A.A., Failla, M., Stella, M.L., Carugo, S., Bombelli, M., Sega, R. and Mancina, G.: Combined effects of hypertension and hypercholesterolemia on radial artery function. *Hypertension*, vol. 29, no. 2, pp. 583–586, 1997.
- [174] Pauca, A.L., O'Rourke, M.F. and Kon, N.D.: Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension*, vol. 38, no. 4, pp. 932–937, 2001.
- [175] Chen, C.H., Nevo, E., Fetters, B., Pak, P.H., Yin, F.C., Maughan, W.L. and Kass, D.A.: Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation*, vol. 95, no. 7, pp. 1827–1836, 1997.
- [176] Karamanoglu, M., O'Rourke, M.F., Avolio, A.P. and Kelly, R.P.: An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *European Heart Journal*, vol. 14, no. 2, pp. 160–167, 1993.

- [177] Fetics, B., Nevo, E., Chen, C.H. and Kass, D.A.: Parametric model derivation of transfer function for noninvasive estimation of aortic pressure by radial tonometry. *IEEE Transactions on Biomedical Engineering*, vol. 46, no. 6, pp. 698–706, 1999.
- [178] Ding, E.H., Fan, W.X., Zhang, R.Y., Zhang, Q., Li, Y. and Wang, J.G.: Validation of the noninvasive assessment of central blood pressure by the SphygmoCor and Omron devices against the invasive catheter measurement. *American Journal of Hypertension*, vol. 24, no. 12, pp. 1306–1311, 2011.
- [179] Lehmann, E.D.: Citation of "validation" references for sphygmocor-based estimates of central aortic blood pressure. *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 4, pp. 1844–1845, 2001.
- [180] Bland, J.M. and Altman, D.G.: Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, vol. 1, no. 8476, pp. 307–310, 1986.
- [181] Hironaka, K., Yano, M., Kohno, M., Tanigawa, T., Obayashi, M., Konishi, M., Umemoto, S. and Matsuzaki, M.: In vivo aortic wall characteristics at the early stage of atherosclerosis in rabbits. *American Journal of Physiology*, vol. 273, no. 3 pt 2, pp. H1142–1147, 1997.
- [182] Mitchell, G.F., Hwang, S.J., Vasan, R.S., Larson, M.G., Pencina, M.J., Hamburg, N.M., Vita, J.A., Levy, D. and Benjamin, E.J.: Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation*, vol. 121, no. 4, pp. 505–511, 2010.
- [183] Bishop, N.J.: Feeding the preterm infant. *Pediatric Nephrology*, vol. 8, no. 4, pp. 494–498, 1994.
- [184] Morgan, J.B. and Kovar, I.Z.: The low birth weight infant and parenteral nutrition. *Nutrition Research Reviews*, vol. 5, no. 1, pp. 115–129, 1992.
- [185] Wiesmann, F., Petersen, S.E., Leeson, P.M., Francis, J.M., Robson, M.D., Wang, Q., Choudhury, R., Channon, K.M. and Neubauer, S.: Global impairment of brachial, carotid, and aortic vascular function in young smokers: direct quantification by high-resolution magnetic resonance imaging. *Journal of the American College of Cardiology*, vol. 44, no. 10, pp. 2056–2064, 2004.

- [186] Sporik, R., Johnstone, J.H. and Cogswell, J.J.: Longitudinal study of cholesterol values in 68 children from birth to 11 years of age. *Archives of Disease in Childhood*, vol. 66, no. 1, pp. 134–137, 1991.
- [187] Jett, P.L., Samuels, M.H., McDaniel, P.A., Benda, G.I., LaFranchi, S.H., Reynolds, J.W. and Hanna, C.E.: Variability of plasma cortisol levels in extremely low birth weight infants. *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 9, pp. 2921–2925, 1997.
- [188] Wilkinson, I. and Cockcroft, J.R.: Cholesterol, lipids and arterial stiffness. *Advances in Cardiology*, vol. 44, pp. 261–277, 2007.
- [189] Delles, C. and Dominiczak, A.F.: Vascular failure or sick vessel syndrome: the cardiovascular continuum is a useful concept for clinical research. *Journal of Hypertension*, vol. 24, no. 11, pp. 2147–2148, 2006.
- [190] van der Meer, R.W., Diamant, M., Westenberg, J.J.M., Doornbos, J., Bax, J.J., de Roos, A. and Lamb, H.J.: Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus. *Journal of Cardiovascular Magnetic Resonance*, vol. 9, no. 4, pp. 645 – 651, 2007.
- [191] Lee, J.M.S., Shirodaria, C., Jackson, C.E., Robson, M.D., Antoniades, C., Francis, J.M., Wiesmann, F., Channon, K.M., Neubauer, S. and Choudhury, R.P.: Multi-modal magnetic resonance imaging quantifies atherosclerosis and vascular dysfunction in patients with type 2 diabetes mellitus. *Diabetes and Vascular Disease Research*, vol. 4, no. 1, pp. 44–48, 2007.
- [192] Hickson, S.S., Butlin, M., Graves, M., Taviani, V., Avolio, A.P., McEniery, C.M. and Wilkinson, I.B.: The relationship of age with regional aortic stiffness and diameter. *Journal of the American College of Cardiology: Cardiovascular Imaging*, vol. 3, no. 12, pp. 1247–1255, 2010.
- [193] 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*, vol. 31, no. 19, pp. 2338–2350, 2010.
- [194] Vlachopoulos, C., Aznaourdis, K. and Stefanadis, C.: Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of the American College of Cardiology*, vol. 55, no. 13, pp. 1318–1327, 2010.

- [195] Rider, O.J., Tayal, U., Francis, J.M., Ali, M.K., Robinson, M.R., Byrne, J.P., Clarke, K. and Neubauer, S.: The effect of obesity and weight loss on aortic pulse wave velocity as assessed by magnetic resonance imaging. *Obesity*, vol. 18, no. 12, pp. 2311–2316, 2010.
- [196] Rodriguez, C., Raposo, B., Martinez-Gonzalez, J., Casani, L. and Badimon, L.: Low density lipoproteins downregulate lysyl oxidase in vascular endothelial cells and the arterial wall. *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 9, pp. 1409–1414, 2002.
- [197] Massaeli, H., Hurtado, C., Austria, J.A. and Pierce, G.N.: Oxidized low-density lipoprotein induces cytoskeletal disorganization in smooth muscle cells. *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 277, no. 5, pp. H2017–H2025, 1999.
- [198] Mitchell, G.F., Parise, H., Benjamin, E.J., Larson, M.G., Keyes, M.J., Vita, J.A., Vasan, R.S. and Levy, D.: Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension*, vol. 43, no. 6, pp. 1239–1245, 2004.
- [199] Lewandowski, A.J., Pitcher, A., Banerjee, R. and Leeson, P.: Arterial stiffness: using simple surrogate measures to make sense of a biologically complex phenomenon. *Hypertension Research*, vol. 35, pp. 155–156, 2012.
- [200] Ónody, A., Csonka, C., Giricz, Z. and Ferdinandy, P.: Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. *Cardiovascular Research*, vol. 58, no. 3, pp. 663–670, 2003.
- [201] Webber, L.S., Srinivasan, S.R., Wattigney, W.A. and Berenson, G.S.: Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. *American Journal of Epidemiology*, vol. 133, no. 9, pp. 884–899, 1991.
- [202] Swee, M.H., Parks, W.C. and Pierce, R.A.: Developmental regulation of elastin production. *Journal of Biological Chemistry*, vol. 270, no. 25, pp. 14899–14906, 1995.
- [203] Pulgar, V.M. and Figueroa, J.P.: Antenatal betamethasone administration has a dual effect on adult sheep vascular reactivity. *Pediatric Research*, vol. 60, no. 6, pp. 705–710, 2006.

- [204] Roghair, R.D., Segar, J.L., Kilpatrick, R.A., Segar, E.M., Scholz, T.D. and Lamb, F.S.: Murine aortic reactivity is programmed equally by maternal low protein diet or late gestation dexamethasone. *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 20, no. 11, pp. 833–841, 2007.
- [205] Dodic, M., Moritz, K. and Wintour, E.M.: Prenatal exposure to glucocorticoids and adult disease. *Archives of Physiology and Biochemistry*, vol. 111, no. 1, pp. 61–69, 2003.
- [206] Seckl, J.R. and Meaney, M.J.: Glucocorticoid programming. *Annals of the New York Academy of Sciences*, vol. 1032, no. 1, pp. 63–84, 2004.
- [207] Fletcher, A.J.W., McGarrigle, H.H.G., Edwards, C.M.B., Fowden, A.L. and Giussani, D.A.: Effects of low dose dexamethasone treatment on basal cardiovascular and endocrine function in fetal sheep during late gestation. *The Journal of Physiology*, vol. 545, no. 2, pp. 649–660, 2002.
- [208] Newnham, J.P., Moss, T.J.M., Nitsos, I. and Sloboda, D.M.: Antenatal corticosteroids: the good, the bad and the unknown. *Current Opinion in Obstetrics and Gynecology*, vol. 14, no. 6, pp. 607–612, 2002.
- [209] Anderson, T.J., Uehata, A., Gerhard, M.D., Meredith, I.T., Knab, S., Delagrang, D., Lieberman, E.H., Ganz, P., Creager, M.A., Yeung, A.C. and Selwyn, A.P.: Close relation of endothelial function in the human coronary and peripheral circulations. *Journal of the American College of Cardiology*, vol. 26, no. 5, pp. 1235–1241, 1995.
- [210] ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 475: Antenatal corticosteroid therapy for fetal maturation. *Obstetrics & Gynecology*, vol. 117, no. 2 pt 1, pp. 422–424, 2011.
- [211] Meadow, W.L., Bell, A. and Sunstein, C.R.: Statistics, not memories: what was the standard of care for administering antenatal steroids to women in preterm labor between 1985 and 2000? *Obstetrics & Gynecology*, vol. 102, no. 2, pp. 356–362, 2003.
- [212] Polyakov, A., Cohen, S., Baum, M., Trickey, D., Jolley, D. and Wallace, E.M.: Patterns of antenatal corticosteroid prescribing 1998–2004. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, vol. 47, no. 1, pp. 42–45, 2007.

- [213] Redheuil, A., Yu, W.C., Mousseaux, E., Harouni, A.A., Kachenoura, N., Wu, C.O., Bluemke, D. and Lima, J.A.C.: Age-related changes in aortic arch geometry: Relationship with proximal aortic function and left ventricular mass and remodeling. *Journal of the American College of Cardiology*, vol. 58, no. 12, pp. 1262–1270, 2011.
- [214] Redheuil, A., Yu, W.C., Wu, C.O., Mousseaux, E., de Cesare, A., Yan, R., Kachenoura, N., Bluemke, D. and Lima, J.A.C.: Reduced ascending aortic strain and distensibility. *Hypertension*, vol. 55, no. 2, pp. 319–326, 2010.
- [215] O'Rourke, M.F. and Hashimoto, J.: Mechanical factors in arterial aging: a clinical perspective. *Journal of the American College of Cardiology*, vol. 50, no. 1, pp. 1–13, 2007.
- [216] Wallace, D.C.: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annual Review of Genetics*, vol. 39, pp. 359–407, 2005.
- [217] Bendeck, M.P. and Langille, B.L.: Rapid accumulation of elastin and collagen in the aortas of sheep in the immediate perinatal period. *Circulation Research*, vol. 69, no. 4, pp. 1165–1169, 1991.
- [218] Wells, S.M., Langille, B.L., Lee, J.M. and Adamson, S.L.: Determinants of mechanical properties in the developing ovine thoracic aorta. *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 277, no. 4, pp. H1385–H1391, 1999.
- [219] Chinoy, M.R., Zgleszewski, S.E., Cilley, R.E. and Krummel, T.M.: Dexamethasone enhances ras-recision gene expression in cultured murine fetal lungs: role in development. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, vol. 279, no. 2, pp. L312–L318, 2000.
- [220] Mulder, E.J.H., de Heus, R. and Visser, G.H.A.: Antenatal corticosteroid therapy: short-term effects on fetal behaviour and haemodynamics. *Seminars in Fetal and Neonatal Medicine*, vol. 14, no. 3, pp. 151–156, 2009.
- [221] Cavalcante, J.L., Lima, J. A.C., Redheuil, A. and Al-Mallah, M.H.: Aortic stiffness: current understanding and future directions. *Journal of the American College of Cardiology*, vol. 57, no. 14, pp. 1511–1522, 2011.

- [222] Sokolis, D.P.: Passive mechanical properties and structure of the aorta: segmental analysis. *Acta Physiologica*, vol. 190, no. 4, pp. 277–289, 2007.
- [223] Moïse, A.A., Wearden, M.E., Kozinetz, C.A., Gest, A.L., Welty, S.E. and Hansen, T.N.: Antenatal steroids are associated with less need for blood pressure support in extremely premature infants. *Pediatrics*, vol. 95, no. 6, pp. 845–850, 1995.
- [224] Nair, G.V. and Omar, S.A.: Blood pressure support in extremely premature infants is affected by different courses of antenatal steroids. *Acta Paediatrica*, vol. 98, no. 9, pp. 1437–1443, 2009.
- [225] Phan-Hug, F., Guimiot, F., Lelièvre, V., Delezoide, A.L., Czernichow, P., Breant, B. and Blondeau, B.: Potential role of glucocorticoid signaling in the formation of pancreatic islets in the human fetus. *Pediatric Research*, vol. 64, no. 4, pp. 346–351, 2008.
- [226] Collaborative Dexamethasone Trial Group. Dexamethasone therapy in neonatal chronic lung disease: an international placebo controlled trial. *Pediatrics*, vol. 88, no. 3, pp. 421–427, 1991.
- [227] Porto, A.M., Coutinho, I.C., Correia, J.B. and Amorim, M.M.: Effectiveness of antenatal corticosteroids in reducing respiratory disorders in late preterm infants: randomised clinical trial. *British Medical Journal*, vol. 342, p. d1696, 2011.
- [228] Miracle, X., Di Renzo, G.C., Stark, A., Fanaroff, A., Carbonell-Estrany, X., Saling, E. and Coordinators Of World Association of Perinatal Medicine Prematurity Working Group: Guideline for the use of antenatal corticosteroids for fetal maturation. *Journal of Perinatal Medicine*, vol. 36, no. 3, pp. 191–196, 2008.
- [229] Fonseca, C.G., Backhaus, M., Bluemke, D.A., Britten, R.D., Chung, J.D., Cowan, B.R., Dinov, I.D., Finn, J.P., Hunter, P.J., Kadish, A.H., Lee, D.C., Lima, J.A.C., Medrano-Gracia, P., Shivkumar, K., Suinesiaputra, A., Tao, W. and Young, A.A.: The Cardiac Atlas Project - an imaging database for computational modeling and statistical atlases of the heart. *Bioinformatics*, vol. 27, no. 16, pp. 2288–2295, 2011.
- [230] Lamata, P., Niederer, S., Nordsletten, D., Barber, D.C., Roy, I., Hose, D.R. and Smith, N.: An accurate, fast and robust method to generate patient-specific cubic hermite meshes. *Medical Image Analysis*, vol. 15, no. 6, pp. 801–813, 2011.

- [231] Gianni, D., McKeever, S., Yu, T., Britten, R., Delingette, H., Frangi, A., Hunter, P. and Smith, N.: Sharing and reusing cardiovascular anatomical models over the web: a step towards the implementation of the virtual physiological human project. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, vol. 368, no. 1921, pp. 3039–3056, 2010.
- [232] Lorell, B.H. and Carabello, B.A.: Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation*, vol. 102, no. 4, pp. 470–479, 2000.
- [233] Rider, O.J., Lewandowski, A., Nethononda, R., Petersen, S.E., Francis, J.M., Pitcher, A., Holloway, C.J., Dass, S., Banerjee, R., Byrne, J.P., Leeson, P. and Neubauer, S.: Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. *European Heart Journal*, vol. 34, no. 4, pp. 292–299, 2012.
- [234] Krittayaphong, R., Boonyasirinant, T., Saiviroonporn, P., Thanapiboonpol, P., Nakyen, S., Ruksakul, K. and Udompunturak, S.: Prognostic significance of left ventricular mass by magnetic resonance imaging study in patients with known or suspected coronary artery disease. *Journal of Hypertension*, vol. 27, no. 11, pp. 2249–2256, 2009.
- [235] Levy, D., Garrison, R.J., Savage, D.D., Kannel, W.B. and Castelli, W.P.: Prognostic implications of echocardiographically determined left ventricular mass in the framingham heart study. *New England Journal of Medicine*, vol. 322, no. 22, pp. 1561–1566, 1990.
- [236] de Simone, G., Verdecchia, P., Pede, S., Gorini, M. and Maggioni, A.P.: Prognosis of inappropriate left ventricular mass in hypertension: the MAVI Study. *Hypertension*, vol. 40, no. 4, pp. 470–476, 2002.
- [237] Kozák-Bárány, A., Jokinen, E., Saraste, M., Tuominen, J. and Valimaki, I.: Development of left ventricular systolic and diastolic function in preterm infants during the first month of life: a prospective follow-up study. *The Journal of Pediatrics*, vol. 139, no. 4, pp. 539–545, 2001.
- [238] Teitel, D.F., Iwamoto, H.S. and Rudolph, A.M.: Effects of birth-related events on central blood flow patterns. *Pediatric Research*, vol. 22, no. 5, pp. 557–566, 1987.
- [239] St. John Sutton, M.G., Gewitz, M.H., Shah, B., Cohen, A., Reichek, N., Gabbe, S. and Huff, D.S.: Quantitative assessment of growth and function of the cardiac chambers in the normal human

- fetus: a prospective longitudinal echocardiographic study. *Circulation*, vol. 69, no. 4, pp. 645–654, 1984.
- [240] Romero, R., Espinoza, J., Goncalves, L.F., Kusanovic, J.P., Friel, L.A. and Nien, J.K.: Inflammation in preterm and term labour and delivery. *Seminars in Fetal and Neonatal Medicine*, vol. 11, no. 5, pp. 317–326, 2006.
- [241] Yu, Q., Horak, K. and Larson, D.F.: Role of T lymphocytes in hypertension-induced cardiac extracellular matrix remodeling. *Hypertension*, vol. 48, no. 1, pp. 98–104, 2006.
- [242] Brower, G., Gardner, J., Forman, M., Murray, D., Voloshenyuk, T., Levick, S. and Janicki, J.: The relationship between myocardial extracellular matrix remodeling and ventricular function. *European Journal of Cardio-Thoracic Surgery*, vol. 30, no. 4, pp. 604–610, 2006.
- [243] Cho, G., Marwick, T.H., Kim, H., Kim, M., Hong, K. and Oh, D.: Global 2-dimensional strain as a new prognosticator in patients with heart failure. *Journal of the American College of Cardiology*, vol. 54, no. 7, pp. 618–624, 2009.
- [244] Borke, W.B., Edvardsen, T., Fugelseth, D., Lenes, K., Ihlen, H., Saugstad, O.D. and Thaulow, E.: Reduced left ventricular function in hypoxemic newborn pigs: a strain Doppler echocardiographic study. *Pediatric Research*, vol. 59, no. 5, pp. 630–635, 2006.
- [245] Henein, M.Y. and Gibson, D.G.: Long axis function in disease. *Heart*, vol. 81, no. 3, pp. 229–231, 1999.
- [246] Jones, C.J., Raposo, L. and Gibson, D.G.: Functional importance of the long axis dynamics of the human left ventricle. *British Heart Journal*, vol. 63, no. 4, pp. 215–220, 1990.
- [247] Morton, G., Schuster, A., Jogiya, R., Kutty, S., Beerbaum, P. and Nagel, E.: Inter-study reproducibility of cardiovascular magnetic resonance myocardial feature tracking. *Journal of Cardiovascular Magnetic Resonance*, vol. 14, p. 43, 2012.
- [248] Pinson, C.W., Morton, M.J. and Thornburg, K.L.: An anatomic basis for fetal right ventricular dominance and arterial pressure sensitivity. *Journal of Developmental Physiology*, vol. 9, no. 3, pp. 253–269, 1987.

- [249] Rudolph, A.M.: The fetal circulation and its adjustments after birth in congenital heart disease. *UCLA Forum of Medical Sciences*, vol. 10, pp. 105–118, 1970.
- [250] Rudolph, A.M.: The changes in the circulation after birth: their importance in congenital heart disease. *Circulation*, vol. 41, no. 2, pp. 343–359, 1970.
- [251] Haddad, F., Hunt, S.A., Rosenthal, D.N. and Murphy, D.J.: Right ventricular function in cardiovascular disease, part I: anatomy, physiology, aging, and functional assessment of the right ventricle. *Circulation*, vol. 117, no. 11, pp. 1436–1448, 2008.
- [252] Augustine, D., Suttie, J.J., Cox, P., Lewandowski, A.J., Holloway, C., Petersen, S.E., Myerson, S., Neubauer, S. and Leeson, P.: CMR right ventricular strain assessment using feature tracking cine images: agreement with echocardiography. *Journal of Cardiovascular Magnetic Resonance*, vol. 14, no. Suppl 1, p. 244, 2012.
- [253] Katz, J., Whang, J., Boxt, L.M. and Barst, R.: Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary hypertension by nuclear magnetic resonance imaging. *Journal of the American College of Cardiology*, vol. 21, no. 6, pp. 1475–1481, 1993.
- [254] Boxt, L.M., Katz, J., Kolb, T., Czegledy, F.P. and Barst, R.: Direct quantitation of right and left ventricular volumes with nuclear magnetic resonance imaging in patients with primary pulmonary hypertension. *Journal of the American College of Cardiology*, vol. 19, no. 7, pp. 1508–1515, 1992.
- [255] Maceira, A.M., Prasad, S.K., Khan, M. and Pennell, D.J.: Reference right ventricular systolic and diastolic function normalized to age, gender and body surface area from steady-state free precession cardiovascular magnetic resonance. *European Heart Journal*, vol. 27, no. 23, pp. 2879–2888, 2006.
- [256] Haddad, F., Doyle, R., Murphy, D.J. and Hunt, S.A.: Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. *Circulation*, vol. 117, no. 13, pp. 1717–1731, 2008.
- [257] Kawut, S.M., Barr, R.G., Lima, J.A., Praestgaard, A., Johnson, W.C., Chahal, H., Ogunyankin, K.O., Bristow, M.R., Kizer, J.R., Tandri, H. and Bluemke, D.A.: Right ventricular structure is

- associated with the risk of heart failure and cardiovascular death: the Multi-Ethnic Study of Atherosclerosis (MESA)–right ventricle study. *Circulation*, vol. 126, no. 14, pp. 1681–1688, 2012.
- [258] Voelkel, N.F., Quaife, R.A., Leinwand, L.A., Barst, R.J., McGoon, M.D., Meldrum, D.R., Dupuis, J., Long, C.S., Rubin, L.J., Smart, F.W., Suzuki, Y.J., Gladwin, M., Denholm, E.M. and Gail, D.B.: Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. *Circulation*, vol. 1114, no. 17, pp. 1883–1891, 2006.
- [259] Hudsmith, L.E., Petersen, S.E., Francis, J.M., Robson, M.D. and Neubauer, S.: Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. *Journal of Cardiovascular Magnetic Resonance*, vol. 7, no. 5, pp. 775–782, 2005.
- [260] Kawut, S.M., Lima, J.A.C., Barr, R.G., Chahal, H., Jain, A., Tandri, H., Praestgaard, A., Bagiella, E., Kizer, J.R., Johnson, W.C., Kronmal, R.A. and Bluemke, D.A.: Sex and race differences in right ventricular structure and function: The Multi-Ethnic Study of Atherosclerosis-right ventricle study. *Circulation*, vol. 123, no. 22, pp. 2542–2551, 2011.
- [261] Todiere, G., Neglia, D., Ghione, S., Fommei, E., Capozza, P., Guarini, G., Dell’Omo, G., Aquaro, G.D., Marzilli, M., Lombardi, M., Camici, P. and Pedrinelli, R.: Right ventricular remodelling in systemic hypertension: a cardiac MRI study. *Heart*, vol. 97, no. 15, pp. 1257–1261, 2011.
- [262] Edstedt Bonamy, A.K., Bengtsson, J., Nagy, Z., De Keyser, H. and Norman, M.: Preterm birth and maternal smoking in pregnancy are strong risk factors for aortic narrowing in adolescence. *Acta Paediatrica*, vol. 97, no. 8, pp. 1080–1085, 2008.
- [263] Antonios, T.F., Raghuraman, R.P., D’Souza, R., Nathan, P., Wang, D. and Manyonda, I.T.: Capillary remodeling in infants born to hypertensive pregnancy: pilot study. *American Journal of Hypertension*, vol. 25, no. 8, pp. 848–853, 2012.
- [264] Shore, A.C. and Tooke, J.E.: Microvascular function in human essential hypertension. *Journal of Hypertension*, vol. 12, no. 7, pp. 717–728, 1994.

- [265] Antonios, T.F., Rattray, F.M., Singer, D.R.J., Markandu, N.D., Mortimer, P.S. and MacGregor, G.A.: Rarefaction of skin capillaries in normotensive offspring of individuals with essential hypertension. *Heart*, vol. 89, no. 2, pp. 175–178, 2003.
- [266] Levy, B.I., Schiffrin, E.L., Mourad, J.J., Agostini, D., Vicaud, E., Safar, M.E. and Struijker-Boudier, H.A.: Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. *Circulation*, vol. 118, no. 9, pp. 968–976, 2008.
- [267] Ungvari, Z., Csiszar, A., Kaminski, P.M., Wolin, M.S. and Koller, A.: Chronic high pressure-induced arterial oxidative stress: involvement of protein kinase C-dependent NAD(P)H oxidase and local renin-angiotensin system. *The American Journal of Pathology*, vol. 165, no. 1, pp. 219–226, 2004.
- [268] Jacobson, A., Yan, C., Gao, Q., Rincon-Skinner, T., Rivera, A., Edwards, J., Huang, A., Kaley, G. and Sun, D.: Aging enhances pressure-induced arterial superoxide formation. *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 293, no. 3, pp. H1344–1350, 2007.
- [269] D'Souza, R., Raghuraman, R.P., Nathan, P., Manyonda, I.T. and Antonios, T.F.: Low birth weight infants do not have capillary rarefaction at birth: implications for early life influence on microcirculation. *Hypertension*, vol. 58, no. 5, pp. 847–851, 2011.
- [270] Pladys, P., Sennlaub, F., Brault, S., Checchin, D., Lahaie, I., Le, N.L., Bibeau, K., Cambonie, G., Abran, D., Brochu, M., Thibault, G., Hardy, P., Chemtob, S. and Nuyt, A.M.: Microvascular rarefaction and decreased angiogenesis in rats with fetal programming of hypertension associated with exposure to a low-protein diet in utero. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, vol. 289, no. 6, pp. R1580–R1588, 2005.
- [271] Pugh, C.W. and Ratcliffe, P.J.: Regulation of angiogenesis by hypoxia: role of the HIF system. *Nature Medicine*, vol. 9, no. 6, pp. 677–684, 2003.
- [272] Tal, R.: The role of hypoxia and hypoxia-inducible factor-1 α in preeclampsia pathogenesis. *Biology of Reproduction*, vol. 87, no. 6, p. 134, 2012.
- [273] Karumanchi, S.A. and Bdolah, Y.: Hypoxia and sFlt-1 in preeclampsia: the "chicken-and-egg" question. *Endocrinology*, vol. 145, no. 11, pp. 4835–4837, 2004.

- [274] Kvehaugen, A.S., Dechend, R., Ramstad, H.B., Troisi, R., Fugelseth, D. and C., S.A.: Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. *Hypertension*, vol. 58, no. 1, pp. 63–69, 2011.
- [275] Smith, L.J., van Asperen, P.P., McKay, K.O., Selvadurai, H. and Fitzgerald, D.A.: Reduced exercise capacity in children born very preterm. *Pediatrics*, vol. 122, no. 2, pp. e287–e293, 2008.
- [276] Grewal, J., McCully, R.B., Kane, G.C., Lam, C. and Pellikka, P.A.: Left ventricular function and exercise capacity. *The Journal of the American Medical Association*, vol. 301, no. 3, pp. 286–294, 2009.
- [277] Barmeyer, A., Müllerleile, K., Mortensen, K. and Meinertz, T.: Diastolic dysfunction in exercise and its role for exercise capacity. *Heart Failure Reviews*, vol. 14, no. 2, pp. 125–134, 2009.
- [278] Kannel, W.B., Wilson, P. and Blair, S.N.: Epidemiological assessment of the role of physical activity and fitness in development of cardiovascular disease. *American Heart Journal*, vol. 109, no. 4, pp. 876–885, 1985.
- [279] Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S. and Atwood, J.E.: Exercise capacity and mortality among men referred for exercise testing. *New England Journal of Medicine*, vol. 346, no. 11, pp. 793–801, 2002.
- [280] Blair, S., Kohl, H.W., Paffenbarger, R.S., Clark, D.G., Cooper, K.H. and Gibbons, L.W.: Physical fitness and all-cause mortality: a prospective study of healthy men and women. *The Journal of the American Medical Association*, vol. 262, no. 17, pp. 2395–2401, 1989.
- [281] Flett, A.S., Hayward, M.P., Ashworth, M.T., Hansen, M.S., Taylor, A.M., Elliott, P.M., McGregor, C. and Moon, J.C.: Equilibrium contrast cardiovascular magnetic resonance for the measurement of diffuse myocardial fibrosis. *Circulation*, vol. 122, no. 2, pp. 138–144, 2010.

APPENDICES

A

LIFESTYLE AND MEDICAL HISTORY QUESTIONNAIRE

The following questionnaire was completed by all participants to provide information on their medical history, their parental medical history, their normal lifestyle, their early life history, and their sociodemographic characteristics.

EVS ID: _____

Early Vascular Study

Subject's Initials

Date of Birth

Study Number

Questions about the subject's medical history

1a Have you ever been told that you have high blood pressure?	No	Yes	
1b Have you ever been treated for high blood pressure?	No	Yes	Don't know
1c If yes, how were you treated (including the name of any medication)			
1d If female , were you pregnant when you had high blood pressure?	No	Yes	
2a Have you ever been told that you have diabetes?	No	Yes	
2b Have you ever been treated for diabetes?	No	Yes	Don't know
2c If yes, how were you treated (including the name of any medication)			
2d If female , were you pregnant when you developed diabetes?	No	Yes	
3a Have you ever been told that you have high cholesterol?	No	Yes	
3b Have you ever been treated for high cholesterol?	No	Yes	Don't know
3c If yes, how were you treated (including the name of any medication)			
4a Has any long term illness been diagnosed?	No	Yes	
4b If yes, what is the illness?			
4c Have you been treated for this condition?	No	Yes	Don't know
4d If yes, how were you treated (including the name of any medication)			
5a Are you taking any other medicines, tablets or pills regularly?	No	Yes	
5b If yes, what are they (if female, include oral contraceptive if normally taken)			
.....		for how long	
.....		for how long	
.....		for how long	

Questions about the subject's family medical history

Have any members of your family ever suffered from (if more than 2 affected siblings continue on blank paper): Please tick all that apply

	mother	m age of onset	father	f age of onset	sibling1	s1 age of onset	sibling2	s2 age of onset
6 angina	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐
7 heart attack	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐
8 high blood pressure	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐
9 stroke	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐
10 diabetes	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐
11 high blood cholesterol	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐	☐	☐ ☐

12a Is there a illness which runs in your family?

No

Yes

Don't know

12b If yes, what is the illness

Questions about the subject's smoking, alcohol and diet

14 What is your normal weekly intake of..

14a Beer, lager or cider pints

14b Red Winewineglasses

White Winewineglasses

14c Sherry, Port and aperitifs..... glasses

14d Spirits pub measures

15a Do you **currently** smoke cigarettes, cigars or a pipe regularly? (e.g. at least 1 cigarette a day for the last 6 months) No Yes

15b If yes, how many cigarettes do you smoke each day at present?cigarettes

how many cigars do you smoke each day at present?cigars

how many ounces of tobacco do you smoke each day at present?ounces

15c Have you in the **past** smoked cigarettes, cigars or a pipe regularly? No Yes

(e.g. at least 1 cigarette per day for a period of 6 months)

15d If yes, how long did you smoke for?months

15e If yes, how many cigarettes did you smoke each day?cigarettes

how many cigars did you smoke each day?cigars

how many ounces of tobacco in a pipe did you smoke each day?..... ounces

15f If you are a smoker or have ever smoked, how old were you when you started smoking?.....years.....

Questions about the subject's exercise

17b For how many hours a week do you take part in these activities?

Questions to ensure a balanced sociodemographic group

18 Are you currently....

married, in a stable relationship

single, divorced, widowed, separated

19a What is the highest educational qualification/training you have obtained?

None GCSE or equivalent A-levels or equivalent Degree or higher

19b Are you

...in full-time paid employment?

...in part-time paid employment?

...caring for the home or children full time?

...a student/apprentice

20a What is/was the name of your most recent job/occupation?

.....

20b What job did or do you actually do?

.....

20c Are you

...a manager working for an employer?

...a foreman or supervisor working for an employer?

...working for an employer/apprentice/student?

...self employed with employees?

...self employed without employees?

Early Life History

13 Where were you born?

14a Were you born prematurely? Yes No

14b What was your gestational age at birth?.....

15 How heavy were you when you were born?

16 How were you fed in early life? Breast-fed Bottle-fed Mixture of both Don't know

16b If **breast-fed**, for how many months?

17 Did your mother have pre-eclampsia when having you? Yes No

17b Or did she have gestational hypertension while pregnant with you? Yes No



PARENTAL SMOKING QUESTIONNAIRE

The following questionnaire was completed by all participants to provide additional information on maternal and paternal smoking prior to, during, and after the pregnancy of interest.

Early Vascular Study ID: _____

Questions about smoking

Please fill out the following questions as accurately as you can.

Please answer questions based on who was in your household when you were growing up. For instance, if instead of a 'father' and 'mother' your household was your 'mother' and a 'stepfather', or a 'main carer' and 'their partner', then please answer the questions titled 'Smoking and your Mother' based on the smoking patterns of your main carer and the questions titled 'Smoking and your Father' based on the secondary care giver in the household.

Smoking and your Mother

	Yes	No
Does your mother currently smoke?	☐	☐
If yes , how many cigarettes does she smoke per day? cigarettes		
At what age did she start smoking? years		
	Yes	No
If no , has she ever smoked?	☐	☐
from what age did she smoke?years		
at what age did she stop smoking?.....years		
how much did she smoke on average per day?		

Early Vascular Study ID: _____

During pregnancy:

	Yes	No
Did she smoke when they were pregnant with you?	☐	☐

	Peri-conception	1 st trimester	2 nd trimester	3 rd trimester	First year
If yes , at what stages of pregnancy did she smoke? (please tick boxes to indicate which stages)	☐	☐	☐	☐	☐

	Peri-conception	1 st trimester	2 nd trimester	3 rd trimester	First year
How many cigarettes did she smoke per day at each stage?					

Did she quit smoking for any length of time during the pregnancy?

	Yes	No
If yes , was nicotine replacement therapy used?	☐	☐

During your childhood:

	Yes	No
Did she smoke when you were a child or teenager?	☐	☐

If **yes**, how old were you when she started (if she has always smoked put 0)
how old were you when she stopped (if she is still smoking put your current age)
how many cigarettes did/does she smoke on average per day?cigarettes
did she smoke inside or outside the home?.....

Early Vascular Study ID: _____

Smoking and your Father

Does your father smoke currently? Yes No
☐ ☐

If **yes**, how many cigarettes are smoked per day? cigarettes
how many cigars are smoked per day? cigars
how many ounces of tobacco are smoked per day? ounces
At what age did they start smoking? Years

During pregnancy:

Did your father smoke when your mother was pregnant with you? Yes No
☐ ☐

	Peri- conception	1 st trimester	2 nd trimester	3 rd trimester	First year
If yes , at what stages of pregnancy did he smoke? (please tick boxes to indicate which stages)	☐	☐	☐	☐	☐

How many cigarettes were smoked per day at each stage?					
---	-------	-------	-------	-------	-------

Did smoking take place **INSIDE** or **OUTSIDE** of the home? (circle one please)

Early Vascular Study ID: _____

During childhood:

Did your father smoke in the years following the pregnancy?

Yes

☐

No

☐

If **yes**, between what ages of your child did he smoke?years

how many cigarettes were smoked on average per day?cigarettes

did smoking take place inside or outside of the home?.....

Other household members?

Yes

☐

No

☐

Did any other members of the household (living in your house) smoke?

If **yes**, what relation were they to you e.g. brother, aunt etc.

.....

	Peri- conception	1 st trimester	2 nd trimester	3 rd trimester	First year	First year until present
Was a smoker present in the home during any stage of pregnancy or your childhood?	☐	☐	☐	☐	☐	☐
Approximately how many cigarettes were smoked per day?						